Abstracts of papers presented at the

EMBO Conference on Fission Yeast: *pombe* 2013

7th International Fission Yeast Meeting
London, United Kingdom, 24 - 29 June 2013

Meeting Organizers:

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Open Biology *http://rsob.royalsocietypublishing.org*

SCHEDULE OVERVIEW

Please always wear your name batches, which are checked for catering.

MONDAY, 24 JUNE

10:00/11:30	Optional Tours to Down House (guided by *Steve Jones*)
From 16:00	Registration
17:30 - 19:30	**Welcome reception with food** *Macmillan & Crush*
19:30 - 21:30	**Session 1: Special lectures** *Beveridge*
21:30-23:00	**PomBase launch party for community curation, desserts** *Macmillan & Crush*

TUESDAY, 25 JUNE

09:00 - 12:10	**Session 2: Epigenetics** *Beveridge*
10:20 - 10:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
	SET UP POSTERS
12:10 - 13:30	LUNCH *Macmillan, Crush & Grand Lobby*
12:30 - 13:00	**Demo of community curation** *Beveridge*
13:30 - 15:30	**Parallel Workshops:**
	Workshop A: Cell cycle *Beveridge*
	Workshop B: Chromatin & gene expression *Chancellors*
	Workshop C: Tools & resources *Senate Room*
15:30 - 16:00	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
16:00 - 17:40	**Session 3: DNA replication** *Beveridge*
18:00 - 19:30	DINNER *Macmillan, Crush & Grand Lobby*
19.30 - 21:30	**Poster Session 1: Odd numbered posters 1-221**
	with DRINKS *Macmillan, Crush & Grand Lobby*

WEDNESDAY, 26 JUNE

09:00 - 12:10	**Session 4: Gene regulation** *Beveridge*
10:20 - 10:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
	PACKED LUNCH, AFTERNOON FREE
20:00 - 21:40	**Session 5: Cell responses to environment** *Beveridge*
21:40 - 22:00	**Stage act** *with magician Tilman Andris Beveridge*
22:00 – 23:00	DRINKS *Macmillan, Crush & Grand Lobby*

SCHEDULE OVERVIEW

THURSDAY, 27 JUNE

09:00 - 12:10	**Session 6: DNA repair & recombination** *Beveridge*
10:20 - 10:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
12:10 - 13:30	LUNCH *Macmillan, Crush & Grand Lobby*
12:30 - 13:00	**Demo of PomBase and tools** *Beveridge*
13:30 - 15:30	**Parallel Workshops:** **Workshop D: DNA metabolism** *Beveridge* **Workshop E: Cell regulation** *Chancellors* **Workshop F: Genome-wide methods & bioinformatics** *Senate Room*
15:30 - 16:00	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
16:00 - 18:00	**Session 7: Cell division** *Beveridge*
18:00 - 19:30	DINNER *Macmillan, Crush & Grand Lobby*
19.30 - 21:30	**Poster Session 2: Even numbered posters 2-222** with DRINKS *Macmillan, Crush & Grand Lobby*

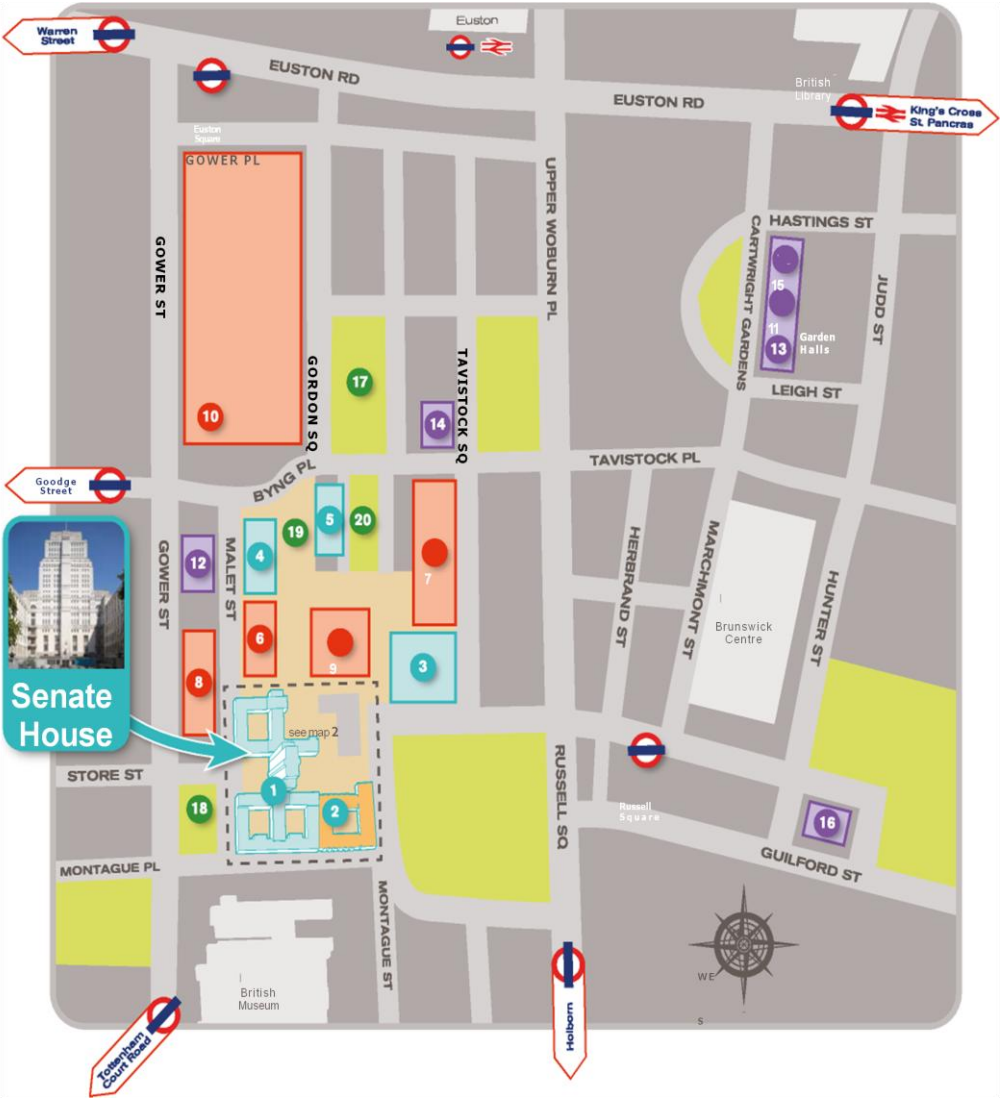
Friday, 28 June

09:00 - 12:10	**Session 8: Chromosomes** *Beveridge*
10:20 - 10:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
12:10 - 13:00	LUNCH *Macmillan, Crush & Grand Lobby*
12:30 - 13:30	**Meet the speakers** *Beveridge*
14:00 - 17:10	**Session 9: Cell morphology** *Beveridge*
15:20 - 15:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
	ALL POSTERS NEED TO BE REMOVED
18:30	Buses leave for Conference Dinner, Malet St Car Park (p. 8)
19:30 – 23:30	CONFERENCE DINNER *aboard the Dixie Queen* Live music by *IMMposters* Close up magician: *Laurent van Trigt* / Poster prizes

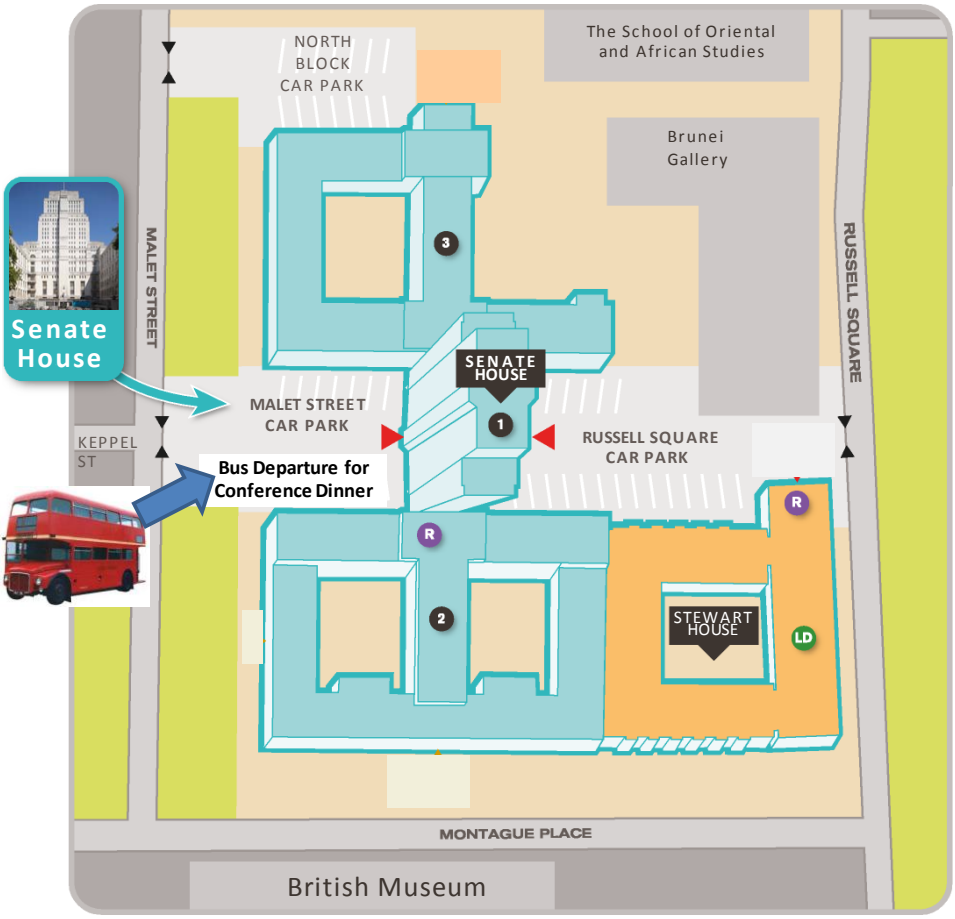
Saturday, 29 June

09:00 - 12:10	**Session 10: Mitosis & meiosis** *Beveridge*
10:20 - 10:50	COFFEE BREAK *Macmillan, Crush & Grand Lobby*
12:10	Meeting close, PACKED LUNCH

Around Senate House:

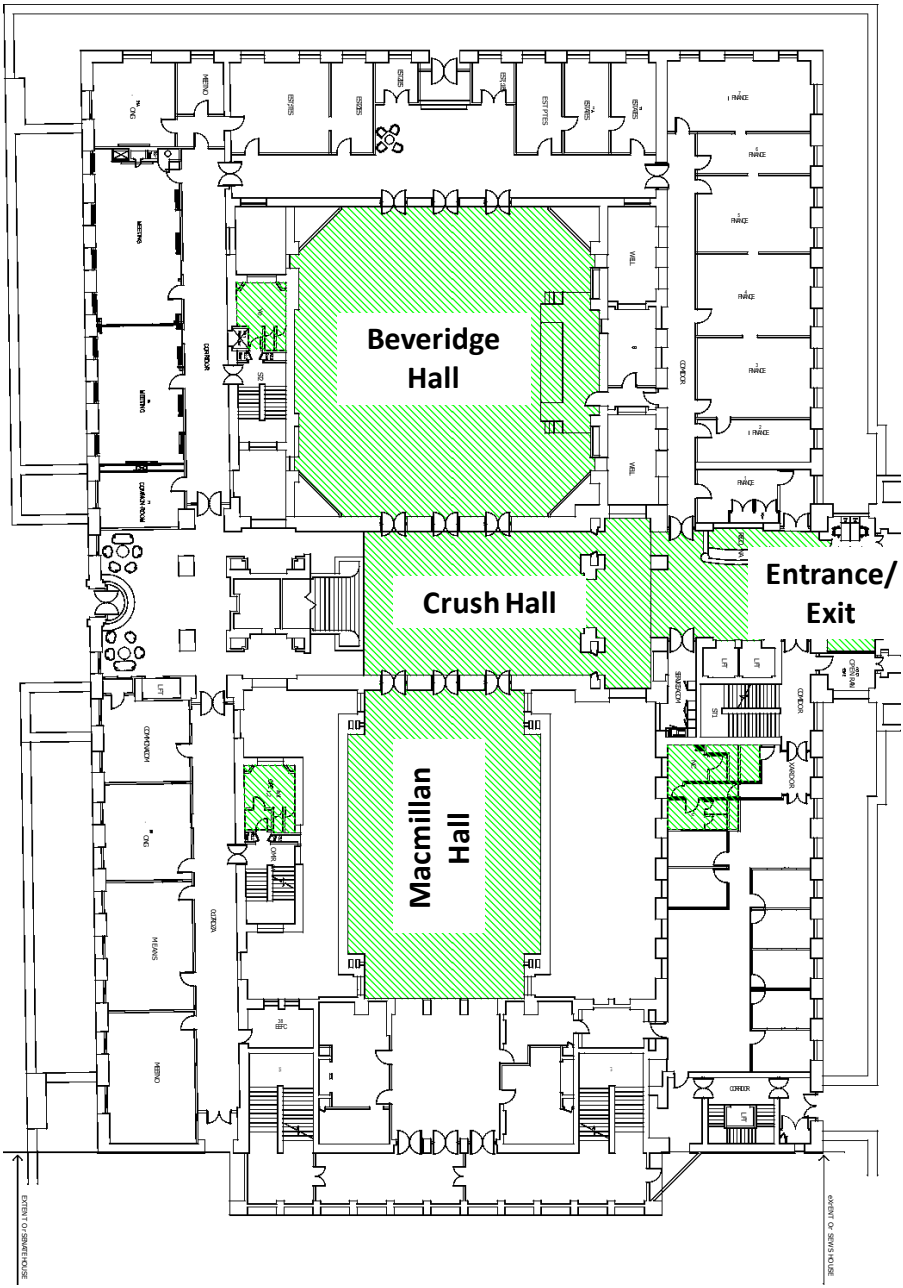


Enter Senate House from Malet Street or Russel Square Car Park. Buses for conference dinner leave from Malet St Car Park at 18:30:

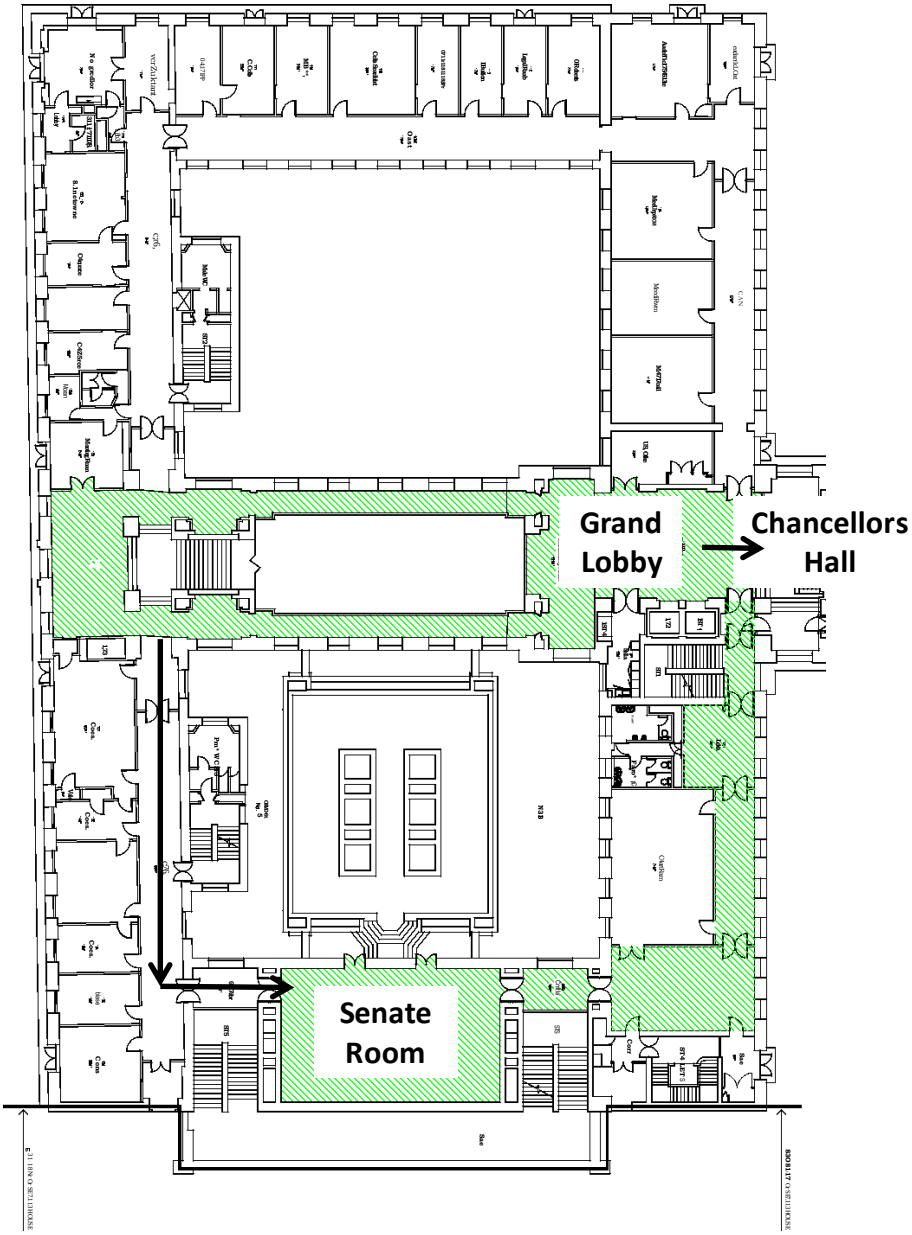


Senate House, our meeting venue, is the administrative centre of the University of London. The *Art Deco* building was constructed between 1932 and 1937. It is commonly said that *Hitler* had ordered against the bombing of Senate House, because he wanted to use it as his Headquarters in London, on account of its fascistic architectural style. The building did make an appropriate appearance in the film, *Batman Begins,* as the heart of corruption in Gotham City, and its brutalist exterior is said to have been the model for the Ministry of Truth in *George Orwell's Nineteen Eighty Four.*

Senate House, Ground Floor:



Senate House, First Floor:



THINGS TO DO CLOSE TO SENATE HOUSE

British Museum, Great Russell Street, London WC1B 3DG
FREE for regular collection, 2 min walk from Senate House. World-class museum showing highlights of human cultural achievements, from Egyptian mummies to cuckoo clocks. Greeks are reminded that artefacts may not be taken back home.
http://www.britishmuseum.org

Wellcome Collection, 183 Euston Road, London NW1 2BE
FREE, 10 min walk from Senate House. Interesting collection on history of medicine, and acclaimed temporal exhibition *Souzou: Outsider Art from Japan*. Nice café and book shop.
http://www.wellcomecollection.org/

London Zoo, Outer Circle Regent's Park, London NW1 4RY
£25.00, about 30 min walk from Senate House. To get there you will cross the pleasant Regent's Park, including a pretty Rose Garden which is in bloom now.
http://www.zsl.org/zsl-london-zoo

The British Library, 96 Euston Rd, London NW1 2DB
FREE for many exhibitions, about 15 min walk from Senate House. A great place to spend some time if you like books, stamps, prints, or music scores.
http://www.timeout.com/london/attractions/british-library

Grant Museum of Zoology, 21 University Street, London WC1E 6DE
FREE, 5 min walk from Senate House. Charming little museum that is packed full of skeletons, animals mounted or preserved in fluid, and glass models.
http://www.ucl.ac.uk/museums/zoology

Waterstone's Book Shop, 82 Gower St, London WC1E 6EQ21
Fantastic book shop on several floors, just 2 min walk from Senate House.

The Foundling Museum, 40 Brunswick Square, London WC1N 1AZ
£7.50, about 10 min walk from Senate House. This museum shows the history of Foundling Hospital, the UK's first children's charity, and celebrates the ways in which artists of all disciplines helped improve children's lives for over 270 years.
http://www.foundlingmuseum.org.uk/

Useful Links
Transport For London Journey Planner:
http://journeyplanner.tfl.gov.uk/user/XSLT_TRIP_REQUEST2?language=en

London Tube map: *http://www.tfl.gov.uk/gettingaround/1106.aspx*

Time Out's 'Cultural' Picks: http://www.timeout.com/london/things-to-do/101-things-to-do-in-london-cultural-highlights

Ask the volunteers or organizers for more information.

EATING AND DRINKING CLOSE TO SENATE HOUSE

Below is just a small selection. There are plenty of restaurants, pubs and cafés in the lively Fitzrovia area (about 10 min walk west of Senate House, past Tottenham Court Road, along *Goodge Street* and other lively side streets).

Another lively area with many restaurants and drinking places is *Covent Garden*, about 15 min walk south of Senate House.

The *Brunswick*, a shopping court about 10 min walk north-east of Senate House also contains several bars and restaurants.

Restaurants

Busaba Eathai, 22 Store St, London WC1E 7BS (Thai Restaurant, 5 min walk from venue)

TAS Restaurant, 22 Bloomsbury St, London WC1B 3QJ (Turkish Restaurant, 5 min walk from venue, just south of British Museum)

Yoisho, 33 Goodge Street, London W1T 2PS (authentic little Japanese Restaurant and drinking place)

ASK, 48 Grafton Way, London W1T 5DZ (Italian Restaurant, 10 min walk, north-west from venue, off Tottenham Court Road)

There are inexpensive Indian and African Restaurants on *Drummond Street*, just west of Euston Train Station, about 10 min walk from venue, e.g. **Chutneys** (124 Drummond St, London NW1 2PA) or **Diwana** (121-123 Drummond St)

Pubs

Jeremy Bentham, 31 University St, London, WC1E 6JL
A favourite with UCL staff and students, about 5 min walk north-west of Senate House. Also serves food.

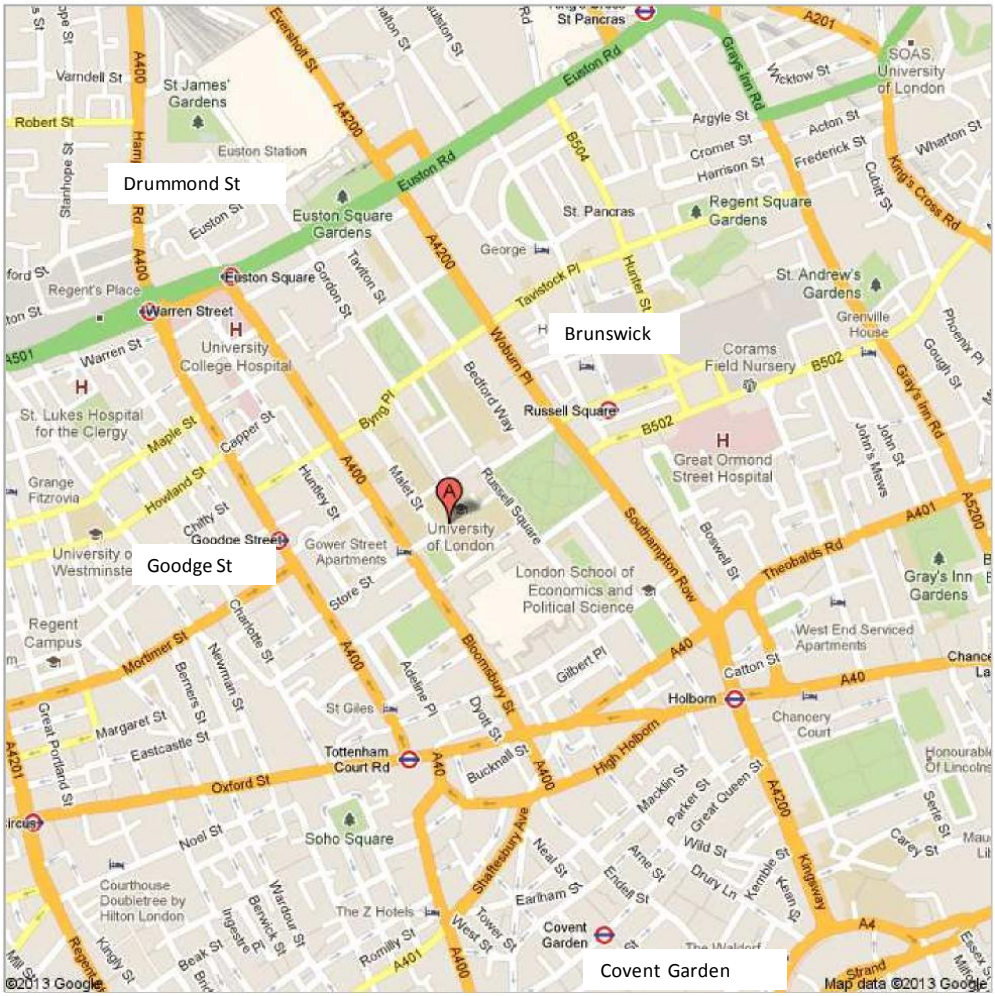
Marlborough Arms, 36 Torrington Place, London WC1E 7LY
Large London pub just south of Jeremy Bentham. Also serves food.

The Marquis Cornwallis, 31 Marchmont Street, London WC1N 1AP
Nice old pub next to Brunswick, 10 min walk north-east of Senate House. Also serves food.

Euston Tap, 190 Euston Road, London, NW1 2EF
Mainly outdoor place to sample the rich diversity of great British beers and ciders (at Cider Tap nearby). Next to Euston Train Station, 10 min walk from venue.

There are of course plenty more places, especially along Tottenham Court Road and Goodge Street, and towards Covent Garden (see map next page).

AREA AROUND SENATE HOUSE



SESSION PROGRAMME

MONDAY, 24 JUNE

19:30-21:30 **Session 1: Special lectures** *Beveridge Hall*

Chair: Paul Nurse

Lecture 1 Roger Highfield
Unsung heroes of science

Lecture 2 Linda Partridge
Ageing *pombe*

Lecture 3 Tim Hunt (*Biochemical Society Lecture*)
Creative science - and how not to teach it

TUESDAY, 25 JUNE

09:00-12:10 **Session 2: Epigenetics** *Beveridge Hall*

Chairs: Rob Martienssen & Geneviève Thon

09:00 **Talk 1: Yota Murakami**
Regulation of RNAi-directed heterochromatin by CTD-phosphorylation of RNA polymerase II

09:20 **Talk 2: Janet Partridge**
Sir2 is required for Clr4 to initiate centromeric heterochromatin assembly in fission yeast

09:40 **Talk 3: Robin Allshire**
Establishing and propagating specialised chromatin states

10:00 **Talk 4: Ruchi Jain (Moazed lab)**
Role of Argonaute Slicer activity in heterochromatin formation

BREAK: SET UP POSTERS

10:50 **Talk 5: Mikel Zaratiegui**
Replication fork barrier and silencing at the Tf LTR

11:10 **Talk 6: Jie Ren (Martienssen lab)**
RNAi promotes heterochromatic silencing through replication-coupled release of RNA pol II

11:30 **Talk 7: Benoit Arcangioli**
The Lysine Specific Demethylases, Lsd1/2, are required for replication fork pauses and mating-type switching in fission yeast

11:50 **Talk 8: Fei Li**
Inheritance of histone methylation in fission yeast

TUESDAY, 25 JUNE, *CONTINUED*

12:30 - 13:00 **Demo of community curation** *Beveridge Hall*

13:30-15:30 **Parallel Workshops A-C**

Workshop A: Cell cycle *Beveridge Hall*

Chairs: Sergio Moreno & Jenny Wu

13:30 **Talk 65: Sergio Moreno**
The fission yeast Greatwall-ENSA(ARPP19)-PP2A pathway links the nutritional environment to mitotic entry

13:45 **Talk 66: Viesturs Simanis**
Analysis of factors governing the asymmetry of Septation Initiation Network (SIN) proteins during mitosis

14:00 **Talk 67**: **Sophie Zaaijer (Cooper lab)**
Rif1 regulates the fate of telomere entanglements during M-phase

14:15 **Talk 68: Robertus de Bruin**
Tolerance of deregulated G1/S transcription requires replication control and genome protection mechanisms

14:30 **Talk 69: Shelley Sazer**
Open vs. Closed Mitosis: What's the Difference and Why does it Matter?

14:45 **Talk 70: Hironori Niki**
Entrainment of cell division by light or temperature in the dimorphic yeast S. japonicus

15:00 **Talk 71: Amar Klar**
Unbiased segregation of chromosome 2 strands to daughter cells

15:15 **Talk 72: Jenny Wu**
Impact of replication origin selection on cellular physiology

Workshop B: Chromatin & gene expression *Chancellors Hall*

Chairs: Pernilla Bjerling & Henry Levin

13:30 **Talk 73: Pernilla Bjerling**
Motifs in the Clr2 protein specific for mating-type silencing

13:45 **Talk 74: Elizabeth Bayne**
Structural analysis of Stc1: insights into the coupling of RNAi and chromatin modification

14:00 **Talk 75: Mario Halic**
Argonaute and Trimmer generate Dicer-independent priRNAs and mature siRNAs to initiate heterochromatin formation

14:15 **Talk 76: Beate Schwer**
The RNA Polymerase II CTD code in fission yeast

TUESDAY, 25 JUNE, *CONTINUED*

14:30 **Talk 77: Sophie Atkinson (Bähler lab)**
The fission yeast non-coding transcriptome

14:45 **Talk 78: Chris Norbury**
Uridylation-mediated RNA turnover

15:00 **Talk 79: Peter Baumann**
New targets and pathways involving the RNA methyltransferase Tgs1

15:15 **Talk 80: Henry Levin**
Tf1 integration increases the expression of adjacent genes and improves resistance to environmental stress

Workshop C: Tools & resources *Senate Room*
Chairs: Edgar Hartsuiker & Charlie Hoffman

13:30 **Talk 81: Shigehiro Kawashima**
Developing fission yeast for chemical biology

13:45 **Talk 82: Charlie Hoffman**
To inhibitors and beyond: PKA-regulated phenotypes in S. pombe for use in genetic and small molecule screens

14:00 **Talk 83: Johanna Sjölander (Sunnerhagen lab)**
Artificial amplification of Sty1 activity in S. pombe, using a conditional chemical protein kinase modulator

14:15 **Talk 84: Dieter Wolf**
Integrated model of gene expression in response to stress

14:30 **Talk 85: Tara Finegan (Carazo-Salas lab)**
Expanding the S.pombe polarity network using a SILAC mass spectrometry approach

14:45 **Talk 86: Hai-Tao Wang (Du lab)**
Screening for overexpression-mediated bypass-of-essential-gene (O-BOE) suppressors in S. pombe

15:00 **Talk 87: Ulrike Endesfelder (Heilemann lab)**
Quantitative single molecule microscopy reveals that CENP-A (Cnp1) deposition occurs during G2 in fission yeast

15:15 **Talk 88: Dan Jeffares (Bähler lab)**
The genomic diversity and population history of S. pombe

16:00-17:40 **Session 3: DNA replication** *Beveridge Hall*
Chairs: Paco Antequera & Hisao Masai

16:00 **Talk 9: Divya Iyer (Rhind lab)**
S-phase DNA damage checkpoint slows replication forks at sites of damage

TUESDAY, 25 JUNE, *CONTINUED*

16:20 **Talk 10: Daochun Kong**
The dual role of Sap1 in DNA replication and checkpoint activation

16:40 **Talk 11: Susan Forsburg**
Replication fork stability is essential to maintain centromere integrity in the absence of heterochromatin.

17:00 **Talk 12: Hisao Masukata**
Replication timing program in fission yeast

17:20 **Talk 13: Hisao Masai**
Regulation of origin firing program in fission yeast

19:30-21:30 **Poster Session 1: ODD numbered posters 1-221**
Macmillan, Crush Hall, and Grand Lobby

WEDNESDAY, 26 JUNE

09:00-12:10 **Session 4: Gene regulation** *Beveridge Hall*
Chairs: Francois Bachand & Chris Norbury

09:00 **Talk 14: Marc Bühler (*EMBO Young Investigator Lecture*)**
Non-coding RNA activity in the fission yeast nucleus

09:20 **Talk 15: Shiv Grewal**
Epigenetic genome control by heterochromatin and RNAi machinery

09:40 **Talk 16: Juan Mata**
A genome-wide view of translational control

10:00 **Talk 17: Monika Gullerova**
Interplay between RNA pol II transcription and cohesion on chromosomal arms of fission yeast

10:50 **Talk 18: Francois Bachand**
The RNA exosome promotes a transcription termination pathway coupled to RNA decay

11:10 **Talk 19: Miriam Sansó (Fisher lab)**
CDK control of RNAPII directionality and transcription termination

11:30 **Talk 20: Lidia Vasiljeva**
Role of non-coding transcription in gene silencing in fission yeast

11:50 **Talk 21: Damien Hermand**
Repression of sexual differentiation by a cis-acting lincRNA (long intergenic non coding RNA) in fission yeast

WEDNESDAY, 26 JUNE, *CONTINUED*

20:00-21:40 **Session 5: Cell responses to environment** *Beveridge Hall*
Chairs: Peter Espenshade & Elena Hidalgo

20:00 **Talk 22: Mitsuhiro Yanagida**
How do cells respond to low glucose concentrations and proliferate?

20:20 **Talk 23: Elena Hidalgo**
Regulation of the stress-dependent gene expression program

20:40 **Talk 24: Simon Labbé**
Molecular control of the meiosis-specific copper transporter Mfc1

21:00 **Talk 25: Beata Grallert**
A Gcn2-dependent G1-S checkpoint in fission yeast

21:20 **Talk 26: Peter Espenshade**
Transcriptional regulation in response to hypoxia

21:40 **Stage Act: magician Tilman Andris** *Beveridge Hall*

THURSDAY, 27 JUNE

09:00-12:10 **Session 6: DNA repair and recombination** *Beveridge Hall*
Chairs: Jo Murray & Kunihiro Ohta

09:00 **Talk 27: Jo Murray**
The Smc5/6 complex and replication stress

09:20 **Talk 28: Nasim Sabouri**
The essential Pfh1 DNA helicase promotes fork progression through G-quadruplex DNA

09:40 **Talk 29: Tony Carr**
Mechanisms of replication-associated genome rearrangement

10:00 **Talk 30: Li-Lin Du**
A proteome-wide visual screen identifies fission yeast proteins localizing to DNA double-strand breaks

10:50 **Talk 31: Hiroshi Iwasaki**
Interactions between Rad51 recombinase and its activator Swi5-Sfr1 complex in fission yeast

11:10 **Talk 32: Michael Boddy**
Combinatorial recognition of SUMO, ubiquitin and DNA provides selectivity in the STUbL-mediated DNA damage response

11:30 **Talk 33: Matthew Whitby**
The role of Fml1 and its partner proteins Mhf1 and Mhf2 in promoting genome stability

11:50 **Talk 34: Kunihiro Ohta**
Spatio-temporal control of meiotic recombination initiation

THURSDAY, 27 JUNE, *CONTINUED*

12:30 - 13:00 **Demo of PomBase and tools** *Beveridge* Hall

13:30-15:30 **Parallel Workshops D-F**

Workshop D: DNA metabolism *Beveridge Hall*

Chairs: Paul Russell & Zoi Lygerou

13:30 **Talk 89: Paul Russell**
The Rad3/ATR-γH2A-Brc1 genome protection module

13:45 **Talk 90: Pierre-Marie Dehé (Gaillard lab)**
Regulation of Mus81-Eme1 Holliday junction resolvase to DNA damage

14:00 **Talk 91: Sarah Lambert**
The Chromatin Assembly Factor 1 promotes homologous recombination by counteracting Rqh1-dependent D-loop dissociation

14:15 **Talk 92: Gerry Smith**
Meiotic crossover control–from DNA breakage to DNA repair

14:30 **Talk 93: Zoi Lygerou**
Stochastic hybrid modeling of DNA re-replication across the genome

14:45 **Talk 94: Atanas Kaykov (Nurse lab)**
Clusters with active replication origins identified on single DNA molecules correspond to nuclear replication foci

15:00 **Talk 95: Ayumu Yamamoto**
Meiotic telomere clustering depends on the telocentrosome, a novel microtubule-organizing center formed at the telomere

15:15 **Talk 96: Katsunori Tanaka**
SUMOylation regulates telomere length homeostasis by targeting the shelterin subunit Tpz1 in fission yeast

Workshop E: Cell regulation *Chancellors Hall*

Chairs: Janni Petersen & Elizabeth Veal

13:30 **Talk 97: Elizabeth Veal**
Responding to stress –the fundamental roles of peroxiredoxins and thioredoxin in coordinating adaptation and survival responses to H_2O_2

13:45 **Talk 98: Mariona Ramos Vecino (Ribas lab)**
Role of glutan synthase Bgs1 in the control of growth polarity

14:00 **Talk 99: Reiko Suguira**
A novel regulatory role for the calcineurin inhibitor DSCR1 (Down syndrome candidate region 1) in p38 MAPK-mediated stress signalling

14:15 **Talk 100: Yusuke Tarumoto (Ishikawa lab)**
Cpc2 facilitates stress-activated MAP kinase-mediated stress response

14:30 **Talk 101: Kaz Shiozaki**
Osmostress signaling by the Wis4-Win1 MAPKKK heteromer stabilized the Mcs4 response regulator

THURSDAY, 27 JUNE, *CONTINUED*

14:45 **Talk 102: Ronit Weisman**
Growth regulation via opposite effects of TORC1 and TORC2 on amino acid homeostasis

15:00 **Talk 103: Dom Helmlinger**
The TOR kinases and the SAGA transcriptional co-activator coordinately control gene expression in response to nutrient availability

15:15 **Talk 104: Janni Petersen**
Full TORC1 inhibition, reduce Wee1 levels and advance mitotic commitment in Fission yeast and mammalian cells

Workshop F: Genome-wide methods & bioinformatics *Senate Room*
Chairs: Gordon Chua & Juraj Gregan

13:30 **Talk 105: Kate Chatfield-Reed (Chua lab)**
Identification of novel upstream regulators of fission yeast transcription factors by synthetic dosage lethality

13:45 **Talk 106: Samuel Marguerat**
Quantitative analysis of fission yeast genome expression at population and single-cell levels

14:00 **Talk 107: Crispin Miller**
Global coordination of the stress response by non-coding transcriptome

14:15 **Talk 108: Danny Bitton (Bähler lab)**
Mind The Gap: Pervasive splicing in the fission yeast transcriptome

14:30 **Talk 109: Punit Prasad**
Chromodomain Helicase DNA binding protein 1 (CHD1) regularly spaces nucleosomes over coding regions in S. pombe

14:45 **Talk 110: Tomáš Pluskal (Yanagida lab)**
Ergothioneine biosynthesis pathway revealed by metabolomic analysis

15:00 **Talk 111: Takashi Sutani (Shirahige lab)**
Condensin complex negates an inhibitory effect of gene transcription on mitotic chromosome segregation

15:15 **Talk 112: Juro Gregan**
Protein kinases required for the proper segregation of chromosomes during meiosis

16:00-17:40 **Session 7: Cell division** *Beveridge Hall*
Chairs: Kathy Gould & Snezhka Oliferenko

16:00 **Talk 35: Jian-Qiu Wu**
Cooperation between formins Cdc12 and For3 for contractile-ring assembly in fission yeast cytokinesis

THURSDAY, 27 JUNE, *CONTINUED*

16: 20 **Talk 36: Mohan Balasubramanian**
Cytokinesis *in vitro* and *in vivo*

16:40 **Talk 37: Issei Mabuchi**
In vitro contraction of cytokinetic ring depends on myosin II but not on actin dynamics

17:00 **Talk 38: Sebastian Mana-Capelli (McCollum lab)**
Identification of SIN pathway targets reveals mechanisms regulating late mitotic events

17:20 **Talk 39: Kathy Gould**
Control of cytokinesis via SIN-mediated phosphoinhibition of formin multimerization

17:40 **Talk 40: Snezhka Oliferenko**
Evolution of division site positioning within the fission yeast clade

19:30-21:30 **Poster Session 2: EVEN numbered posters 2-222**
Macmillan, Crush Hall, and Grand Lobby

FRIDAY, 28 JUNE

09:00-12:10 **Session 8: Chromosomes** *Beveridge Hall*
Chairs: Robin Allshire & Toru Nakamura

09:00 **Talk 41: Paco Antequera**
Base composition and nucleosomal organization of the *S. pombe* genome

09:20 **Talk 42: Karl Ekwall**
A SNF2 chromatin remodeling factor controlling nuclear organization

09:40 **Talk 43: Christian Haering**
Quantitative analysis of chromosome condensation in fission yeast

10:00 **Talk 44: Takeshi Sakuno (Watanabe lab)**
Casein kinase 1 regulates kinetochore-microtubule attachment during meiosis I through Aurora B activation

10:50 **Talk 45: Shigeaki Saitoh**
Epigenetic mechanism stabilizing chromosomes with multiple centromeres

11:10 **Talk 46: Hani Ebrahimi (Cooper lab)**
New insights into role of nuclear position in replicating heterochromatin

FRIDAY, 28 JUNE, *CONTINUED*

11:30 **Talk 47: Junko Kanoh**
Roles of *S. pombe* "sub"telomeres

11:50 **Talk 48: Toru Nakamura**
Regulation of ATM/ATR kinase signaling at fission yeast telomeres

12:30-13:30 **Meet the speakers** *Beveridge Hall*
Opportunity to talk with the plenary speakers you want to meet

14:00-17:10 **Session 9: Cell morphology** *Beveridge Hall*
Chairs: Fred Chang & Iva Tolić-Nørrelykke

14:00 **Talk 49: Sophie Martin (*EMBO Young Investigator Lecture*)**
A new actomyosin focus guides cell-cell fusion in *S. pombe*

14:20 **Talk 50: Rafael Carazo Salas**
A high-throughput microscopy-based genomic survey of the machineries that control and link cell shape, microtubules and cell cycle progression

14:40 **Talk 51: Nicolas Minc**
Symmetry breaking in spore germination

15:00 **Talk 52: Fred Chang**
Shaping the fission yeast cell wall

BREAK: REMOVE ALL POSTERS

15:50 **Talk 53: Fulvia Verde**
Spatial regulation of Cdc42 GTPase in the control of cell morphogenesis

16:10 **Talk 54: Dimitrios Vavylonis**
Model of fission yeast shape driven by membrane-bound growth factors and the cytoskeleton

16:30 **Talk 55: Phong Tran**
Multiple mechanisms for chromosome segregation

16:50 **Talk 56: Iva Tolić-Nørrelykke**
Pivoting of microtubules around the spindle pole accelerates kinetochore capture

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18:30 Buses leave for Conference Dinner, Malet Street Car Park (p. 8)

19:30–23:30 **Conference Dinner** aboard the **Dixie Queen**,
The boat leaves from *Tower Bridge Pier* at 19:30 sharp!!
Live music by **IMMposters** (featuring **Chris Norbury**)
Close up magician: **Laurent van Trigt**
Poster prizes

23:30 Buses back to Senate House

SATURDAY, 29 JUNE

09:00-12:10 **Session 10: Mitosis and meiosis** *Beveridge Hall*
Chairs: Jonathan Millar & Yoshi Watanabe

09:00 **Talk 57: Ursula Fleig**
Inositol pyrophosphates regulate fission yeast mitosis

09:20-**Talk 58: Ngang Heok Tang (Toda lab)**
The TACC-TOG microtubule-associated proteins connect microtubule plus ends to the kinetochores

09:40 **Talk 59: Silke Hauf**
Determinants for robustness in spindle assembly checkpoint signaling

10:00 **Talk 60: John Meadows**
Clp1-mediated localisation of the chromosome passenger complex to Klp9 (kinesin-6) sharpens the anaphase switch

10:50 **Talk 61: Iain Hagan**
A PP1/PP2A phosphatase relay controls mitotic progression

11:10 **Talk 62: Yuki Aoi (Yamamoto lab)**
Cuf2/Sms5 boosts the transcription of APC/C activator Fzr1/Mfr1 to terminate the meiotic division cycle

11:30 **Talk 63: Kayoko Tanaka**
Meiosis-specific regulation of the microtubule organising centre

11:50 **Talk 64: Yasushi Hiraoka**
Nuclear membrane proteins that affect chromosome movements

12:10 Meeting close

Unsung heroes of science

Roger Highfield

Science Museum Group, UK

Roger Highfield was born in Wales, raised in north London and became the first person to bounce a neutron off a soap bubble. He was the Science Editor of The Daily Telegraph for two decades and the Editor of New Scientist between 2008 and 2011. Today, he is the Director of External Affairs at the Science Museum Group. Roger has written seven books, including the bestsellers *The Arrow of Time and Can Reindeer Fly*? He also had thousands of articles published in newspapers and magazines. His latest book, with Martin Nowak, is *SuperCooperators*.

Here's why he became hooked on science
"A combination of factors got me hooked on science. It probably began with my father, who taught mathematics at technical college. Then there was the shiny, optimistic vision of science in the 1960s, which was incredibly seductive. Like every schoolboy, I could not get enough of the Apollo moon landings and the remarkable escape in Apollo 13 – which, if anything, seems even more incredible all these decades later. I was lucky that at school my chemistry teacher – Glyn James – always went beyond what we were supposed to be taught, and strayed into subjects like quantum mechanics. His efforts, plus all the dangerous demonstrations and explosions allowed at that time, led me to study chemistry at university. My doctorate was inspired by the thought of being able to jet off to Grenoble a couple of times each year to bounce neutrons off soap bubbles, and to work my way through one menu compris after another – and gallons of wine – on civil service expenses"

Ageing *pombe*

Linda Partridge

University College London, UK and

Max Planck Institute for Biology of Ageing, Germany

Professor Linda Partridge works on the biology of ageing. Her research is directed to understanding both how the rate of ageing evolves in nature and the mechanisms by which healthy lifespan can be extended in laboratory model organisms. Her work has focussed in particular on the role of nutrient-sensing pathways, such as the insulin/insulin-like growth factor signalling pathway, and on dietary restriction. Her current work is directed to developing pharmacological treatments that ameliorate the human ageing process to produce a broad-spectrum improvement in health during ageing.

She is the recipient of numerous awards, including giving the Royal Society Croonian Lecture in 2009 and a DBE for services to science. She is a Fellow of the Royal Society, the Academy of Medical Sciences, the European Academy of Sciences and the American Academy of Arts and Sciences. She is the Director of the UCL Institute of Healthy Ageing, as well as founding director of the Max Planck Institute for Biology of Ageing in Cologne.

Biochemical Society Lecture

Creative science - and how not to teach it

Tim Hunt

CRUK London Research Institute, UK

Tim Hunt was, until his retirement in 2010, a 'principal scientist' (note, not THE principal scientist) at Cancer Research UK, Clare Hall Laboratories, in South Mimms, Hertfordshire. Tim was born in 1943 and grew up in Oxford, moving to Cambridge to read Natural Sciences in 1961. He obtained his Ph.D. from the Department of Biochemistry in Cambridge in 1968. He spent almost 30 years in Cambridge, working in the Department of Biochemistry on the control of protein synthesis and the cell cycle, but with spells in the USA; he was a postdoctoral Fellow at the Albert Einstein College of Medicine in 1968---70 and spent summers at the Marine Biological Laboratory, Woods Hole from 1977 until 1985, teaching laboratory courses and doing research. In 1982, he discovered cyclins, which turned out to be components of "Key Regulator(s) of the Cell Cycle" This led to a share of the Nobel Prize in Physiology or Medicine in 2001, together with Lee Hartwell and Paul Nurse. Tim Hunt was chairman of the council of EMBO (European Molecular Biology Organisation) from 2006 - 2010. In 2011, he was appointed a member of the ERC (European Research Council) Scientific Council.

ABSTRACTS

Regulation of RNAi-directed heterochromatin by CTD-phosphorylation of RNA polymerase II

Takuya Kajitani[a], Damien Hermand[b], Chikashi Obuse[a], Stewart Shuman[c], Yota Murakami[a]

[a] *Hokkaido University, Japan*
[b] *University of Namur, Belgium*
[c] *Memorial Sloan-Kettering Cancer Center, USA*

Presented by: Murakami, Yota

Transposable elements on the genome are suppressed by two mechanisms: heterochromatin-dependent transcriptional gene silencing (TGS) and RNAi-dependent post-transcriptional gene silencing (PTGS). Recent study using fission yeast revealed the interdependency between heterochromatin formation and RNAi function. Interestingly, the exosome-dependent RNA quality control mechanism also functions at the heterochromatin.

We have been isolated many factors involved in the RNAi-dependent heterochromatin in fission yeast and found that transcriptional machinery including RNA polymerase II regulated TGS and PTGS in the heterochromatin. Phosphorylation of C-terminal repeats of the largest subunit of RNA polymerase II (CTD) is known to control the initiation and the elongation of transcription as well as the processing and transportation of the nascent RNA. We analyzed roles of the phosphorylation of the second and the seventh serine of CTD and found that the each CTD-phosphorylation differentially regulates PTGS and TGS in the RNAi-dependent heterochromatin. This observation prompts us to speculate that the CTD-phosphorylation functions as a "molecular switch" of heterochromatin-dependent gene silencing. In addition, based on our analysis about other factors related to transcription, the regulatory mechanism of heterochromatic gene silencing by transcriptional machinery will be discussed.

Sir2 is required for Clr4 to initiate centromeric heterochromatin assembly in fission yeast

Benjamin Alper, Godwin Job, Rajesh Yadav, Sreenath Shanker, Brandon Lowe, Janet Partridge

St. Jude Children's Research Hospital, Memphis, TN, USA

Presented by: Partridge, Janet

Heterochromatin assembly in fission yeast depends on the Clr4 histone methyltransferase which targets H3K9. We show that the histone deacetylase Sir2 is required for Clr4 activity at telomeres, but acts redundantly with Clr3 histone deacetylase to mainten centromeric heterochromatin. However, Sir2 is critical for Clr4 function during de novo centromeric heterochromatin assembly. We identified new targets of Sir2 and tested if their deacetylation is necessary for Clr4-mediated heterochromatin establishment. Sir2 preferentially deacetylates H4K16Ac and H3K4Ac, but mutation of these residues to mimic acetylation did not prevent Clr4-mediated heterochromatin establishment. Sir2 also deacetylates H3K9Ac and H3K14Ac. Strains bearing H3K9 or H3K14 mutations exhibit heterochromatin defects. H3K9 mutation blocks Clr4 function, but why H3K14 mutation impacts heterochromatin was not known. Here we demonstrate that recruitment of Clr4 to centromeres is blocked by mutation of H3K14. We suggest that Sir2 deacetylates H3K14 to target Clr4 to centromeres. Further, we demonstrate that Sir2 is critical for de novo accumulation of H3K9me2 in RNAi deficient cells. These analyses place Sir2 and H3K14 deacetylation upstream of Clr4 recruitment during heterochromatin assembly.

Establishing and propagating specialised chromatin states

Robin Allshire

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland, UK

Presented by: Allshire, Robin

Overview: Centromeres are the chromosomal region where kinetochores assemble. DNA sequence alone does not dictate centromere location. 'Epigenetic' cues promote specialised chromatin assembly on which kinetochores are built.

Heterochromatin: In fission yeast siRNAs, generated from outer repeat transcripts guide Clr4 methyltransferase to homologous chromatin to methylate histone H3 on lysine 9 allowing binding of Swi6 & other proteins. This outer repeat heterochromatin flanks the central domain where the centromere specific histone H3 variant, CENP-A, is deposited in place of canonical H3 to direct kinetochore assembly. Our analyses show that Sir2 HDAC acts together with RNAi-mediated nucleation to extend heterochromatin over repeat elements (Buscaino et al. in press).

CENP-A chromatin: Kinetochore assembly at specific loci is directed by the deposition of the histone H3 variant CENP-A in place of canonical H3. RNAi, Clr4 methyltransferase & Swi6/HP1 are required to establish CENP-A chromatin over centromeric central domain DNA (Folco et al. 2008). Tethering of Clr4 methyltransferase to a euchromatic locus allows the establishment and maintenance of synthetic heterochromatin independently of RNAi. This synthetic heterochromatin can substitute for centromeric outer repeats, the normal targets of RNAi at centromeres, and allow the assembly of CENP-A chromatin to assemble kinetochores and form fully functional centromeres (Kagansky et al. 2009).

Kinetochore Transcription: The CENP-A chromatin underlying the kinetochore is transcribed. This transcription may play a role in the deposition of CENP-A chromatin in place of H3 within the central domain. Factors that preserve stable H3 chromatin during transcription paly a role in preventing promiscuous CENP-A deposition. Related processes may destabilize H3 nucleosomes within centromere regions and thereby contribute to CENP-A and kinetochore assembly (Choi et al. 2011 & 2012).

Role of Argonaute Slicer activity in heterochromatin formation

Ruchi Jain, Danesh Moazed

Department of Cell Biology, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA

Presented by: Jain, Ruchi

Centromeric repeats in S. pombe are transcribed and give rise to siRNAs, which are bound to Argonaute (Ago1) in the RNA induced transcriptional silencing (RITS) complex. siRNA-RITS targets complementary nascent RNA transcripts and recruits the RNA-dependent RNA polymerase (RDRC) and the CLRC histone H3 lysine 9 (H3K9) methyltransferase complexes, which together form a positive feedback loop for the formation and maintenance of heterochromatin. Initially, double-stranded siRNAs are loaded onto Ago1 but are converted into the active single-stranded form after cleavage of the siRNA passenger strand by Slicer, an endo-ribonuclease found in the PIWI domain of many Argonaute proteins. Slicing leads to the release of the siRNA passenger strand and formation of an active siRNA-guided RITS complex. However, it has been a challenge to understand if slicing is required further downstream in the pathway. Slicing of the nascent RNA may be required to provide free 3′-OH ends that serve as templates for RDRC-dependent siRNA amplification, or may play a direct role in silencing by promoting transcription termination. To address this problem, we identified an abundant Dicer-independent small RNA that originates from the processing of 35S pre-rRNA (called rr-sRNA). rr-sRNA loads onto Ago1 and bypasses the requirement for slicing to form the Argonaute-guide siRNA complex. Furthermore, rr-sRNA is incorporated into the RITS complex. Using strains engineered with reporter genes that have binding sites complementary to rr-sRNA, we show that rr-sRNA can promote H3K9 methylation and silencing of the reporter gene. This silencing can bypass the requirement for Dicer, RDRC, and Ago1 Slicer, indicating that while Slicer activity is critical for steps leading to siRNA generation, it is dispensable for siRNA-mediated H3K9 methylation and heterochromatin formation.

Replication fork barrier and silencing at the Tf LTR

Mikel Zaratiegui, Vincent Tournier, Jake Jacobs, Jesus Rosado

Rutgers University, USA

Presented by: Zaratiegui, Mikel

Eukaryotic genomes show the accumulation of repetitive elements, in the form of large arrays of tandem repeats (Satellite Repeats) as well as proliferation of numerous Transposons. These two types of DNA elements share no sequence identity, but they are often packaged in Heterochromatin. The mechanisms by which the cell recognizes these very diverse elements and coats them in Heterochromatin are largely unknown. Recently several pathways involving small RNA were implicated in the sequence recognition that allows for Heterochromatin deposition. We have discovered in fission yeast that both Satellite Repeats and Transposons share another characteristic: they are difficult to replicate, and failure of Heterochromatin deposition destabilizes replication intermediates at the repetitive elements. This suggests a mechanism for the loss of genome integrity observed when Heterochromatin is compromised.

In order to disentangle the cross-regulation of DNA replication, Transcriptional silencing and Recombination inhibition by Heterochromatin, we are using as a model the Long Terminal Repeat promoter (LTR) of the Tf transposon in fission yeast. This promoter shows all the hallmarks of Heterochromatin in a small element. The DNA binding factor CENP-B recruits the Heterochromatin deposition machinery to the LTR. The LTR has a DNA replication barrier dependent on the DNA binding factor Sap1. CENP-B and Sap1 counteract each other, and a proper balance of their activities is necessary to prevent DNA damage at the LTR, maintain genome integrity and silence the promoter. We have completed a genetic screen for modifiers of this counteraction, and found the implication of several DNA polymerase and Histone genes. We are currently characterizing the implication of these genes in the progression of the replication fork through the LTR, and their consequences on transcription and recombination inhibition.

RNAi promotes heterochromatic silencing through replication-coupled release of RNA pol II

Jie Ren[a], Mikel Zaratiegui[b], Stephane Castel[a], Danielle Irvine[c], Anna Kloc[c], Li Fei[d], W. Zacheus Cande[e], Francisco Antequera[f], Benoit Arcangioli[g], Robert Martienssen[a]

[a] *Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA*
[b] *Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, New Jersey 08854, USA*
[c] *Murdoch Children's Research Institute, University of Melbourne, Melbourne, Victoria 3052, Australia*
[d] *Department of Biology, New York University, New York, New York 10003, USA*
[e] *Molecular and Cellular Biology, University of California Berkeley, Berkeley 94720, USA*
[f] *Instituto de Biologıa Funcionaly Genomica, CSIC/Universidad de Salamanca, Salamanca 37007, Spain*
[g] *Institut Pasteur, Paris, France*

Presented by: Ren, Jie

Heterochromatin comprises tightly compacted repetitive regions of eukaryotic chromosomes and has widespread roles in chromosome integrity, stability and silencing. The inheritance of heterochromatin requires RNA interference (RNAi), which guides histone modification on the two daughter strands upon DNA replication. However, the underlying mechanism is poorly understood. In *Schizosaccharomyces pombe*, the alternating arrangement of origins of replication and non-coding RNA transcribed during S phase in heterochromatic pericentromeric region provokes the collision of RNA polymerase with replication machinery. We propose that it is resolved by co-transcriptional RNAi, allowing replication to complete and couple the spreading of heterochromatin with fork progression. In the absence of RNAi, stalled forks are repaired by homologous recombination without histone modification (1). The molecular basis of this model and its genome-wide impact will be further investigated, which may explain the participation of RNAi and DNA replication in *S. pombe* and many other systems of heterochromatin inheritance.

(1) Zaratiegui et al. Nature 2011, 479:135-8

The Lysine Specific Demethylases, Lsd1/2, are required for replication fork pauses and mating-type switching in fission yeast.

Benoit Arcangioli

Pasteur Institute, Paris, France

Presented by: Arcangioli, Benoit

In the fission yeast *Schizosaccharomyces pombe* a chromosomal imprinting event controls the asymmetric pattern of mating-type switching. The orientation of DNA replication at the mating- type locus is instrumental in this process. We have recently shown that the replication fork pause at the mat1 locus (MPS1), essential for imprint formation, depends on the lysine specific demethylase, Lsd1. We also demonstrate that either Lsd1 or Lsd2 amine oxidase activity is required for these processes, working upstream of the imprinting factors Swi1 and Swi3 (homologs of mammalian Timeless and Tipin, respectively). Furthermore, we found that Lsd1/2 complex controls the replication fork terminators, within the rDNA repeats. These findings reveal a novel role for the Lsd1/2 demethylases in controlling replication fork progression, imprint formation and ensuing asymmetric cell divisions. To have a better understanding of the imprinting process in fission yeast, we are now searching for suppressors.

Inheritance of histone methylation in fission yeast

Fei Li[a], Rob Martienssen[b], Haijin He[a]

[a] *Department of Biology, New York University, New York, NY 10003, USA*
[b] *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA*

Presented by: Li, Fei

Post-translational modifications of histones, such as histone methylation, are crucial for the control of chromatin structure and gene expression. These modifications can be faithfully transmitted through many generations. However, during S phase of the cell cycle, DNA replication disrupts the chromatin at the nucleosome level and poses a major challenge for inheritance of these epigenetic marks. In fission yeast, histone H3 lysine 9 (H3K9) methylation is enriched in heterochromatin and is stably inherited from generation to generation. Clr4, a member of the SUV39 family of histone methyltransferases, is responsibly for catalyzing H3K9 methylation. Clr4's activity is mediated by RNA interference (RNAi) in a cell cycle-dependent manner. During S phase, heterochromatin repeats are briefly transcribed at S phase and processed into small RNAs by RNAi machinery. The small RNAs in turn promote H3K9 methylation by Clr4. Using Tandem Affinity Purification (TAP) system and mass spectrometry analysis, we previously identified that Dos2 (also known as Raf2/Cmc2/Clr7), a key silencing factor, interacts Cdc20, a DNA polymerase epsilon subunit, Mms19, a transcription regulator, and Rik1. We demonstrated that the complex is important for H3K9 methylation, small RNA generation, and heterochromatin silencing. Recently, we further showed that Dos2 also interacts with Cdc27, a DNA polymerase delta subunit. Disruption of Cdc27 results in severe loss of heterochromatin silencing and H3K9 methylation. Our findings reveal an important role of DNA replication components in inheritance of histone methylation. We propose that Cdc27 functions together with Cdc20 to recruit the histone modification complex to heterochromatin to mediate H3K9 methylation during DNA replication.

The S-phase DNA damage checkpoint slows replication forks at sites of DNA damage

Divya Iyer, Nick Willis, Nick Rhind

University of Massachusetts Medical School, Worcester MA, USA

Presented by: Iyer, Divya

The S-phase DNA damage checkpoint slows replication and allows cells to replicate damaged DNA, but how slowing relates to damage tolerance remains unclear. The standard explanation—that checkpoints delay cell-cycle progression until damage can be repaired—does not seem to apply in the case of DNA replication. Instead, replication proceeds in the presence of the damage, but at a slower rate. Replication slowing can be achieved by inhibition of origin firing, slowing of fork progression or a combination of both. Bulk assays suggest that slowing in fission yeast is primarily achieved through a decrease in the rate of fork progression on damaged DNA. For example, blocking Hsk1 activity, which is required for origin firing, is not sufficient to cause the delay seen in response to DNA damage. Genetic analysis shows that fork slowing involves negative regulation of recombination. Moreover, the degree of slowing is proportional to the amount of damage present. These observations suggest that the checkpoint acts locally on forks as they encounter damage. We have used single-molecule DNA combing to rigorously show that the checkpoint regulates individual replication forks. We are currently investigating how the checkpoint regulates fork components to achieve replication slowing.

Another key question is how forks replicate quickly through damaged DNA in the absence of the checkpoint. The MMS-induced lesions we use are believed to stop replicative polymerases. Nonetheless, in the absence of the checkpoint, replication forks pass them without any apparent delay. This bypass seems to be mainly dependent on recombination and translesion-polymerase-based synthesis. We are working on how forks bypass polymerase-stalling lesions and what effect this bypass has on genomic stability.

The dual role of Sap1 in DNA replication and checkpoint activation

Yuan Zhang, Yu Hua & Daochun Kong

College of Life Sciences, Peking University, China

Presented by: Kong, Daochun

We find that Sap1 is a pre-RC component that is required for loading Cdc18 to DNA origins. The further studies of Sap1 protein indicated that the function of Sap1 appeared to be regulated by phosphorylation. We also recently found that Sap1 might function in checkpoint activation. The studies in Dr. Paul Nurse's lab indicate that Cdc18 has a role in the intra-S phase checkpoint activation and Cdc18 interacts with ATR/ATRIP and anchors it to chromatin for its activation in the fission yeast. However, it remains unclear how Cdc18 loads ATR/ATRIP to chromatin DNA because Cdc18 itself does not bind to DNA. In our study, we found that the sap1ts5 cells were sensitive to HU treatment at permissive temperature, and when Sap1ts5 dissociated from chromatin at restrictive temperature, the cells got premature entry into mitosis. This result suggests that Sap1 participates in checkpoint activation. We further found that Sap1 interacted with Cdc18 and RPA and was able to anchor Cdc18-Rad3/Rad26ATR/ATRIP to RPA-bound single-stranded DNA for the activation of Rad3/Rad26ATR/ATRIP. Thus, we conclude that the role of Sap1 in checkpoint is to recruit Cdc18 and subsequently Rad3/Rad26ATR/ATRIP to RPA-bound ssDNA in chromatin for the activation of intra S-phase checkpoint.

Replication fork stability is essential to maintain centromere integrity in the absence of heterochromatin.

Pao-Chen Li[a], Ruben Petreaca[b], Amanda Jensen[b], Ji-Ping Yuan[b], Marc Green[b], Susan Forsburg[b]

[a] *University of Southern California (now at Gladstone Institute, UCSF), USA*
[b] *University of Southern California, USA*

Presented by: Forsburg, Susan

Replication fork stability mechanisms including the intra-S checkpoint and the fork protection complex are important to maintain genome integrity during replication stress, including naturally occurring stresses such as repetitive sequences, highly transcribed genes, or pause sites. We hypothesized that the repetitive sequences found in the outer repeats of the centromere might be a source of replication stress that is normally protected by heterochromatin.

Heterochromatin is established by binding of heterochromatin associated proteins such as Swi6 and Chp1 to methylated histone H3 K9. Previous studies showed that heterochromatin is cold for recombination, consistent with the DNA being packaged and relatively inaccessible. In *swi6* or *chp1*mutants, we observe an increased rate of recombination in the outer repeats of the centromere, as well as increased chromosome loss.

We find that chromosome loss and increased recombination in the outer repeats in heterochromatin mutants is severely aggravated by loss of replication fork stability mechanisms. We isolated several strains in a *swi6 mrc1* background that showed a balanced translocation between ChrIII and a non-essential minichromosome. This generated strains of fission yeast with four essential chromosomes that can be stably transmitted through meiosis and maintained in a wild type background. Thus, the synthetic defect between heterochromatin mutants and replication fork mutants can be used to model chromosome instability (CIN) such as seen in malignant cells.

Replication timing program in fission yeast

Hisao Masukata[a], Atsutoshi Tazumi[a], Ami Kishimoto[a], Toru Takigawa[a],Minako Kawakita[a], Tetsuya Handa[a], Shiho Ogawa[a], Tatsuro Takahashi[a],Takuro Nakagawa[a], Junko Kanoh[b]

[a] *Graduate School of Science, Osaka University, Japan*
[b] *Institute for Protein Research, Osaka University, Japan*

Presented by: Masukata, Hisao

DNA replication initiates at a large number of chromosome loci known as replication origins in eukaryotes. Initiation of replication is tightly regulated for maintenance of genome stability. Replication origins do not fire simultaneously but the individual origins replicate at distinct time points during S phase. Control of replication timing is common in eukaryotes and it is correlated with alterations of gene expression during development, although the underlying mechanisms remain elusive. We investigated the mechanism that determines a pre-fixed program of replication timing in fission yeast. We found that subsets of late origins in the internal chromosome regions are associated with two copies of telomeric repeat. Telomeric repeats are required for late timing control. Telomere-binding protein Taz1, a fission yeast counterpart of human TRF1 and TRF2, binds to the internal telomeric repeats and required for the timing control. The Taz1-mediated pathway prevents the DDK-dependent Sld3 loading for the initiation at late origins in early S phase. Genome-wide analysis revealed that about half of late origins including those in sub-telomeres are dependent on Taz1. Rif1 that interacts with Taz1 plays key roles in control of both Taz1-dependent and independent late origins. Interestingly, sub-telomeric late origins are further regulated by a shugoshin protein (Sgo2). Sgo2, which is required for spindle assembly checkpoint at centromeres in M phase, localizes at sub-telomeres in interphase and participates in replication timing control of sub-telomeric origins. These results suggest that replication timing program consists of several mechanisms relating with genome maintenance at the telomeres and centromeres.

Regulation of origin firing program in fission yeast

Yutaka Kanoh[a], Motoshi Hayano[a], Seiji Matsumoto[a], Michie Shimmoto[a],Claire Renard-Guillet[b], Katsuhiko Shirahige[b], Hisao Masai[a]

[a] *Tokyo Metropolitan Institute of Medical Science, Japan*
[b] *Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan*

Presented by: Masai, Hisao

DNA replication of the entire genome proceeds under a defined temporal and spatial program, which is dictated by the chromatin structures and organization and is also under checkpoint regulation. However, how this replication program is regulated has not been known.

Cdc7 is a conserved serine-threonine kinase that plays a crucial role in determination of the origin firing program. We discovered that various mutations or physiological conditions can bypass the requirement of Hsk1 kinase (the fission yeast homologue of Cdc7) for DNA replication at least partly by changing the origin firing program. Random screening of suppressors for hsk1 mutation led to identification of genes, deletion of which permits the growth of hsk1Δ. Among them, mrc1Δ and cds1Δ, checkpoint regulators, show firing at the late/ dormant origins in the presence of HU. This is due to increased initiation potential in these checkpoint mutants. Mrc1 binds selectively to early-firing origins in a manner independent of Hsk1 and Cdc45, and physically interacts with Hsk1. Mutational analyses indicate that Mrc1 regulates firing of early origins in a checkpoint-independent manner as well.

One of the most efficient bypass mutations was identified as rif1 (Rap1-interacting factor), known to bind to telomeres and regulate telomere length. In rif1Δ cells, extensive deregulation of origin firing was observed along with downregulation of many early-firing origins. Rif1 binds to telomere and centromere core as well as to many sites on the chromosome arms which do not precisely overlap with the origins. About half of Rif1-regulated dormant origins are not activated in checkpoint mutants, whereas 90% of checkpoint-regulated origins are activated also in rif1Δ. The Rif1-regulated dormant origins tend to be associated with Rif1 binding sites in its closer proximity. Cdc45 loading but not pre-RC formation is affected by Rif1. Potential mechanisms of Rif1-mediated origin firing program will be discussed.

EMBO Young Investigator Lecture

Non-coding RNA activity in the fission yeast nucleus

Marc Bühler

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Presented by: Bühler, Marc

Heterochromatin is classically perceived to be refractory to transcription because of its compact structure. Our work has challenged this paradigm as we could demonstrate that heterochromatic transcripts can be synthesized even when heterochromatin is normally packaged. However, mechanisms exist that ensure highly efficient and specific elimination of heterochromatic RNAs.

Recently, we demonstrated that specificity for this RNA degradation pathway is provided by one of the best-known heterochromatin proteins, HP1. Our results not only reveal novel mechanistic insights into heterochromatin silencing but also offer a possible novel function for the many short-lived, low abundant ncRNAs that are just being uncovered. In contrast to the emerging theme that ncRNAs can serve as a scaffold to assemble, recruit, or guide chromatin-modifying complexes to their respective targets, we discovered that they may also function as repellents. I will discuss our latest insights into this ncRNA function at the meeting.

Epigenetic genome control by heterochromatin and RNAi machinery

Shiv Grewal

Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Presented by: Grewal, Shiv

RNAi is a conserved mechanism in which small interfering RNAs (siRNAs) guide the degradation of cognate RNAs, but also promote heterochromatin assembly at repetitive DNA elements such as centromeric repeats. However, the full extent of RNAi functions and its endogenous targets have not been explored. We show that in the fission yeast *Schizosaccharomyces pombe*, RNAi and heterochromatin factors cooperate to silence diverse loci, including sexual differentiation genes, genes encoding transmembrane proteins, and retrotransposons that are also targeted by the exosome RNA degradation machinery. In the absence of the exosome, transcripts are processed preferentially by the RNAi, revealing siRNA clusters and corresponding increase in heterochromatin modifications across large domains containing genes and retrotransposons. Interestingly, the generation of siRNA clusters and heterochromatin assembly by RNAi is triggered by a mechanism involving the canonical poly(A) polymerase Pla1 and an associated RNA surveillance factor Red1, which also activate the exosome. More importantly, siRNA production and heterochromatin modifications at these target loci are regulated by environmental growth conditions, and by developmental signals that induce gene expression during sexual differentiation. These analyses uncover interplay between RNAi and the exosome that is conserved in higher eukaryotes, and show that differentiation signals modulate RNAi silencing to regulate developmental genes.

Yamanaka, S., Mehta, S., Reyes-Turcu, F., Zhuang, F., Fuchs, R., Rong, Y., Robb, G., and Grewal, S.I.S. (2012) RNAi triggered by specialized machinery silences developmental genes and retrotransposons. Nature 493: 557-560.

Zofall, M., Yamanaka, S., Reyes-Turcu, F., Zhang, K., and Grewal, S.I.S. (2011). RNA elimination machinery targeting meiotic mRNAs promotes facultative heterochromatin formation. Science 335: 96-100.

A genome-wide view of translational control

Caia Duncan, Cristina Cotobal, Juan Mata

Department of Biochemistry, University of Cambridge, Cambridge, CB2 1QW, UK

Presented by: Mata, Juan

Ribosomal profiling provides a genome-wide view of translation at single nucleotide resolution. We have applied this approach to fission yeast cells and used it to estimate translation rates and to identify translation start sites, uORFs, potential frameshifting events and translation of annotated non-coding RNAs. I will present these data as well as additional experiments addressing how translation is regulated in response to environmental and developmental cues.

Interplay between RNA pol II transcription and cohesion on chromosomal arms of fission yeast

Monika Gullerova, Margarita Schlackow, Shweta Bhardwaj

University of Oxford, UK

Presented by: Gullerova, Monika

Polyadenylation of pre-mRNAs, a critical step in eukaryotic gene expression, is mediated by cis elements, collectively called the polyadenylation signal. We demonstrate that the canonical AATAAA motif, originally identified in humans, is the most frequent and functional polyadenylation signal in *Schizosaccharomyces pombe*. Using RNA-Seq analysis of datasets from cells grown under various physiological conditions, we observe heterogeneity of cleavage sites and the sequence elements around them. Our bioinformatic and experimental genome-wide results can be accessed in a user-friendly database Pomb(A).

Sister chromatids of duplicated chromosomes must be correctly aligned and held together by a ring shaped protein called cohesin, prior to their physical separation in mitosis. Cohesin also plays an important role in transcription termination. We show that convergent genes lacking cohesin are generally associated with longer overlapping mRNA transcripts.
Cohesive cohesin is known to associate with centromeres and telomeres. We performed extensive DNA FISH experiments using single locus probes and demonstrate that cohesion on chromosomal arms is more stable at the sites, where cohesin loader Mis4 co-localizes with cohesin subunit Rad21 in comparison to sites with Rad21 only. Furthermore, two separate Mis4/Rad21 sites are in close proximity in cis creating long-range chromatin loops. These sites correlate with highly transcribed genes. Finally, we demonstrate that even short-term (45min) loss of transcription causes displacement of Mis4 and Rad21 from chromatin on chromosomal arms and centromeres.

Overall our data suggest interplay between RNA pol II transcription and cohesion on chromosomal arms of fission yeasts.

The RNA exosome promotes a transcription termination pathway coupled to RNA decay

Jean-Francois Lemay[a], Marc Larochelle[a], Samuel Marguerat[b], Jürg Bähler[b], Francois Bachand[a]

[a] *RNA Group, University of Sherbrooke, Canada*
[b] *Department of Genetics, Evolution & Environment, University College London, UK*

Presented by: Bachand, Francois

The transcription cycle is composed of three essential steps that consist of initiation, elongation, and termination. Termination is probably the least understood of these three steps in eukaryotic cells. For protein-coding genes, termination is normally initiated by the endonucleolytic cleavage of the nascent mRNA, which generates an entry point for a 5'-3' exonuclease-dependent termination pathway known as the torpedo model. In contrast to this 5'-3' termination pathway, we provide evidence for a new mechanism of transcription termination that depends on the 3'-5' exonuclease activity of the RNA exosome. Accordingly, transcriptome-wide analysis of fission yeast cells depleted for exosome subunits reveal widespread accumulation of 3'-extended transcripts from coding and noncoding genes, whereas mature RNA levels are not reduced. Importantly, the detection of read-through RNAs in exosome-deficient cells strongly correlates with a genome-wide increase in RNA polymerase II density at the 3' end of genes, consistent with transcription termination defects in the absence of a functional exosome. We show that RNA exosome subunits are present along transcribed genes and are recruited in a transcription-dependent manner, supporting a direct role for the exosome in promoting transcription termination. Our results also indicate that the exonucleolytic activity, but not the endonucleolytic function of the Dis3 catalytic subunit is required for exosome-dependent termination. These findings support a reverse torpedo model in which the 3'-5' exonucleolytic activity of the RNA exosome promotes the release of RNAPII and the concomitant degradation of the nascent transcript.

CDK control of RNAPII directionality and transcription termination

Miriam Sansó[a], Danny Bitton[b], Rebecca Levin[c], Nicholas Hertz[c], Kevan M. Shokat[c], Jürg Bähler[b], Jason C. Tanny[d], Robert P. Fisher[a]

[a] *Department of Structural and Chemical Biology, Mount Sinai School of Medicine, New York, USA*
[b] *Department of Genetics, Evolution & Enviroment, University College London, UK*
[c] *Howard Hughes Medical Institute and Department of Cellular and Molecular Pharmacology, University of California, San Francisco, USA*
[d] *Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada*

Presented by: Sansó, Miriam

Transcription by RNA polymerase II (RNAPII) is coordinated with RNA processing in part by cyclin-dependent kinases (CDKs). Coherent gene expression also depends on 1) efficient termination once RNAPII reaches the end of a transcription unit, to prevent interference with adjacent genes and perhaps facilitate reinitiation; and 2) mechanisms that favor productive sense over divergent antisense elongation at bidirectional promoters. Cdk9, the catalytic subunit of positive transcription elongation factor b (P-TEFb), has two known, conserved substrates: the elongation factor Spt5 and the RNAPII carboxyl-terminal domain (CTD). However, the precise functions and most targets of Cdk9 remain unknown. During the transcription cycle, multiple CDKs phosphorylate the CTD in the repeated sequence YSPTSPS to recruit mRNA-processing factors. It was unknown if Cdk9 also acts directly on these factors to regulate processing. To address this question, we took a chemical-genetic approach—labeling with analog-selective Cdk9 in extracts—to define P-TEFb-dependent phosphoproteomes of fission yeast and human cells. This analysis revealed enrichment of proteins that function in RNA metabolism, including a human 5'-end decapping complex and the 5'-3' exonuclease Xrn2 required for transcription termination by the "torpedo" pathway. In parallel, analysis by strand-specific RNA-seq revealed genome-wide increases in read-through and divergent antisense transcription after allele-specific inhibition of analog-sensitive Cdk9 in *S. pombe*. Increased antisense transcription correlated with decreased sense transcription at neighboring loci. Strains with mutations in cdk9 and dhp1, which encodes the Xrn2 ortholog, were synthetically sick; and analysis of individual genes confirmed roles for both Cdk9 and Dhp1 in preventing read-through. Our data suggest conserved functions of CDKs in termination and antisense suppression, through direct phosphorylation of factors that limit nonproductive RNA synthesis.

Role of non-coding transcription in gene silencing in fission yeast

Sneha Shah, Sina Wittmann, Cornelia Kilchert, Lidia Vasiljeva

Department of Biochemistry, Oxford, UK

Presented by: Vasiljeva, Lidia

Non-coding (nc) transcription has been recently identified as a typical feature of eukaryotic genomes. It is becoming apparent that nc transcription plays an important regulatory role in gene expression. A number of different scenarios where either act of nc transcription or ncRNA regulate expression of a protein-coding gene has been described in various model systems, however the mechanistic details are not very well understood. We describe a novel ncRNA transcript involved in repression of a protein-coding gene in response to nutrients. The ncRNA is expressed specifically under repressive nutrient conditions and is degraded by the exosome complex. Surprisingly, in the absence of the exosome, increased levels of H3K9me2 are detected across this locus. Interestingly, blocking nc transcription by deleting nc promoter leads to loss of H3K9me as well as loss of Ago1 recruitment. We hypothesize that there are several redundant mechanisms involved in repression of the expression of the protein-coding genes mediated via nc transcription to allow for dynamic regulation of gene expression in response to various environmental cues.

Repression of sexual differentiation by a cis-acting lincRNA (long intergenic non coding RNA) in fission yeast

Sylvain Fauquenoy[a], Valerie Migeot[a], Olga Khorosjutina[b], Karl Ekwall[b], Damien Hermand[a]

[a] *NARC (Namur Research College), rue de Bruxelles 61, 5000 Namur, Belgium*
[b] *Karolinska Institute, Hälsovägen 7, S-141 83 Huddinge, Sweden*

Presented by: Hermand, Damien

Mammalian long non-coding RNAs have been recognized as a pivotal layer of regulation in the specification of cellular identities with the emerging theme that they regulate cell fate by directly acting at the chromatin level. Specifically, cis-acting long non coding RNAs recruit chromatin modifiers or remodelers to locally modulate the epigenetic status of their neighbouring target gene.
The switch from cellular proliferation to differentiation occurs to a large extent through specific programs of gene expression. In fission yeast, the master regulator of sexual differentiation, ste11, is induced by environmental conditions leading to mating and meiosis.

Here, we show that ste11 transcription is repressed in rich media by the expression of rse1 (repressor of ste11 expression), a 2.2 kb long intergenic non coding RNA (lincRNA) which is transcribed on the reverse strand upstream of the ste11 promoter. Using engineered diploid cells; we show that res1 acts in an allele specific manner, which is confirmed by the fact that ectopic expression of res1 has no effect on ste11. Genome-wide expression profiling reveals an overexpression of the Ste11-dependent targets while the rest of the transcriptome is unaffected.

Deletion of rse1 suppresses the requirement of both the Rst2 transcription factor and nitrogen starvation for the induction of ste11 transcription and results in elevated PolII occupancy and histone H3 acetylation at the ste11 promoter. Our data indicate the rse1 lincRNA represses the expression of ste11 by recruiting the Hos2 histone deacetylase, which is also supported by the fact that an hypoacetylated histone H3 K14R mutant suppresses the rse1 deletion phenotype. Taken together, these results show that the control of cell differentiation by a cis-acting lincRNAs is likely an ancient mechanism. The recent discovery of several hundreds of long non coding RNA in fission yeast supports that this type of regulation is more widely used than previously anticipated.

How do fission yeast cells respond to low glucose concentrations and proliferate?

Mitsuhiro Yanagida[a], Shigeaki Saitoh[b]

[a] *Okinawa Institute of Science and Technology Graduate University, Japan*
[b] *Kurume University, Japan*

Presented by: Yanagida, Mitsuhiro

The fission yeast *Schizosaccharomyces pombe* cells rapidly proliferate in medium containing low glucose concentrations (~4.4 mM) equivalent to that in human blood (Pluskal et al, 2011). We explored the mechanism for efficient cell division under low glucose, and found that the gene expression of the major high-affinity glucose transporter Ght5 is de-repressed under low-glucose and distributes along the whole cell surface, enabling the greatly enhanced uptake of glucose. Ssp1protein kinase similar to calcium, calmodulin-dependent kinase kinase (CaMKK), and Sds23, an inhibitor of 2A-like phosphatases, synergistically regulate transcriptional de-repression of the ght5+ gene through the nuclear exclusion of a zinc-finger transcription factor for Ght5, upon glucose limitation. TORC2 and related kinases also become essential for proper trafficking and localization of Ght5 onto the plasma membrane. Ght5 newly synthesized in low glucose locates along the cell tip, where Ght5 was devoid in high glucose, suggesting that newly synthesized Ght5 in low glucose is possibly tethered to specific surface components with the aide of TOR signalling. These results shed light on higher eukaryotic glucose consumption. We discuss overall cellular regulations upon the transfer from ample glucose to limited glucose environment.

Regulation of the stress-dependent gene expression program in fission yeast

Jorge Fernández-Vázquez[a], Itzel Vargas-Pérez[a], Miriam Sansó[a], Damien Hermand[b], Miguel Rodríguez-Gabriel[c], José Ayté[a], Sebastian Leidel[d], Elena Hidalgo[a]

[a] *Universitat Pompeu Fabra, Spain*
[b] *Academie Universitaire Louvain, France*
[c] *Centro de Biología Molecular-SO, Spain*
[d] *Max Planck Institute for Molecular Biomedicine, Germany*

Presented by: Hidalgo, Elena

Microorganisms are invariably exposed to abrupt changes in their environment, and consequently display robust, high plasticity gene programs to respond to stress. In *Schizosaccharomyces pombe*, the Sty1 MAP pathway is activated in response to diverse stress conditions, such as osmotic and oxidative stress, heat shock, or nitrogen deprivation. The MAP kinase Sty1 and its main substrate, the transcription factor Atf1, engage a wide gene expression program aimed to allow cell survival by decreasing and repairing the damage exerted. Searching for mediators of this wide gene expression program, we isolated several chromatin-related mutant strains very sensitive to oxidative stress conditions. Among them, strains lacking the histone acetyl transferases (HAT) Gcn5 or Sin3/Elp3, were sensitive to H2O2. Gcn5, a component of the SAGA complex, is recruited to stress promoters in a transcription factor dependent manner and mediates eviction of nucleosomes positioned downstream of promoters, allowing efficient Pol II progression along the genes. Other chromatin regulators required for efficient transcription of stress genes will be described. The role of Sin3/Elp3, a component of the Elongator complex, in promotion of stress tolerance seems to be completely different from that of Gcn5, since no direct recruitment of this HAT to stress genes has been detected. Instead, Elongator mediates the modification of some tRNAs, modulating translation efficiency. Cells lacking Sin3/Elp3 cannot modify the uridine wobble nucleoside of certain tRNAs, and other tRNA modifying activities such as Ctu1-Ctu2 are also essential for normal tolerance to H2O2. A plasmid over-expressing a particular tRNA complements the stress-related phenotypes of Sin3/Elp3 mutant cells. In conclusion, SAGA and Elongator regulate the stress gene expression program at the level of transcription and translation, respectively.

Molecular control of the meiosis-specific copper transporter Mfc1

Simon Labbé[a], Jude Beaudoin[a], Raphael Ioannoni[a], Stephane Mailloux[a],Samuel Plante[a], Luis López-Maury[b], Jürg Bähler[c], Christopher J. Chang[d]

[a] *Département de Biochimie, Faculté de médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, J1E 4K8, Canada.*
[b] *IBVF, CSIC-Universidad de Sevilla, Spain*
[c] *Department of Genetics, Evolution & Environment, University College London, UK*
[d] *Department of Chemistry and Howard Hughes Medical Institute, University of California, Berkeley, California, USA*

Presented by: Labbé, Simon

Mfc1 is a meiosis-specific protein that mediates copper transport during the meiotic program in *Schizosaccharomyces pombe*. Although the mfc1+ gene is induced at the transcriptional level in response to copper deprivation, the molecular determinants that are required for its copper starvation-dependent induction are unknown. In the present study, we show that mfc1+ transcriptional induction is exclusively detected after treatment with a copper chelator and not by iron or zinc chelators. Analysis of regions in the promoter of mfc1+ reveal that two TCGGCG regulatory elements containing CGG triplets are required for the induction of mfc1+ in response to copper starvation. We consistently find that Mca1, a putative member of the Zn(2)Cys(6) binuclear cluster class of regulators which are known to bind repeated cis-acting elements containing CGG triplets, is necessary to mount a maximal transcriptional response of mfc1+. Microscopic analyses reveal that a functional Mca1-Cherry protein localizes to the nucleus during the course of vegetative growth of diploid cells and co-localizes with chromosomes during the meiotic process of differentiation. Although mca1Δ/mca1Δ cells exhibit normal progression under basal and copper-replete conditions, these mutant cells undergo a meiotic block at metaphase I under conditions of copper starvation. Binding studies reveal that the N-terminal 150-residue segment of Mca1 expressed as a fusion protein in E. coli specifically binds to the TCGGCG sequences of mfc1+ promoter region. Taken together, these results have identified cis and trans-acting elements involved in molecular control of the meiosis-specific copper transporter Mfc1.

A Gcn2-dependent G1-S checkpoint in fission yeast

Cathrine A. Bøe[a], Jon Halvor Knutsen[a], Erik Boye[a], Beata Grallert[a]

Department of Cell Biology, Oslo University Hospital, The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway

Presented by: Grallert, Beata

Cell-cycle progression is governed by checkpoint mechanisms to ensure that previous cell-cycle phases have been correctly completed and that there is no DNA damage. Entry into S phase is carefully regulated and, in most organisms, under the control of a G1-S checkpoint. We are studying cell-cycle progression in G1-S in fission yeast. We have previously described a checkpoint that delays formation of the pre-replicative complex at chromosomal replication origins after exposure to ultraviolet light (UVC). The delay is fully dependent on the Gcn2 kinase, previously known for its role in regulating translation in response to starvation. The only known target of Gcn2 is the translation initiation factor eIF2α, which inhibits translation in its phosphorylated form.

One of the aspects of the checkpoint we explored was the molecular nature of the signal initiating the response. We have shown that the checkpoint is not a general DNA-damage checkpoint. More recently we found that that the G1-S delay depends on damage to the DNA as opposed to other macromolecules and that the activating signal derives not from the initial DNA damage, but from a repair intermediate(s). Surprisingly, we find that activation of Gcn2 is not dependent on the processing of DNA damage and that activated Gcn2 alone is not sufficient to delay entry into S phase in UVC-irradiated cells. Thus, the G1-S delay is dependent upon at least two different inputs.

We are also trying to understand the mechanism by which Gcn2 delays the cell cycle. One possibility we are pursuing is that it is through the phosphorylation of eIF2α and resulting effects on translation. We have identified the mRNAs that continue to be associated with polysomes after UV irradiation and find that some of these encode proteins required for DNA repair. Thus Gcn2 might be required for the checkpoint through its role in regulating selective translation.

Transcriptional Regulation in Response to Hypoxia

Peter Espenshade

Johns Hopkins University School of Medicine, USA

Presented by: Espenshade, Peter

Cells require environmental oxygen for essential metabolic processes and thus possess mechanisms to sense and adapt to changes in oxygen supply. Sterol regulatory element binding proteins (SREBPs) are membrane-bound transcription factors that control cellular lipid homeostasis. The fission yeast SREBP, called Sre1, additionally acts as an oxygen-responsive transcription factor that is required for adaptation to a low oxygen environment. Our studies defined two independent mechanisms by which oxygen controls Sre1 activity and identified molecular machinery essential for SREBP pathway function. Interestingly, Sre1 does not control all oxygen-dependent gene expression, suggesting that other oxygen-responsive factors exist.

The Smc5/6 complex and replication stress

Gokhan Akman, Jennifer Whitwood, Jo Murray

Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, E. Sussex, BN1 9RQ, UK

Presented by: Murray, Jo

Smc (Structural Maintenance of Chromosomes) complexes play crucial roles in chromosome maintenance and segregation. Cohesin and Condensin respectively mediate sister chromatid cohesion and chromosome condensation at mitosis. Smc5/6 is also an essential complex and is involved in the response of cells to DNA damage and stalling of replication forks.

Smc5/6 has been most studied in relation to DNA repair but its repair functions likely reflect a more fundamental role in the regulation of chromosome structure. Smc5/6 has been shown to regulate cohesin after DNA damage and to prevent chromosome mis-segregation and fragmentation. In budding yeast Smc5/6 is required to reinforce genome-wide loading of cohesin after DNA damage. In *S. pombe* we showed that Smc5/6 is required for cohesin removal from chromosome arms after DNA damage or replication inhibition. Thus SMC complexes functionally interact and this is critical for accurate chromosome segregation and genome stability.

In both budding and fission yeast, Smc5/6 is required for efficient homologous recombination (HR) after DNA damage or replication inhibition. We initially showed in *S. pombe* that the complex is required to resolve recombination intermediates after HR-dependent restart at collapsed forks and this is important for chromosome segregation. We also identified a second requirement at stable stalled replication forks, which is important for replication restart. DNA combing analysis shows that in hypomorphic smc6 mutants the profile of origin firing is altered in HU and that upon release replication restart is delayed. This correlates with changes in Rpa and Rad52 chromatin association and implicates the Smc5/6 complex as acting at the stalled fork. We hypothesise that the Smc5/6 complex is required to keep stalled forks primed for restart.

The essential Pfh1 DNA helicase promotes fork progression through G-quadruplex DNA

Nasim Sabouri[a], John A Capra[b], Matthew L Bochman[c], Virginia A Zakian[c]

[a] *Department of Medical Biochemsitry and Biophysics, Umeå University, Umeå, Sweden*
[b] *Department of Biomedical informatics, Vanderbilt University, Nashville, TN, USA*
[c] *Department of Molecular Biology, Princeton University, Princeton, NJ, USA*

Presented by: Sabouri, Nasim

Pif1 family enzymes comprise a 5'-3' DNA helicase family that is found in essentially all eukaryotes and some prokaryotes. This helicase family plays a major role both in nuclear and mitochondrial DNA maintenance. *S. pombe*, like humans, encodes a single Pif1 family protein, Pfh1. Pfh1 is essential for both nuclear and mitochondrial genome replication. Pfh1 promotes fork movement in the ribosomal DNA, the mating type locus, telomeres, highly transcribed RNA Polymerase II genes and RNA Pol III genes. In addition, Pfh1 resolves converged replication forks and suppresses DNA damage at all of these sites. G-quadruplex structures are stable non-canonical secondary DNA structures that have been associated with genomic features, such as telomeres and promoter regions. To identify regions with the potential to form G4 DNA genomewide in fission yeasts, we performed a computational search across the four available fission yeast genomes. By performing ChIP-seq, we observed that Pfh1 significantly binds these motifs in *S. pombe*. Cells depleted of Pfh1 showed an increased fork pausing at G4 motifs. In addition, we observed an increased number of double strand breaks near G4 motifs in Pfh1-depleted cells. We conclude that Pfh1 facilitates fork progression through G4 motifs and therefore preserves genome stability.

Mechanisms of replication-associated genome rearrangement

Antony M. Carr, Izumi Miyabe, Ken'ichi Mizuno, Saed Mohebi, Johanne M. Murray

Genome Damage and Stability centre, School of Life Sciences, University of Sussex, Brighton, BN1 9RQ, UK

Presented by: Carr, Tony

Impediments to DNA replication are known to induce gross chromosomal rearrangements (GCR) and copy number variations (CNV). GCRs/CNVs underlie human genomic disorders and are a feature of the replication stress promoted by oncogene-driven proliferation, contributing to both cancer development and therapy resistance.

When stress arrests DNA replication, the replisome remains associated with the DNA fork structure (paused/stalled fork) and is protected by the ATR-dependent inter-S phase checkpoint. Stalled forks efficiently resume when the stress is relieved. However, if the replisome dissociates from the fork (collapsed fork), or the fork structure breaks (broken fork), replication restart can proceed by either homologous recombination or microhomology-primed re-initiation (FoSTeS/MMBIR).

I will describe work where we ascertain the consequences of replicating a region of DNA with a fork that has been correctly restarted by homologous recombination. We identified an entirely novel mechanism of chromosomal rearrangement: recombination-restarted forks show an exceptionally high propensity to execute a U-turn at small inverted repeats (up to 1:40 replication events). This correlates with the replication fork generated by restart replicating in a non-canonical manner.

The error-prone nature of restarted forks likely contributes to the generation of GCRs and gene amplification in cancer as well as to non-recurrent CNVs in genome disorders.

A proteome-wide visual screen identifies fission yeast proteins localizing to DNA double-strand breaks

Yang Yu, Jing-Yi Ren, Jia-Min Zhang, Fang Suo, Xiao-Feng Fang, Fan Wu, Li-Lin Du

National Institute of Biological Sciences, Beijing, China

Presented by: Du, Li-Lin

DNA double-strand breaks (DSBs) are a major threat to genome integrity. Proteins involved in DNA damage checkpoint signaling and DSB repair often relocalize and concentrate at DSBs. To systematically identify proteins targeted to DSBs in the fission yeast *Schizosaccharomyces pombe*, we performed a visual screen using an ORFeome library expressing about 95% of the fission yeast proteins in YFP-tagged form. We found 51 proteins that, when expressed from the nmt1 promoter on the ORFeome plasmids, were able to form a distinct nuclear focus at an HO endonuclease-induced DSB. The majority of these proteins have known connections to DNA damage response, but few have been visualized at a specific DSB before. Among the screen hits, 37 can be detected at DSBs when expressed from native promoters. We classified them according to the focus emergence timing of the endogenously tagged proteins. Eight of these 37 proteins are yet unnamed. We named these eight proteins DNA-break-localizing proteins (Dbls) and performed preliminary functional analysis on two of them, Dbl1 (SPCC2H8.05c) and Dbl2 (SPCC553.01c). We found that Dbl1 and Dbl2 contribute to the normal DSB targeting of checkpoint protein Rad26 (homolog of human ATRIP) and DNA repair helicase Fml1 (homolog of human FANCM), respectively. As the first proteome-wide inventory of DSB-localizing proteins, our screen result will be a useful resource for understanding the mechanisms of eukaryotic DSB response.

Interactions between Rad51 recombinase and its activator Swi5-Sfr1 complex in fission yeast

Kentaro Ito, Yasuhiro Tsutsui, Hiroshi Iwasaki

Tokyo Institute of Technology, Japan

Presented by: Iwasaki, Hiroshi

Two sub-pathways function in the Rad51-mediated homologous recombination repair (HRR) in fission yeast; one is the Rad55-Rad57 complex-dependent and the other is the Swi5-Sfr1 complex-dependent. Any single mutants of the four genes, rad55, rad57 swi5 and sfr1 show a moderate HRR-defect while double mutants with combinations of rad55/rad57 and swi5/sfr1 show the severe HRR-defect, which are very similar to that of rad51Δ mutants. The moderate HRR-defect of the single mutants of rad55, rad57, swi5 and sfr1 is suppressed by over-expression of Rad51. Therefore, if a rad51 mutant complements HRR-defect of rad51Δ mutants and overproduction of the rad51 mutant cannot suppress HRR-defect rad51Δ rad57Δ, such rad51 mutants are expected to be specifically defective in the Swi5-Sfr1-dependent sub-pathway of Rad51-mediated HRR. We tried to isolate and indeed we did such rad51 mutants. In the meeting, we will discuss about the functional interactions between Rad51 and the Swi5-Sfr1 complex, based on the characterizations of the isolated Rad51 mutants and the crystal structure of the Swi5-Sfr1 complex.

Combinatorial recognition of SUMO, ubiquitin and DNA provides selectivity in the STUbL-mediated DNA damage response

Minghua Nie[a], Aaron Aslanian[a], John Prudden[a], Johanna Heideker[a], Ajay Vashisht[b], James Wohlschlegel[b], John Yates 3rd[a], Jeff Perry[a], Eros Lazzerini-Denchi[a], Michael Boddy[a]

[a] *The Scripps Research Institute, La Jolla, CA, USA*
[b] *University of California, Los Angeles, USA*

Presented by: Boddy, Michael

Protein modification by SUMO and ubiquitin critically impacts genome stability via effectors that "read" their signals using SUMO interaction motifs (SIMs) or ubiquitin binding domains (UBDs), respectively. A novel mixed SUMO and ubiquitin signal is generated by the SUMO-targeted ubiquitin ligase (STUbL), which ubiquitinates SUMO conjugates. We determine that the "ubiquitin-selective" segregase Cdc48-Ufd1-Npl4 also binds SUMO via a SIM in Ufd1, and can thus act as a selective receptor for STUbL targets. In this regard, we define key cooperative DNA repair functions for Cdc48-Ufd1-Npl4 and STUbL; thus revealing a new signaling mechanism involving dual recruitment by SUMO and ubiquitin for Cdc48-Ufd1-Npl4 functions in maintaining genome stability. Prompted by our fission yeast analyses, we tested for analogous functions of the mammalian STUbL, RNF4, in the DNA damage response, and for potential cooperation with the Cdc48 homologue, p97. Finally, we describe a novel target selection mechanism, involving dual recognition of SUMO and DNA, utilized by RNF4 in the mammalian DNA damage response.

The role of Fml1 and its partner proteins Mhf1 and Mhf2 in promoting genome stability

Sonali Bhattacharjee, Fikret Osman, Laura Feeney, Rachael Barton, Jacqueline Neo, Matthew Whitby

Department of Biochemistry, University of Oxford, UK

Presented by: Whitby, Matthew

Homologous recombination (HR) can promote genome stability through its capacity to restart collapsed replication forks and faithfully repair DNA double strand breaks (DSBs). However HR can also cause genome instability by recombining "inappropriate" homologous sequences, especially if the recombination intermediates are resolved to form crossovers. In fission yeast the DNA helicase Fml1, which is related to human FANCM and budding yeast Mph1, plays important roles in limiting crossing over during DSB repair and promoting Rad51 mediated recombination at blocked replication forks. Recent studies from the Wang and Meetai laboratories have identified a conserved protein complex called MHF, consisting of two small histone-fold containing proteins Mhf1 and Mhf2, that interacts with FANCM. In vitro MHF stimulates DNA binding and reversal of model replication forks by FANCM. In vivo it is required together with FANCM for resistance to DNA interstrand crosslinking agents, promotion of FANCD2 monoubiquitination following DNA damage, and suppression of sister chromatid exchange. To further investigate the importance of MHF to the function of FANCM family members we have performed a genetic analysis of mhf1Δ and mhf2Δ mutants in fission yeast. Both mutants exhibit DNA repair and recombination phenotypes that are similar to a fml1Δ mutant. Moreover, mutations in Fml1, which impede its interaction with MHF, confer phenotypes that are consistent with the notion that MHF performs an accessory role to Fml1. Intriguingly Mhf1 and Mhf2 are components of the constitutive centromere-associated network in vertebrates, and consistent with this we observe that fission yeast mhf1Δ and mhf2Δ mutants exhibit phenotypes indicative of centromere dysfunction. Importantly these phenotypes are not shared by fml1Δ suggesting that MHF has a function at centromeres that is distinct from its role in promoting Fml1 activity in DNA repair and recombination.

Spatio-temporal control of meiotic recombination initiation

Masaru Ito[a], Tomoichiro Miyoshi[a], Shintaro Yamada[b], Takatomi Yamada[a],Kazuto Kugou[a], Arisa Oda[b], Hisao Masai[c], Kunihiro Ohta[a]

[a] *Dept. of Life Sciences, Grad. Sch. of Arts and Sciences, the Univ. of Tokyo, Japan*
[b] *Dept. of Biophys. and Biochem., Grad. Sch. of Science, the Univ. of Tokyo, Japan*
[c] *Genome Dynamics Project, Tokyo Metropol. Inst. of Med. Sci., Japan*

Presented by: Ohta, Kuni

Higher order chromosome structures called “axes” and “loops” are established in early stage of meiosis. Meiotic recombination is initiated by programed DNA double-strand breaks (DSBs) at recombination hotspots often formed in loop regions. After premeiotic S phase, DSB are formed by Rec12/Spo11 protein with help of its partner proteins, which are mainly localized at chromosome axes. DSB sites in loops are assumed to form “tethered-loop axis complex” to activate Rec12/Spo11. Positioning of DSB sites is possibly regulated by histone modifications such as histone H3K4 trimetylation (H3K4me3) and H3K9 actylation (H3K9ac). However, precise molecular mechanisms are left unsolved. We demonstrate how *S. pombe* DSB formation is controlled spatially and temporally. Rec12 and its partners form two distinct subcomplexes, DSBC (Rec6-12-14) and SFT (Rec7-15-24). Among them, Mde2, whose expression is strictly regulated by the replication checkpoint, plays a pivotal role in axis-loop interaction. Mde2 is localized at DSB sites in loops, and bridges loop and axis by interacting with Rec15 on axis. Rec10, the major component of axis, provides a docking platform for Rec15 binding to axes. Mde2 can inter with Rec14 in DSBC. Interaction of Mde2 to Rec14 in axis-loop complex may lead to final activation of DSB formation. These results suggest Mde2 functions as a central coupler that tethers DSB sites to axes, in liaison with the meiotic replication checkpoint.

We also studied histone modification patterns specific for recombination hotspots, and found that DSB sites are often accompanied with higher H3K9ac and lower H3K4me3. This suggests that combination of multiple histone marks may determine the position of DSB sites in *S. pombe*. Introduction of a point mutation at H3K9 or deletion of the unique H3K4 methyltransferase, their impacts on DSB efficiency differ from one site to another. We will discuss diversification and functions of histone marks for hotspot positioning.

Cooperation between formins Cdc12 and For3 for contractile-ring assembly in fission yeast cytokinesis

Valerie Coffman[a], Jennifer Sees[b], David Kovar[b], Jian-Qiu Wu[a]

[a] *The Ohio State University, Columbus, OH 43210, USA*
[b] *The University of Chicago, Chicago, IL 60637, USA*

Presented by: Wu, Jian-Qiu

Cytokinesis is the final step of the cell-division cycle that partitions cellular components from a mother cell into two daughter cells. The contractile ring consisting of actin filaments and many other proteins is required for cytokinesis in fungi and animal cells. Formins are a family of proteins that promote nucleation and rapid elongation of linear actin filaments. The formin For3 is involved in polarized growth in fission yeast. During cell division, it relocalizes to the cleavage site but has no known function in cytokinesis. Here we show that For3 but not the formin Cdc12 activity is responsible for node condensation into clumps in cdc12 mutants. For3 localization at the division site depends on F-BAR protein Cdc15, and for3 deletion is synthetic lethal with mutations defective in contractile-ring formation, suggesting that For3 plays a role during contractile-ring assembly. For3 is essential in cells expressing NH2-terminal truncations of Cdc12, which depend on actin filaments for localization to the division site. By tetrad fluorescence microscopy, we find that the double mutants of for3 deletion and cdc12 truncations are severely defective in contractile-ring assembly since the Cdc12 truncations cannot localize to cell equator efficiently. Together, these data reveal that For3 plays a role in contractile-ring assembly in cooperation with Cdc12.

Cytokinesis *in vitro* and *in vivo*

Mohan Balasubramanian[a,b,c], Evelyn Yaqiong Tao[a,b], Mithilesh Mishra[b], Jun Kashiwazaki[d], Yinyi Huang[c], Jacky Junqi Huang[a,b], Tomoko Takagi[d], Dhivya Subramaniam[a,b], Tang Xie[b], Issei Mabuchi[d]

[a] *Department of Biological Sciences, National University of Singapore, Singapore*

[b] *Temasek Life Sciences Laboratory, Singapore*
[c] *Mechanobiology Institute, Singapore*
[d] *Gakushuin University, Tokyo, Japan*

Presented by: Balasubramanian, Mohan

The mechanism of cell division is conserved in many eukaryotes, from yeast to man. A contractile ring of filamentous actin and myosin II motors generates the force to bisect a mother cell into two daughters. The actomyosin ring is among the most complex cellular machines, comprising over 150 proteins. Understanding how these proteins organize themselves into a functional ring with appropriate contractile properties remains one of the great challenges in cell biology. We use the fission yeast *S. pombe*, which divides employing an actomyosin based contractile ring, as model to understand actomyosin ring dependent cytokinesis mechanisms. I will present recent work using novel probes to investigate actin dynamics, a synthetic rewiring approach to assemble medial actomyosin rings, and a new permeabilized cell system to understand actomyosin ring constriction in vitro. These studies shed light on the mechanism of medial assembly of the actomyosin ring as well as the mechanism of ring constriction and force generation.

In vitro contraction of cytokinetic ring depends on myosin II but not on actin dynamics

Mithilesh Mishra[a], Jun Kashiwazaki[b], Tomoko Takagi[b], Ramanujam Srinivasan[c], Yinyi Huang[c], Mohan Balasubramanian[a], Issei Mabuchi[b]

[a] *Temasek Life Sciences Laboratory, The National University of Singapore, Singapore*
[b] *Department of Life Science, Faculty of Science, Gakushuin University, Tokyo, Japan*
[c] *Mechanobiology Institute, The National University of Singapore,, Singapore*

Presented by: Mabuchi, Issei

Cytokinesis in many eukaryotes involves the contraction of an actomyosin-based contractile ring (CR). However, the detailed mechanism of CR contraction is not fully understood. We establish for the first time an experimental system to study contraction of the CR to completion in vitro. We show that CR of permeabilised fission yeast cells undergo rapid contraction in an ATP- and myosin-II-dependent manner in the absence of other cytoplasmic constituents. The rate of contraction in vitro was much faster than that of the CR in live cells suggesting that the ring contraction in these cells is somehow suppressed. Actin disassembly occurs during contraction so that actin is not detectable after contraction, but neither actin polymerisation nor its disassembly is required for contraction of CR. Addition of exogenous actin cross-linking proteins blocks CR contraction. Using CRs generated from fission yeast cytokinesis mutants, we show that not all proteins required for assembly of the ring are required for its contraction in vitro. Our work provides the beginnings in the definition of a minimal contraction-competent contractile ring apparatus.

Identification of SIN pathway targets reveals mechanisms regulating late mitotic events.

Sebastian Mana-Capelli[a], Sneha Gupta[a], Janel McLean[b], Kathleen Gould[b], Dannel McCollum[a]

[a] *University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology, USA*
[b] *Vanderbilt University, Department of Cell and Developmental Biology, USA*

Presented by: Mana-Capelli, Sebastian

The Septum Initiation Network (SIN) regulates multiple functions during late mitosis including actomyosin ring and septum assembly, mitotic exit, spindle elongation, nuclear positioning, and inhibition of the interphase polarity pathway called the MOR. To determine how the SIN regulates these many late mitotic pathways, we employed both a proteomics and candidate based approach that allowed us to identify multiple substrates of the SIN effector kinase, Sid2. Many of these targets have functions consistent with being effectors of the SIN. We have begun to characterize candidate SIN targets as a way of elucidating the detailed molecular mechanisms of SIN regulation. For example we show that the MOR pathway components Nak1 kinase and an associated protein Sog2 are phosphorylated by Sid2 to inhibit interphase polarized growth and cell separation until cytokinesis is complete. Sid2 phosphorylation of Nak1 causes removal of Nak1 from the SPBs, which may both relieve Nak1 inhibition of the SIN, and block MOR signaling by preventing interaction of Nak1 with the scaffold protein Mor2. We show that the SIN is also involved in regulating anaphase spindle elongation and telophase nuclear positioning via inhibition of Klp2, a minus end directed kinesin-14. Sid2 phosphorylation of Klp2 blocks localization of Klp2 to microtubules in anaphase/telophase to promote efficient spindle elongation and telophase nuclear positioning. Phosphorylation of Klp2 blocks its loading onto microtubules by inhibiting its interaction with Mal3 (EB1 homolog). Together these studies provide a foundation for building a systems level understanding of control of late mitotic events by the SIN.

Control of cytokinesis via SIN-mediated phosphoinhibition of formin multimerization

K. Adam Bohnert[a], Agnieszka Grzegorzewska[b], Alaina Willet[a], Yujie Li[b],Jennifer A. Sees[b], Craig W. Vander Kooi[c], David R. Kovar[b], Kathleen L. Gould[a]

[a] *Dept. of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37212, USA*
[b] *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA*
[c] *Department of Molecular and Cellular Biochemistry and Center for Structural Biology, University of Kentucky, Lexington, KY 40536, USA*

Presented by: Gould, Kathy

Many cell cycles terminate with cytokinesis, the process by which daughter cells physically separate. Most eukaryotes accomplish this task by forming and constricting a medial actomyosin-based cytokinetic ring (CR). In the fission yeast *Schizosaccharomyces pombe*, a conserved signaling pathway termed the septation initiation network (SIN) regulates this machinery. However, which integral CR components are directly targeted by the SIN was not known. Here, we report that the cytokinetic formin Cdc12 is hyperphosphorylated during cytokinesis, and that the SIN kinase Sid2 participates in this modification. Consistent with formin phosphorylation constituting a key SIN signal, eliminating Sid2-mediated Cdc12 phosphorylation severely compromises node/Mid1-independent cytokinesis as well as challenges to CR maintenance. In these contexts, abnormal Cdc12 clustering accompanies CR fragmentation. Indeed, during late cytokinesis, Sid2-mediated phosphorylation counteracts multimerization of a novel Cdc12 domain involved in F-actin bundling. Collectively, our findings broaden the scope of formin functions and interactions during cytokinesis and illustrate their complex post-translational control.

Evolution of division site positioning within the fission yeast clade

Ying Gu, Candice Yam, Snezhana Oliferenko

Temasek Life Sciences Laboratory, 1 Research Link, NUS, 117604 Singapore

Presented by: Oliferenko, Snezhka

Fission yeast cells divide in the middle. A commonly used model system *Schizosaccharomyces pombe* appears to utilize both positive and negative cues to assemble the actomyosin ring precisely at the cellular equator. I will show our recent data on division ring assembly in the sister organism, *Schizosaccharomyces japonicus*, and will discuss how the division site selection strategies have diverged within the fission yeast clade.

Base composition and nucleosomal organization of the *Schizosaccharomyces pombe* genome

Luis Quintales, Ignacio Soriano, Enrique Vázquez, Francisco Antequera

Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas (CSIC)/Universidad de Salamanca, Campus Miguel de Unamuno 37007-Salamanca, Spain

Presented by: Antequera, Paco

Nucleosomes are the basic units of chromatin. They facilitate the packaging of the eukaryotic genome inside the nucleus and modulate the access of regulatory proteins to DNA either directly or through the epigenetic modification of histones. To study the contribution of nucleosomes to genome regulation in *S. pombe*, we have generated high-resolution maps that allow precise localisation of individual nucleosomes. Our results show that regular nucleosomal arrays extend upstream from the nucleosome depleted region present at the 5' end of many genes and downstream from the transcription termination site. Nucleosome positioning is also present along genes that show low or undetectable levels of transcription. Different patterns are obtained depending on whether the genomic position used to generate the aggregated nucleosomal profiles is localized inside the transcribed units or at their 5' or 3' end. These results indicate that regular nucleosomal arrays are widespread along the genome and do not necessarily represent a specific feature of the 5' end of the genes. We have also found that the nucleosomal profile overlaps with periodic oscillations in the A+T content of the underlying DNA sequence, which suggests a stable association between nucleosomes and some specific regions of DNA. The implications of these observations for the organization and the evolution of the genome will be discussed.

A SNF2 chromatin remodeling factor controlling nuclear organization

Karl Ekwall

Center for Biosciences, Department of Biosciences and Nutrition, Karolinska Institute, Sweden

Presented by: Ekwall, Karl

The chromosomes of eukaryotes are organized into structurally and functionally discrete domains. This implies the presence of insulator elements that separate adjacent domains, allowing them to maintain different chromatin structures. We have earlier shown that the chromatin remodeler Fft3, belonging to the Etl1/SMARCAD/Fun30 family of SNF2 ATP-dependent remodeling enzymes, is essential for maintaining a proper chromatin structure at centromeres and subtelomeres in *S. pombe*.

I will present data supporting a general role for the Fft3 remodeling enzyme in nuclear organization. Fft3 is localized to insulator elements and inhibits euchromatin assembly in silent chromatin domains at centromeres and subtelomeres. To map chromatin at the nuclear periphery, we have set up the DNA adenine methyl-transferase identification (DamID) genome-wide method. Using this we have shown that peripheral chromatin in fission yeast, in contact with inner nuclear membrane proteins, mainly contains repressed genes i.e. the fission yeast nuclear periphery shows similar properties to those of metazoan cells, despite the absence of a nuclear lamina. We show that the change in chromatin structure at centromeres and subtelomeres in fft3D cells is accompanied by a dramatic change of the nuclear organization. These regions lose their peripheral association detected by DamID and move inside the nucleus. At the same time, we observe an increase of RNA Pol II association, increased gene expression and active chromatin marks. Moreover, we found that Fft3 is physically interacting with TFIIIC, a part of the RNA Pol III transcription complex, at tRNA genes. The tRNA genes are normally located at the nuclear envelope, and this association is lost in fft3D cells. Thus, our data shows that Fft3 plays an important role in mediating the tight association between specific chromatin domains and the nuclear envelope in fission yeast.

Quantitative analysis of chromosome condensation in fission yeast

Boryana Petrova[a], Christoph Schiklenk[a], Kota Miura[b], Jean-Karim Hériché[c], Christian H. Haering[a]

[a] *Cell Biology & Biophysics Unit and Structural & Computational Biology Unit, EMBL Heidelberg, Germany*
[b] *Centre for Molecular and Cellular Imaging, EMBL Heidelberg, Germany*
[c] *Cell Biology & Biophysics Unit, EMBL Heidelberg, Germany*

Presented by: Haering, Christian

Chromosomes undergo extensive conformational rearrangements in preparation for their segregation during cell divisions. Insights into the molecular mechanisms behind this still poorly understood condensation process requires the development of new approaches to quantitatively assess chromosome formation in vivo.

Here, we present a live cell microscopy-based chromosome condensation assay in the fission yeast *Schizosaccharomyces pombe*. By automatically tracking the 3D distance changes between fluorescently marked chromosome loci at high temporal and spatial resolution, we analyze chromosome condensation during mitosis and meiosis and deduct defined parameters to describe condensation dynamics.

We demonstrate that this method can determine the contributions of condensin, topoisomerase II and Aurora kinase to mitotic chromosome condensation. We furthermore show that the assay is able to identify proteins required for mitotic chromosome formation de novo with high accuracy by isolating new condensin mutants that are specifically defective in pro-/metaphase condensation.

To identify yet unknown components of the mitotic chromosome condensation machinery, we started to screen a newly generated collection of more than 1,000 conditional fission yeast mutants using a highly automated version of the live-cell condensation assay. We will present the initial characterization of the first candidates isolated from this screen.

Casein kinase 1 regulates kinetochore-microtubule attachment during meiosis I through Aurora B activation

Takeshi Sakuno[a], Shigehiro Kawashima[b], Yoshinori Watanabe[a]

[a] *Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan*
[b] *Graduate School of Pharmaceutical Sciences, ERATO Kanai Life-Science Catalysis Project, University of Tokyo, Japan*

Presented by: Sakuno, Takeshi

During meiosis I, sister kinetochores become mono-oriented, and homologous chromosomes (homologs) are connected via reciprocal recombination (chiasmata); thus homologs are captured by spindles emanating from opposite poles. The monopolar attachment of sister kinetochores requires proper kinetochore geometry (side-by-side configuration of sister kinetochores), which is established by cohesion at the core centromere. Mono-polar attachment also needs Ark1 (Aurora B) activity at centromeres for destabilizing erroneous attachments of the sister kinetochores and thereby facilitating re-orientation. Fission yeast homologs of casein kinase 1 δ/ε (CK1) reportedly facilitate monopolar attachment; however, the underlying molecular mechanism remains elusive.

Here, we elucidate the function of CK1 in detail by using the meiosis-specific shut-off mutant CK1 (CK1-so). In CK1-so mutants, a high incidence of erroneous attachment is observed during anaphase I, while the core centromeric cohesion is preserved intact. Erroneous attachments in CK1-so cells are significantly suppressed by the simultaneous overexpression of Ark1 and another subunit of the Aurora B complex, Pic1 (INCENP). CK1 phosphorylates Pic1 in vitro at the IN box, a region required for Aurora B activation. Accordingly, the expression of a phosphomimetic Pic1 mutant protein suppresses the incorrect attachment of CK1-so cells. Our data imply that CK1 is dispensable for the canonical mono-orientation pathway that depends on core centromeric cohesion, but is required for the full activation of Ark1, a correction mechanism for chromosome mal-attachment.

Epigenetic mechanism stabilizing chromosomes with multiple centromeres

Shigeaki Saitoh, Hiroshi Sato

Institute of Life Science, Kurume University, Kurume, Fukuoka, Japan

Presented by: Saitoh, Shigeaki

In eukaryotes, genetic information is organized into a set of a certain number of chromosomes. Each chromosome contains a single centromere, and the presence of multiple centromeres on a chromosome is predicted to cause fatal loss of genetic information. To explore the mechanism restricting the number of the centromere per chromosome, we made a dicentric chromosome by artificial chromosome fusion and analyzed how the dicentric chromosome was stabilized. Unlike to the previous observation that a dicentric chromosome caused abnormal mitosis in budding yeast, the presence of the dicentric chromosome resulted in interphase cell cycle arrest in fission yeast. While most of cells with the dicentric chromosome were inviable, 0.7 - 0.2 % of the cells survived to from colonies. In >70 % of the survivors, the dicentric chromosome was converted into the monocentric chromosome by “centromere inactivation”, whereby one of the centromere lost the ability for kinetochore formation without accompanying alteration in the DNA sequence. An inactivated centromere was found to be covered with Swi6-containing heterochromatin. Kinetochore-deficient mutations increased the probability of centromere inactivation, implicating that centromere inactivation may be initiated by disorganization of the kinetochore, and subsequently heterochromatinized. As the inactivated centromere was found to regain its activity frequently in heterochromatin-deficient mutants, heterochromatin appears important for preventing reactivation of once-inactivated centromeres. I will also discuss why the fission yeast genome consists of three chromosomes. We generated a strain in which all three chromosomes were fused into a single chromosome containing a centromere derived from the chromosome III. While single-chromosome cells maintained high viability, cell cycle progression was significantly delayed in these cells. Our findings may shed a light into the mechanism determining chromosome architecture.

New insights into the role of nuclear position in replicating heterochromatin.

Hani Ebrahimi, Devanshi Jain, Julia P. Cooper

CRUK London Research Institute, London, UK

Presented by: Ebrahimi, Hani

Heterochromatic DNA regions sequester inside distinct domains in the eukaryotic nucleus. The study presented here is unraveling the crucial roles of subnuclear organization in promoting the replication and faithful inheritance of heterochromatin.

We have shown that in telomerase deficient *S. pombe*, survivors arise in which the canonical telomeric sequences are replaced with heterochromatic rDNA repeats. This alternative mechanism for maintaining linear chromosomes requires establishment of heterochromatin and is therefore named HAATI (Heterochromatin amplification-mediated and telomerase independent). Recently we have identified a component of the nuclear envelope (Bqt4) that is also essential for maintenance of HAATI chromosomes. In order to decipher the role of this nuclear envelope component in maintaining linear chromosome ends devoid of canonical telomeres, we have first investigated the role of Bqt4 in faithful inheritance of heterochromatin in cells with wild-type telomeres. Absence of Bqt4 causes spreading of heterochromatin into neighboring euchromatic regions. We find that Bqt4 is required for tethering of all heterochromatin regions to the nuclear membrane; moreover, the role of Bqt4 in nuclear membrane tethering is most pronounced during the cell cycle phase in which a given heterochromatic region is replicated. In accord with a role for Bqt4 in heterochromatin replication, bqt4Δ cells are sensitive to agents that cause DNA damage during replication. Artificially tethering such regions to the nuclear membrane in a bqt4Δ setting rescues heterochromatin boundary formation without rescuing S-phase hypersensitivity, suggesting that Bqt4 has separable roles in promoting heterochromatin boundary inheritance and in heterochromatic DNA replication per se.

Roles of *S. pombe* "sub"telomeres

Yuki Nishihara[a], Takuto Ban[a], Kazumi Miyasato[a], Kojiro Ishii[b], Hisao Masukata[c], Junko Kanoh[a]

[a] *Institute for Protein Research, Osaka University, Japan*
[b] *Graduate School of Frontier Biosciences, Osaka University, Japan*
[c] *Graduate School of Science, Osaka University, Japan*

Presented by: Kanoh, Junko

Telomere consists of the telomere repeat and the subtelomere regions. Recent studies have uncovered the functions of the telomere repeat region, such as the maintenance of telomere DNA length, the protection of chromosomal ends, and the regulation of chromosome movements in mitosis and meiosis. However, little is known about the physiological roles of subtelomeres. *S. pombe* is an ideal organism for the analyses of subtelomere because it has only four subtelomeres on chromosomes I and II. *S. pombe* subtelomeres contain approximately 50 kb-long highly homologous DNA sequences, and the heterochromatin is formed at those homologous regions. Deletion of those homologous regions did not have an obvious effect on the vegetative cell growth and the telomere DNA length; however, the deletion resulted in the formation of heterochromatin at the subtelomere-adjacent regions (approximately 50 kb-long) that contain only non-essential genes and in the defective mating and spore viability. Moreover, our ChIP-Chip analyses have shown that the spindle assembly checkpoint protein Sgo2 is localized at the subtelomere and its adjacent region, which we named "SL (Sgo2 localization) region", in interphase. Deletion of Sgo2 caused the abnormal activation of the late replication origins at the SL region in the HU-arrested cells, suggesting that Sgo2 regulates the timing of DNA replication at the SL regions. Other data will be presented.

Regulation of ATM/ATR kinase signaling at fission yeast telomeres

Bettina Moser, Ya-Ting Chang, Jennifer Harland, Olga Raguimova,

Toru Nakamura

Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, USA

Presented by: Nakamura, Toru

Stable maintenance of telomeres is critical to preserve genomic integrity. The GT-rich telomeric repeats are bound by the shelterin complex (composed of Taz1, Rap1, Poz1, Tpz1, Ccq1 and Pot1) and extended by telomerase (composed of Trt1, Est1 and TER1 RNA) in fission yeast. While the shelterin complex is necessary to prevent the DNA damage checkpoint kinases ATM/Tel1 and ATR/Rad3 from fully activating DNA damage responses at telomeres, these kinases play essential roles in telomere maintenance. Recently, we have shown that Tel1/Rad3-dependent phosphorylation of Ccq1 Threonine-93 (T93) is essential for promoting Ccq1-Est1 interaction and telomerase recruitment.

In addition to Ccq1, we found that Tpz1 undergoes Tel1/Rad3-dependent phosphorylation. Much like Ccq1 phosphorylation, Tpz1 is hyper-phosphorylated in the absence of telomerase inhibitors (Taz1, Rap1 and Poz1), or cells carrying short telomeres due to defects in telomerase function. Based on chromatin immunoprecipitation (ChIP) analysis, we have determined that Taz1, Rap1 and Poz1 are required to limit accumulation of the Rad3-Rad26 (ATR-ATRIP) complex to telomeres. Intriguingly, Tel1/Rad3-dependent Tpz1 phosphorylation was abolished in ccq1 deletion mutant cells or cells carrying mutations (in either Tpz1 or Ccq1) that specifically disrupt Tpz1-Ccq1 interaction. Furthermore, we found that Ccq1 directly interacts with Cut5 (TopBP1) through the same N-terminal BRCT domains within Cut5. Therefore, it appears that Ccq1 could function as an adaptor to promote Tel1/Rad3-dependent Tpz1 phosphorylation, much like Crb2 promotes Chk1 phosphorylation. We have also found that disrupting Tpz1-Ccq1 interaction results in reduced accumulation of Ccq1 at telomeres and strong activation of the G2 checkpoint, which could be reversed by disrupting Cut5-Crb2 interaction. Taken together, we therefore hypothesize that Cut5-Ccq1 interaction might compete with Cut5-Crb2 interaction to prevent activation of Chk1 at telomeres.

EMBO Young Investigator Lecture

A new actomyosin focus guides cell-cell fusion in *S. pombe*

Omaya Dudin, Sophie Martin

University of Lausanne, Switzerland

Presented by: Martin, Sophie

Cell-cell fusion is a highly regulated process, essential for zygote formation and tissue development in eukaryotes. Fission yeast cells of opposite mating type fuse to create a diploid zygote in response to pheromone signaling. This fusion process depends on Fus1, an actin nucleator of the formin family. How the two partner cells coordinate cell wall degradation and plasma membrane fusion at sites precisely facing each other is unknown. We show that an actin focus forms in a Fus1-dependent manner at the incipient fusion site, and disassembles post-fusion. While this focus contains the actin-binding protein Crn1, other actin patch and actin ring components are absent. Instead, both type-V myosins Myo52 and Myo51 independently form dots, which co-localize with the actin focus. Myo52 concentration into a distinct dot, albeit not its localization at the projection site, depends on Fus1 and on the presence of a partner cell. Type-V myosins are together essential for fusion, and play non-redundant roles in post-fusion remodeling. Using a microfluidics device to wash out external factors around cell pairs during the fusion process, we define several phases in actin focus formation and cell fusion. This starts with a reversible phase during which Myo52 dots are unstable and disappear upon washout. Myo51 localization coincides with focus stabilization. Remarkably, myosin V dots are asymmetric during this phase, with the P partner displaying higher dot mobility, and later accumulation of Myo51, than the M partner. The latest phase is defined by accumulation of the plasma membrane protein Prm1, essential for fusion. Together, these results suggest that a novel actin structure may be used by type-V myosins to precisely deliver membranous components to the incipient fusion site. The actin focus may form in response to external washable factors, likely pheromones, first in M cells, and in turn guide the location of focus formation in the P cell, for precisely juxtaposed fusion.

A high-throughput microscopy-based genomic survey of the machineries that control and link cell shape, microtubules and cell cycle progression

Veronika Graml[a,b], Xenia Studera[a,b], Jonathan Lawson[a], Anatole Chessel[a], Marco Geymonat[a], Miriam Bortfeld-Miller[b], Laura Wagstaff[a], Thomas Walter[c], Eugenia Piddini[a], Rafael E. Carazo Salas[a,b]

[a] *The Gurdon Institute, University of Cambridge, Cambridge, CB2 1QN, UK*
[b] *Institute of Biochemistry, ETH Zurich, HPM G16.2, Zurich, CH-8093, Switzerland.*
[c] *Institut Curie, Centre for Computational Biology, 75248 Paris, France*

Presented by: Carazo Salas, Rafael

The genomes of most organisms remain black boxes. For instance the *S. pombe* genome consists of ~4'900 protein coding genes of which 26.1% are essential and, like for all other major model eukaryotes, despite its sequencing over 10 years ago most of its genes and protein products have still undefined function - 40% have one only inferred from homology and 20% are completely unknown. Classical genetic screening with *S. pombe* has allowed discovery of many molecules and pathways controlling basic processes like the cytoskeleton, cell morphogenesis and the cell cycle, thanks to the genetic tractability, simple histology and uniform proliferative pattern of S. pombe. Recently a genome-wide collection of knockout haploid *S. pombe* strains became available, opening the possibility to unrestrictedly capitalize on and potentiate that discovery power, using ultrasensitive image-based phenotypic screening strategies.

To accomplish this, in the ERC-funded project SYSGRO we have pioneered a live cell, 3D fluorescence image-based phenotypic profiling pipeline adapted to screen yeast mutant libraries, using automated high-resolution spinning disk confocal microscopy and large-scale, quantitative multiparametric image analysis. With this approach we recently completed a genome-wide screen that discovered 100s of mostly conserved genes involved in controlling and linking cell shape, microtubules and cell cycle progression. Capitalizing on this technology several other screens are ongoing in our group, including automated large-scale epistasis screens. Our vision with this resource is to create an incremental quantitative phenotypic database to store, analyze, visualize and share with the community the biological big image datasets we are generating, and provide fundamental new insights into how genes direct and coordinate multiple biological processes in cells and how diverse processes are co-regulated.

Here I present the development of the pipeline and results from our first screens.

Symmetry breaking in spore germination

Daria Bonazzi[a], Maryse Romao[b], Rima Seddiki[a], Matthieu Piel[b], Arezki Boudaoud[c], Nicolas Minc[a]

[a] *Institut Jacques Monod, France*
[b] *Institut Curie/UMR144, Paris, France*
[c] *Laboratoire Joliot-Curie, ENS, Université de Lyon, France*

Presented by: Minc, Nicolas

The cross-talk between cell polarity and mechanics serves as a central paradigm for cellular morphogenesis. The rod-shape fission yeast *S. pombe* is an excellent model to study mechanisms regulating polarity and morphogenesis. In these cells, spatial landmarks at the cortex direct actin assembly and cell wall synthesis for turgor-driven polarized growth at cell tips. In here, we investigate how these cells become rod in the first place. We monitor germinating spores that exit quiescence to regenerate vegetative cells by quantitative time-lapse microscopy. Rounded spores exhibit an initial near-isotropic growth phase and then break symmetry to stabilize a single polarized growth axis, in a process termed outgrowth. We observe that the polarisome assembles in a single cap long before outgrowth. Strikingly, the polarisome wanders around the rounded spore yielding localized growth sites that fail to progress, disassemble and reform at a new site around the spore surface. Quantitative volume measurements reveal that polarisome stabilization and outgrowth happen when the spore has doubled its volume. This size sensing is independent of cell cycle progression, initial spore size, and is maintained in cytoskeleton, polarity or secretion mutants. Electron microscopy reveals that outgrowth is concomitant with the local rupture of the outer spore wall, a thin and highly rigid cell wall structure deposited at sporulation and essential for spore resistance. Mutants in spore wall assembly outgrow at smaller sizes, and local photoablation of this outer spore wall hastens outgrowth. Mechanical models that compute the stress on the spore wall predict size-dependent symmetry breaking, and suggest positive feedback loops between growth and polarity. These studies document a ubiquitous morphogenetic process in unicellular organisms, and bring fundamental knowledge in general polarization processes.

Shaping the fission yeast cell wall

Fred Chang, Zhou Zhou, Erdinc Atilgan

Columbia University, New York, USA

Presented by: Chang, Fred

We are interested in how fission yeast cells determine their cell shape and dimensions. I will present some recent work in the lab on how forces may shape the cell wall.

During cytokinesis, we find that although the actin-myosin cytokinetic ring is not critical for ingression of the septum, it globally coordinates cell wall assembly at the septum so that the closing hole of the septum is round and centered. In cells with defective rings, the growth of the septum is disorganized so that the septum hole is non-circular and displaced. By examining cells with septum of altered shape, we find that the growth of the septum correlates with local curvature in a ring-dependent manner. This property suggests how the ring may correct for defects in septum shape. This coordinated growth of the septum may contribute to the fidelity and efficiency of cell division.

In other studies, physical measurements of the cell wall contribute to quantitative models for how fission yeast cells are shaped into rods. For instance, these models explain how after cell division, the rounded new end of the cell may be formed by turgor pressure inflating the cell wall of the septum.

Spatial regulation of Cdc42 GTPase in the control of cell morphogenesis

Suarez Illyce[a], Maitreyi Das[b], Fulvia Verde[a]

[a] *University of Miami Miller School of Medicine, USA*
[b] *University of Tennessee, Knoxville, USA*

Presented by: Verde, Fulvia

Cdc42 GTPase is a key morphology control factor that promotes cell polarization by regulating actin-cable polymerization and exocyst localization. Activated Cdc42 displays dynamic oscillations, which are anticorrelated at the two cell tips in fission yeast (Das et al, Science, 2012). These oscillations are due to positive and delayed negative feedbacks and competition for Cdc42 activators. This elegant mechanism spatially controls Cdc42 distribution, regulating the pattern of cell growth and defining cellular dimensions, such as cell diameter.
In this presentation, we discuss the function of the cytoskeleton in Cdc42 GTPase oscillations at the cell cortex. Furthermore, we address the role of phosphorylation in the control of Cdc42 Guanine Exchange Factor (GEF) Gef1 localization at the cell cortex and in modulating Cdc42 dynamics at the cell tips. We show that Gef1 phosphorylation regulates cell width, functionally cooperating with Cdc42 GTPase Activating Protein (GAP) Rga4 in determining the extent of Cdc42 activation at the growth zone.

Model of fission yeast shape driven by membrane-bound growth factors and the cytoskeleton

Dimitrios Vavylonis, Tyler Drake

Department of Physics, Lehigh University, Bethlehem PA, USA

Presented by: Vavylonis, Dimitrios

We developed mathematical and computational models to investigate how fission yeast cells use the cellular polarization machinery consisting of signaling molecules and the actin and microtubule cytoskeleton to regulate their shape. Many studies identify active Cdc42, found in a cap at the inner membrane of growing cell tips, as an important regulator of local cell wall remodeling, likely through control of exocyst tethering and the targeting of other polarity-enhancing structures. First, we show that a computational model with Cdc42-dependent local cell wall remodeling under turgor pressure predicts a relationship between spatial extent of growth signal and cell diameter that is in agreement with prior experiments. Second, we model the consequences of feedback between cell shape and distribution of Cdc42 growth signal at cell tips. We show that stability of cell diameter over successive cell divisions places restrictions on their mutual dependence. We argue that simple models where the spatial extent of the tip growth signal relies solely on geometrical alignment of confined microtubules might lead to unstable width regulation. Third, we study a computational model that combines a growth signal with an intrinsic length scale (as, for example, by a reaction-diffusion mechanism) with an axis-sensing microtubules system that places landmarks at positions where microtubule tips touch the cortex. A two-dimensional implementation of this model leads to stable cell diameter for a wide range of parameters. Changes to the parameters of this model reproduce straight, bent, and bulged cell shapes, and we discuss how this model is consistent with other observed cell shapes in mutants. Our work provides an initial quantitative framework for understanding the regulation of cell shape in fission yeast, and a scaffold for understanding this process on a more molecular level in the future.

Multiple mechanisms for chromosome segregation

Judite Costa[a], Chuanhai Fu[a], Tianpeng Li[a], Viky Syrovatkina[a], Kathleen Scheffler[b], Phong Tran[b]

[a] *University of Pennsylvania, USA*
[b] *Institut Curie, France*

Presented by: Tran, Phong

Proper chromosome segregation is accomplished by the bipolar mitotic spindle, which is composed of microtubules, motors, MAPs and other regulatory proteins. Spindle bipolarity is accomplished by kinesin-5 Cut7 (Hagan and Yanagida, 1992 Nature). However, recent work from our team reveals an additional mechanism for bipolarity, which increases the efficiency of Cut7 function.

We identify the novel Psr1 and Psr2. Both localize to the SPB throughout the cell cycle. Their SPB localization is dependent on Sad1. Deletion of either leads to transient monopolar spindle and high frequency of chromosome mis-segregation. Psr1 shows interaction with Mal3, the +TIP microtubule tracker. This suggests a model where Psr1 is first recruited to the SPB by Sad1, then it captures microtubule plus ends emanating from the opposite SPB by binding to Mal3, leading to an antiparallel organization of microtubules. Cut7 may then crosslink and slide the microtubules apart, forming the initial bipolar spindle. Transient monopolar spindle defects may lead to subsequent chromosome segregation defects.

Interestingly, Psr1 also has a role in clustering centromeres to the SPB at interphase (Hou et al, 2012 J Cell Biol). Psr1 deletion leads to declustered centromeres from the SPB, suggesting a second model, that centromere declustering may lead to subsequent chromosome segregation defects.

To distinguish the relative contribution of the monopolar spindle or the centromere declustering defects on subsequent chromosome segregation, we uncouple these two pathways using Psr2 and chimeras. Surprisingly, individual defects do not lead to chromosome mis-segregation. We uncover a third model, where altered microtubule dynamics in Psr1 or Psr2 deletion may contribute to chromosome mis-segregation.

Pivoting of microtubules around the spindle pole accelerates kinetochore capture

Iana Kalinina, Amitabha Nandi, Benjamin Lindner, Nenad Pavin, Iva Tolić-Nørrelykke

MPI-CBG, Dresden, Germany

Presented by: Tolić-Nørrelykke, Iva

For a mother cell to divide its genetic material equally between the two daughter cells, the chromosomes have to attach to microtubules, which will pull them apart. The linkers between chromosomes and microtubules are kinetochores, protein complexes on the chromosome. The central question, how microtubules find kinetochores, is still under debate. Here we show in fission yeast that mitotic kinetochores are captured by microtubules pivoting around the spindle pole body, instead of growing directly towards the kinetochores. This pivoting motion of microtubules is random and independent of ATP-driven motor activity. By introducing a theoretical model, we show that the observed random movement of microtubules and kinetochores is sufficient to explain the process of kinetochore capture. Our theory predicts that the speed of capture depends mainly on how fast microtubules pivot, which was confirmed experimentally by speeding up and slowing down microtubule pivoting. Similarly to mitosis, microtubule pivoting facilitates kinetochore capture in meiosis I. Thus, pivoting motion allows microtubules to explore space laterally, as they search for targets such as kinetochores.

Inositol pyrophosphates regulate fission yeast mitosis

Boris Topolski, Ursula Fleig

Institut für funktionelle Genomforschung der Mikroorganismen, Heinrich Heine Universität, Düsseldorf, Germany

Presented by: Fleig, Ursula

Inositol pyrophosphates are signaling molecules involved in numerous cellular processes. A suppressor analysis of mal3-1, which encodes a mutant version of the microtubule-plus-end binding protein Mal3, identified the *S. pombe* Asp1 protein, which belongs to the highly conserved Vip1 family of 1/3 inositol polyphosphates. Vip1 proteins have a dual domain structure consisting of a N-terminal "rimk"/ATP-grasp superfamily domain and a C-terminal domain which resembles histidine acid phosphatases. While no enzymatic activity could be demonstrated for the latter domain, we have shown that the Asp1 N-terminal domain generates inositol pyrophosphates in vitro and this enzymatic activity is modulated negatively by the C-terminal part of the molecule. This enabled us to generate *S. pombe* strains that had Asp1 generated inositol pyrophosphate concentrations above or below the wild-type level. Interestingly, we found a direct correlation between the resistance to microtubule-destabilizing drugs and increased intracellular inositol pyrophosphate concentrations. Furthermore above wild-type inositol pyrophosphate amounts fully rescued the abnormal spindle phenotypes observed for nmt81::gfp-atb2+ strains, while asp1+ deletion strains had a substantial number of metaphase-like- spindle that showed consecutive cycles of spindle breakage. Thus fine tuning of Asp1 generated inositol pyrophosphates regulates spindle midzone stability probably by controlling the dynamics of microtubule plus ends. The role of inositol pyrophosphates in mitosis is diverse: apart from its effects on spindle microtubules, microtubule-kinetochore interactions and kinetochore targeting of components of the Mal2-Sim4-Mis6 kinetochore complex are also modulated by these high energy molecules.

The TACC-TOG microtubule-associated proteins connect microtubule plus ends to the kinetochores

Takashi Toda, Ngang Heok Tang

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, London WC2A 3LY, UK

Presented by: Tang, Ngang Heok

Microtubules emanate from the spindle pole body (SPB) and capture the chromosomes at kinetochores during mitosis. Microtubules are intrinsically dynamic and bind to a cohort of microtubule-associated proteins (MAPs) that in turn regulate microtubule dynamics. However, how MAPs regulate kinetochore-spindle microtubule attachment and mitotic progression remain largely unknown.

Our recent studies have unveiled that two different MAPs play crucial roles in connecting the microtubule to the kinetochore in mitosis. The Dis1/TOG protein, which localises to the kinetochore in early mitosis, plays crucial roles in capturing the kinetochore via the plus end of spindle microtubules and maintaining microtubule stability in the spindle-kinetochore interface. On the other hand, the Alp7/TACC-Alp14/TOG complex localises to the SPB and microtubules during early mitosis and subsequently to the kinetochores in mid mitosis. Binding of this complex to the kinetochore is required for proper spindle-kinetochore attachment and accurate anaphase progression. Both Dis1 and Alp7-Alp14 complex bind to the ubiquitous internal loop of an outer kinetochore protein, Ndc80. We propose that the sequential loading of Dis1 and Alp7-Alp14 to the Ndc80 plays a key role in the establishment of stable bipolar attachment and ordered mitotic progression.

Determinants for robustness in spindle assembly checkpoint signaling

Silke Hauf[a], Stephanie Heinrich[a], Eva-Maria Geissen[b], Susanne Trautmann[c], Julia Kamenz[a], Christian Widmer[d], Philipp Drewe[d], Michael Knop[e], Nicole Radde[b], Jan Hasenauer[f]

[a] *Friedrich Miescher Laboratory of the Max Planck Society, Tuebingen, Germany*
[b] *Institute for Systems Theory and Automatic Control, University of Stuttgart, Germany*
[c] *EMBL, Heidelberg, Germany*
[d] *Memorial Sloan-Kettering Cancer Center, New York, USA*
[e] *ZMBH and EMBL, Heidelberg,Germany*
[f] *Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum, Munich, Germany*

Presented by: Hauf, Silke

The spindle assembly checkpoint is a highly conserved signaling pathway that safeguards the proper distribution of chromosomes into daughter cells. This checkpoint detects kinetochores that fail to make proper contact to microtubules and prevents anaphase as a consequence. Despite the crucial importance of this pathway, it is unknown to which extent checkpoint signaling is robust to perturbations. We varied the expression of checkpoint proteins in fission yeast, and analyzed the outcome on checkpoint activity in single cells. We created a framework for interpreting these results by accurately quantifying checkpoint and target proteins on the single cell level. We found that, for core checkpoint proteins, a mere 20 % reduction in abundance can suffice to shift the checkpoint out of the robustness zone and to provoke errors in signaling. Under such conditions, genetically identical cells split into two populations, where some cells maintain a checkpoint response, whereas others fail. Our combined in vivo imaging and in silico analysis suggests that stoichiometric inhibition of the anaphase activator Slp1 can explain both the physiological robustness as well as the population split outside the robustness zone. Our results highlight that checkpoint protein abundance is an important determinant in specifying the checkpoint response and that some checkpoint proteins need to be kept within exceptionally tight windows of abundance to ensure robust signaling.

Clp1-mediated localisation of the chromosome passenger complex to Klp9 (kinesin-6) sharpens the anaphase switch

John C. Meadows[a], Theresa C. Lancaster[a], Graham J. Buttrick[a], Alicja M. Sochaj[b], Kevin G. Hardwick[b], Jonathan B.A. Millar[a]

[a] *Division of Biomedical Cell Biology, Warwick Medical School, University of Warwick, Coventry, CV7 4AL, UK*
[b] *Wellcome Trust Centre for Cell Biology, Institute of Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK*

Presented by: Meadows, John

It is generally thought that the timing of anaphase onset is dictated solely by the spindle assembly checkpoint (SAC). Components of the checkpoint include Mad1, Mad2, Mad3(BubR1), Bub3 and the Bub1, Mph1(Mps1), and Aurora B kinases. The checkpoint is activated in early mitosis when individual kinetochores are not bound to spindle microtubules. This causes Mad2 to undergo a conformational change at the kinetochore which triggers its association with Mad3 and Cdc20 to form the mitotic checkpoint complex (MCC), a potent inhibitor of the APC/C. When all sister kinetochores are bi-oriented the SAC is switched off by a mechanism which requires the association of type-1-phosphatase (PP1) to both the Spc7 (KNL1) kinetochore protein and Klp5/Klp6 (kinesin-8) motors (Meadows et al., 2011, Developmental Cell 20, 739-750). In fission yeast, as in most eukaryotes, the chromosome passenger complex (CPC), which is composed of the Ark1 (Aurora B), Pic1 (INCENP), Nbl1 (Borealin) and Bir1 (Survivin) proteins, re-localises from centromeres to the spindle midzone at anaphase onset. We find that this localisation depends on Clp1 phosphatase-mediated interaction of CPC with Klp9 (kinesin-6), a homologue of mammalian MKLP2. Although Klp9 has a known role in spindle elongation we now show that, in cells lacking Klp9, kinetochores congress to form a metaphase plate but anaphase onset and APC/C activation is delayed by a mechanism that requires Sgo2 and some (Mad3, Bub1 and Mph1), but not all (Mad1 and Mad2), components of the SAC. Finally, we show that the C-terminal 38 amino acids and motor activity of Klp9 are differentially required for the timing of anaphase onset and rate of anaphase spindle elongation respectively. These data indicate that Clp1-mediated localisation of CPC to Klp9 (MKLP2) at the spindle midzone sharpens the anaphase switch by terminating an APC/C-inhibitory pathway that is distinct from the SAC.

A PP1/PP2A phosphatase relay controls mitotic progression

Agnes Grallert, Elvan Boke, Iain Hagan

CRUK Cell Division Group, Paterson Institute for Cancer Research, University of Manchester, UK

Presented by: Hagan, Iain

Extensive protein phosphorylation transforms cellular architecture for chromosome transmission and cytokinesis. Activation of a network of mitotic kinases is accompanied by the simultaneous reduction of counteracting phosphatase activities to ensure that sufficient substrate molecules accumulate phosphate to drive mitotic transitions. Phosphatase activity must then be restored to promote subsequent mitotic transitions and mitotic exit. We will describe a mitotic phosphatase relay in which Protein Phosphatase 1 (PP1) de-represses PP2A activity. Previous work from the Yanagida lab established that phosphorylation of T316 by Cdk1-Cyclin B inhibits Dis2 (PP1) phosphatase activity [1,2]. This inhibition prevents PP1(Dis2) from reactivating PP2A holoenzymes until declining Cdk1/Cyclin B activity allows PP1(Dis2) to auto-reactivate. Auto-reactivated PP1 then drives the reactivation of PP2A-B55(Pab1). Reactivated PP2A-B55(Pab1) counteracts Polo(Plo1) kinase repression of PP2A-B56(Par1) to promote PP2A-B56(Par1) reactivation by PP1(Dis2). This simple phosphatase relay counterbalances the mitotic kinase cascades to ensure a staged control of mitotic progression.

1 Yamano, H., Ishii, K. & Yanagida, M. Embo J 13, 5310-5318 (1994).
2 Ishii, K., Kumada, K., Toda, T. & Yanagida, M. Embo J 15, 6629-6640 (1996).

Cuf2/Sms5 boosts the transcription of APC/C activator Fzr1/Mfr1 to terminate the meiotic division cycle

Yuki Aoi[a], Kunio Arai[b], Masaya Miyamoto[b], Yuji Katsuta[b], Akira Yamashita[a], Masamitsu Sato[c], Masayuki Yamamoto[a]

[a] *Kazusa DNA Research Institute, Japan*
[b] *Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan*
[c] *Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University-TWIns, Japan*

Presented by: Aoi, Yuki

The number of nuclear divisions during meiosis is strictly limited to two, to enable generation of haploid gametes from diploid germ line cells after one round of DNA replication. Although the precise mechanism remains unknown, this special form of nuclear division seems to be achieved by adjusting the anaphase-promoting complex/cyclosome (APC/C) activity to degrade cyclin. Through analysis of suppressors of a meiosis II-defective mutant, we identified the fzr1+/mfr1+ and cuf2+/sms5+ genes as possible factors required for the termination of meiotic divisions. fzr1+ encodes a meiosis-specific APC/C activator, and cuf2+ encodes a transcription factor expressed during meiosis. We noticed that fzr1Δ and cuf2Δ cells exhibited similar novel phenotypes, represented by ectopic spindle assembly and abnormal chromosome segregation induced after the second meiotic division, which implied that the cells entered a meiosis-III like division. Analysis of Cuf2 indicated that it was required for proper transcription of fzr1+ and could bind to the fzr1+ promoter region, indicating that fzr1+ is a critical target of Cuf2. Consistently, the Fzr1 protein level decreased and cyclin Cdc13 was stabilized after meiosis II in cuf2Δ cells. Taken together, we conclude that meiosis can potentially undergo an additional nuclear division if cyclin-dependent kinase (CDK) activity persists after meiosis II, and that Cuf2 is essential to terminate the M-phase cycle timely by boosting expression of Fzr1, which in turn ensures complete degradation of Cdc13, to produce functional gametes.

Meiosis-specific regulation of the fission yeast microtubule organising centre

Charlotta Funaya[a], Yvonne Connolly[b], Agnes Grallert[b], Duncan Smith[b], Claude Antony[a], Kayoko Tanaka[c]

[a] *Electron Microscopy Core Facility, EMBL, Germany*
[b] *Paterson Institute for Cancer Research, UK*
[c] *Department of Biochemistry, University of Leicester, UK*

Presented by: Tanaka, Kayoko

Fission yeast pheromone signaling triggers dramatic rearrangement of microtubule architecture. The parallel cytoplasmic microtubule (MT) bundles generated from the interphase MT organizing centres (iMTOC) are converted into a radial MT array emanating from the spindle pole body (SPB). During meiotic prophase the radial MT array, aided by the dynein-dynactin complex, directs "horsetail" nuclear movements that are necessary for efficient meiotic recombination. We previously showed that Hrs1/Mcp6 is both necessary and sufficient to convert interphase MT bundles into the radial MT array by scaffolding a specialised radial MT organising centre (rMTOC). The rMTOC lasts only during meiotic prophase and it is rapidly removed when meiosis I spindle formation takes place.

Immuno-gold labelling / TEM as well as electron tomography showed that the rMTOC is located at a cytoplasmic region in the vicinity of the SPB. γ-tubulin is found accumulated in the rMTOC during meiotic prophase. This is in a striking contrast to the vegetative cell cycle where majority of the γ-tubulin signal is found at the nucleoplasmic side throughout the cell cycle. Therefore we predict a specialised mechanism that places γ-tubulin to the cytoplasmic side at the start of meiotic differentiation.

At the transition of meiotic prophase to meiosis I onset, the rMTOC disappears and meiosis I spindle formation starts. γ-tubulin is then found at the nucleoplasmic side of the SPB that acts as a spindle pole, indicating that γ-tubulin is translocated again to the nucleoplasmic side. Collectively, translocation of γ-tubulin occurs twice during the meiotic differentiation: nucleoplasmic side to cytoplasmic at early stage of meiotic prophase and cytoplasmic side to the nucleoplasmic side at onset of meiosis I spindle formation. We aim to find out the molecular mechanism that regulates the γ-tubulin translocation during the meiotic differentiation.

Fission yeast nuclear membrane proteins that affect chromosome movements

Yoshie Tange[a], Asakawa Haruhiko[a], Hui-Ju Yang[a], Yuji Chikashige[b], Tokuko Haraguchi[b], Yasushi Hiraoka[a]

[a] *Graduate School of Frontier Biosciences, Osaka University*
[b] *National Institute of Infromation and Communications Technology*

Presented by: Hiraoka, Yasushi

Inner nuclear membrane (INM) proteins and nuclear pore complexes (NPCs) can be important for spatial organization of chromosomes within the nucleus. In the fission yeast *S. pombe*, an INM protein Sad1 provides has an active role in telomere movement in meiosis, providing a clear example of directly affecting chromosome movements within the nucleus. We have been studying INM and NPC proteins that may affect chromosome behaviors in *S. pombe*. In *S. pombe*, Ima1, Lem2 and Man1 are conserved INM proteins (homologous to human Samp1, Lem2 and Man1, respectively). Cells deleted of any one of these genes (ima1Δ, lem2Δ and man1Δ) are viable although cells deleted of all three genes are inviable. *S. pombe* has NPC components identified, 13 out of 33 components are non-essential for mitotic viability. We examined chromosome behaviors in cells of ima1Δ, lem2Δ and man1Δ as well as cells deleted of a non-essential NPC component by following chromosome loci marked by lacO/lacI-GFP in living cells. These experiments found INM and NPC proteins that affect nuclear architectures and chromosome stability.

The fission yeast Greatwall-ENSA(ARPP19)-PP2A pathway links the nutritional environment to mitotic entry

Nathalia Chica, Livia Pérez-Hidalgo, Javier Botet, Ana Elisa Rozalén, Angela Rubio-Tenor, Sergio Moreno

Instituto de Biología Funcional y Genómica (IBFG), CSIC/Universidad de Salamanca. 37007 Salamanca, Spain

Presented by: Moreno, Sergio

Entry into mitosis in fission yeast requires the activity of the Cdc2/Cdc13 cyclin complex. The Wee1/Cdc25 switch ensures proper timing of mitotic entry by regulating Cdc2 activity. In addition to Cdc2/Cdc13, an opposing phosphatase might contribute to the switch. According to other biological models, a suitable candidate is the protein phosphatase type 2A (PP2A) that dephosphorylates Cdk1 substrates, including Cdc25 and Wee1.

Using a genome wide synthetic lethal screening with wee1-50 and cdc2-3w, we have identified one of the catalytic subunits of PP2A, Ppa2, and some of its activators (Ypa1 and Ypa2). Further dissection of this pathway, including the fission yeast orthologues of Greatwall and ENSA-ARPP19 (Ppk18 and Igo1), suggests that this pathway connects the nutritional environment (in particular nitrogen) to the mitotic switch. We will present evidence that Ppk18-Igo1-PP2A may connect TOR signalling to the mitotic switch.

Analysis of factors governing the asymmetry of Septation Initiation Network (SIN) proteins during mitosis

Viesturs Simanis[a], Paulina Wachowicz[a], Anastasia Chasapi[b], Ioannis Xenarios[b], Daniel Schmitter[c], Daniel Sage[c], Michael Unser[c]

[a] *Cell cycle control laboratory, EPFL School of Life Sciences, ISREC, CH1015 Lausanne, Switzerland*
[b] *Vital-IT/Swiss-Prot groups, Center for Integrative Genomics UNIL/SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland*
[c] *Biomedical Imaging Laboratory, EPFL-STI, CH1015 Lausanne, Switzerland*

Presented by: Simanis, Viesturs

The Septation Initiation Network is a group of protein kinases and their regulators, which have been implicated in many events, including assembly and contraction of the contractile actin ring during mitosis, synthesis of the division septum, the cytokinesis checkpoint, reformation of the EMTOC at the end of mitosis, regulation of the G2-M transition, inhibition of the MOR, to shut down cell elongation during mitosis. Failure of SIN signalling results in elongated multinucleated cells; increased SIN signalling results in multiple rounds of septum formation without cytokinesis, and can trigger septum formation form any point in the cell cycle. Understanding how the SIN is regulated will provide insights into how cell cycle events are coordinated.

Association with the SPB plays an important role in SIN signalling. The core kinases of the SIN and their regulatory subunits all associate with the SPB during mitosis. During anaphase, these associations become asymmetric, with active signalling presumed to be limited to the new SPB. Since mutants that perturb the asymmetric distribution of SIN proteins affect the regulation of cytokinesis, it has been proposed that this asymmetry is critical for SIN regulation.
We developed an automated software package that tracks the SPBs via a reference tag, and determines the intensity of the SPB-associated SIN protein signals over time. We have used this to analyse very large populations of wild-type cells, and examine mutants for their effects upon SIN protein localisation and regulation. In parallel, we have used Boolean modelling techniques to develop a model of the SIN, which incorporates all published SIN regulators, and the data from our image analysis; we have used it to perform in silico genetics to guide our wet-lab analysis of how the SIN is regulated. Our analysis of the SIN and its regulators will be presented.

Rif1 regulates the fate of telomere entanglements during M-phase

Sophie Zaaijer, Julie P Cooper

Cancer Research UK London Research Institute, UK

Presented by: Zaaijer, Sophie

The protection of chromosome ends, and in turn genome stability, is intimately linked with the ability of telomeres to replicate and segregate with high fidelity. We have previously shown that Taz1, the fission yeast ortholog of mammalian TRF1 and TRF2, is important for promoting passage of the replication fork through telomere sequences. Deletion of taz1+ results in stalled telomeric replication forks that fail to resume. These defective replication intermediates lead to telomere entanglements that fail to be resolved at mitosis at cold temperatures, leading taz1Δ cells to be cold sensitive. In taz1Δ cells grown in the cold entangled telomeres become apparent as DNA masses persisting between the separating sister chromatids during M-phase. These masses are associated with a persistent Swi7Polα focus, consistent with the idea that the entanglements originate from stalled replication forks; Rad11RPA and Rad22Rad52¬ are also seen between the segregating chromosome masses.

Rif1, a conserved replication/repair protein implicated in the regulation of telomere length and overhang processing, sparked our interest when we observed that rif1+ deletion suppresses taz1Δ cold sensitivity. We have found that Rif1 plays a role in removal of telomeric entanglements rather than the fork-stalling events that generate them. Although Rif1 is reported to bind telomeres in a Taz1-dependent manner, we observe Rif1 at the mid-zone during anaphase in wild type and taz1Δ cells. Artificially recruiting Rif1 to a taz1Δ telomere is lethal in the cold, indicating that Rif1 is transiently present at a taz1Δ telomere. We will present a model where Rif1 regulates the fate of the ssDNA overhang generated by prolonged replication fork stalling and in turn, the final steps of sister chromatid resolution.

Tolerance of deregulated G1/S transcription requires replication control and genome protection mechanisms

Catia Caetano[a], Sarah Farmer[a], Claire Dovey[b], Ollie Limbo[b], Paul Russell[b], Robertus de Bruin[a]

[a] *MRC Laboratory for Molecular Cell Biology and UCL Cancer Institute, University College London, London, WC1E 6BT, UK*
[b] *Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA*

Presented by: de Bruin, Rob

The primary regulation of cell proliferation in most eukaryotic cells is imposed during the G1-S transition of the cell cycle. Activation of G1/S transcription is required to drive entry into S phase, which commits a cell to a new division cycle. Uncontrolled cell proliferation is an invariable characteristic of human cancer and deregulated expression of G1/S cell cycle genes is found in virtually every type of cancer. While the accelerated entry into S-phase caused by deregulated G1/S transcription undoubtedly contributes to uncontrolled cell division in cancer cells, it might also have other consequences that drive tumor development. We investigate this question in the fission yeast *Schizosaccharomyces pombe*.

In fission yeast the transcriptional repressor Nrm1 controls G1/S-transcription. Constitutive G1/S transcription in nrm1Δ cells increases spontaneous DNA damage associated with replication fork collapse, activating a Chk1-dependent cell cycle checkpoint. The Nrm1-regulated homology-directed repair (HDR) protein Ctp1CtIP/Sae2 is especially critical in nrm1Δ cells. Hyperaccumulation of the replication origin-licensing factor Cdc18Cdc6 is well tolerated in nrm1Δ cells, but this depends on coordinated up-regulation of the S-phase CDK cyclin Cig2cyclinA that prevents catastrophic over-replication. Consequently, elevated levels of G1/S transcription create a delicate balance of high levels of licensing factors and cyclin/CDK that, when slightly disrupted, can cause replication stress leading to genomic instability.

Overall this study reveals how cells cope with the deregulated activities of G1/S transcription factors, which creates specific cellular requirements for replication control and genome protection mechanisms. Since deregulated G1/S transcription is found in nearly all types of cancers our future work will explore how these vulnerabilities could be exploited to identify potential anti-cancer drug targets.

Open vs. closed mitosis: what's the difference and why does it matter?

Jae Min, Heetae Jeong, Brigitte Lovell, Shelley Sazer

Baylor College of Medicine, USA

Presented by: Sazer, Shelley

During the open mitosis of animal cells, the nuclear pores disassemble and the nuclear envelope (NE) is reorganized into the endoplasmic reticulum (ER), resulting in mixing of nucleoplasm and cytoplasm. However, in the closed mitosis of yeast the NE does not break down. The structural and functional differences between open and closed mitosis are poorly understood but influence chromosome segregation, the distribution of proteins within the NE/ER membrane system, the Ran GTPase gradient, and nucleocytoplasmic transport.

In animal cells, the nuclear lamina provides a structural scaffold for the nuclear envelope and tethers heterochromatin and a specialized set of proteins to the inner nuclear membrane. In the fission yeast *S. pombe*, heterochromatic chromosomal domains, including telomeres, are also enriched at the nuclear periphery, despite the absence of lamin proteins or a fibrous nuclear scaffold. However, we found that Lem2, a DNA-binding integral inner nuclear membrane protein, performs some lamina-like functions in fission yeast. We will present data consistent with the following model: Lem2 provides a docking site for telomeric heterochromatin at the inner NE in interphase. Lem2 is not required for viability in mitotic cells, but is required in the absence of other telomere tethering proteins. The telomere clustering at the spindle pole body in early meiosis, which is necessary for recombination and spore formation, is dependent on Lem2.

Comparative genome analysis indicates that ancient versions of DNA-binding membrane proteins similar to Lem2 were present in the last eukaryotic common ancestor, which gave rise to the diversity of present-day cells. We are exploring the evolutionary history of nuclear organization to better understand the differences between open and closed mitosis throughout the tree of life.

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Entrainment of cell division by light or temperature in the dimorphic yeast *Schizosaccharomyces japonicus*

Sho Okamoto[a], Kanji Furuya[b], Shingo Nozaki[a], Keita Aoki[a], Hironori Niki[a]

[a] *Microbial Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, Japan*
[b] *Department of Mutagenesis, Radiation Biology Center, Kyoto University, Japan*

Presented by: Niki, Hironori

Many organisms on Earth have adapted themselves to the alternating light and temperature cycles. Many fungi respond to light and regulate fungal development and behavior. A prominent part of the light spectrum that affects fungi is blue light. A blue light activated photoreceptor has been identified in *Neurospora crassa* as the product of the *wc-1* and *wc-2* genes. Orthologs of WC-1 and WC-2 have hitherto been found only in filamentous fungi and not in yeast, with the exception of the basidiomycete pathogenic yeast Cryptococcosis. We found orthologs of WC-1 and WC-2 in the genome of the fission yeast *Shizosaccharomyces japonicus* and refer to them here as Wcs-1 and Wcs-2, respectively. Here, we report that the response to light of *Sz. japonicus* depends on Wcs-1 and Wcs-2. *Sz. japonicus* changes from yeast to hyphae in response to environmental stresses. After incubation at 30 °C, a colony of yeast was formed, and then hyphal cells extended from the periphery of the colony. When light cycles (12-h of light and 12-h of dark) were applied throughout hyphal growth, dark- and bright-colored stripes were distinctly formed in the hyphal zone. Septate hyphae were enriched at the dark-colored stripes. Thus, cytokinesis in growing hyphal cells is synchronously activated by light. Genetic analyses showed that Wcs1 and Wcs2 are certainly responsible for the stripe formation via blue-light sensing. In addition, alteration of temperature induced a similar response of the hyphal cells to light. After temperature cycles of 30 °C for 12 h and 35 °C for 12 h during incubation in the dark, patterning with dark- and bright-colored stripes was observed. Stripes caused by the temperature cycles were formed in all*wcs* gene mutants. Both responses to light and temperature, which are daily external cues, lead the growing hyphal cells to synchronously activate the cell division cycle. A dual sensing mechanism of external cues leads organisms to adapt to daily changes of environmental alteration.

Unbiased segregation of fission yeast chromosome 2 strands to daughter cells

Amar Klar

NCI Frederick, USA

Presented by: Klar, Amar

Inheritance of a strand- and site-specific imprint confers developmental asymmetry to fission yeast sister cells in the mating/cell-type switching phenomenon (Klar 1987). Curiously, location of DNA strands with respect to each other at the centromere is fixed, and as a result, their selected segregation to specific sister chromatid copies normally occurs in eukaryotic cells. The yeast system provides a unique opportunity to determine the significance of such biased strand distribution to sister chromatids. The mat1 imprint, employed by yeast for mat1 switching, is also a "hotspot" of mitotic recombination in diploid cells. Angehrn and Gutz (1968) and Egel (1981) reported that 2% cell mitoses produce homozygosis of all markers located distal to the mat1 locus. Curiously, if one the mat1 allele is switching defective due to a cis-acting mutation, the hotspot recombination is correspondingly reduced. Why does the wild-type mat1 locus present in one of the homologs not cause efficient recombination in the diploid cell when the other mat1 allele is compromised in switching ability? We found that this recombination is very unusual in that it does not require general recombination functions. We propose that transiently broken chromatid arms in S/G2 phase are simply swapped between homologs, much like the topoisomerase activity that exchanges two DNA partners without involving the DNA synthesis machinery. We also exploited unusual hotspot recombination features to investigate whether there is selective segregation of strands such that oldest Watson-containing strands co-segregate in the diploid cell at mitosis. Results bearing on this very unusual recombination mechanism will be presented.

1. Angehrn P, Gutz H (1968) Genetics 60: 158. Abstract
2. Egel R (1981) Cold Spring Harbor Symp Quant Biolg 45: 1003-1007

Impact of replication origin selection on cellular physiology

Blanca Gomez-Escoda, Pei-Yun Jenny Wu

Institute of Genetics and Development of Rennes, CNRS UMR 6290, France

Presented by: Wu, Pei-Yun Jenny

Maintenance of genome integrity requires the accurate duplication and transmission of genetic information. This is ensured by multiple layers of control that specify the activation of replication origins across the genome and regulate the S phase checkpoint. The program of replication changes during development and differentiation as well as in pathologies such as cancer. However, the physiological consequences of undergoing S phase with specific replication programs remain surprisingly unknown. We have shown in fission yeast that changing origin selection in pre-meiotic S phase has a significant impact on the organization of meiotic recombination. Interestingly, our recent results show that replication stress in checkpoint-defective cells, a situation often encountered in cancer, alters the replication program, with the activation of a number of new sites of DNA synthesis across the genome. We are now investigating the characteristics of these deregulated sites, the mechanisms by which they are activated in these conditions, and the potential effect of their usage on genome stability.

Motifs in the Clr2 protein specific for mating-type silencing

Daniel Steinhauf[a], Alejandro Rodriguez[a], Dimitris Vlachakis[b], Gordon Virgo[a], Carolina Kristell[a], Ida Olsson[a], Erik Bongcam-Rudloff[c], Sophia Kossida[b], Pernilla Bjerling[a]

[a] *Dept. of Medical Biochemistry and Microbiology (IMBIM), University of Uppsala Box 582, SE-751 23 Uppsala, Sweden*
[b] *Bioinformatics & Medical Informatics Team, Foundation for Biomedical Research, Academy of Athens, Greece*
[c] *Department of Animal Breeding and Genetics, Swedish University of Agriculture Sciences, Sweden*

Presented by: Bjerling, Pernilla

Schizosaccharomyces pombe is a well-established model for the process of heterochromatin formation. Several components involved in heterochromatin formation have been identified, but the exact sequence of events for initiation remains to be elucidated. Crucial factors involved are RNA transcribed from repeated sequences together with methyltransferase Clr4 and histone deacetylases, for example Clr3, found in the SHREC complex. Clr2 is another crucial factor essential for heterochromatin formation found in the SHREC complex. The exact function of Clr2 has been difficult to pinpoint since no homologous proteins or conserved domains with known functions has been identified in the protein.

Using a bioinformatics approach three conserved motifs in Clr2 were identified, that were named C2SM1-3. C2SM3 corresponds to the previously annotated Clr2 domain (IPR018839). Eight amino acids were mutated in these motifs and integrated at the endogenous clr2 locus. Surprisingly these amino acids were essential for transcriptional repression in the mating-type region, but dispensable for pericentromeric silencing. Several of the mutated strains show unstable silencing in the mating-type region, switching between on and off states, indicative of an establishment or maintenance defect. When tested for silencing in the central core centromere and rDNA the mutant displayed weak effects. In silico modelling suggests that the introduced mutations cause instability to the Clr2 protein. Moreover, a subnuclear localisation of the Clr2 protein is demonstrated. Finally, we show that all the SHREC components are necessary for targeted silencing by tethering of Clr4.

We have identified critical amino acids in the Clr2 protein providing us with the necessary tool to pinpoint the exact function of Clr2 in heterochromatin formation. In addition, these mutations indicate partly separate mechanisms of action for Clr2 in the mating-type as compared to the pericentromeric region.

Structural analysis of Stc1: insights into the coupling of RNAi and chromatin modification

Chao He[a], Sreerekha Pillai[b], Francesca Taglini[b], Fudong Li[a], Ke Ruan[a], Jiahai Zhang[a], Jihui Wu[a], Yunyu Shi[a], Elizabeth Bayne[b]

[a] Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China.
[b] Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, UK

Presented by: Bayne, Liz

Non-coding RNAs can modulate gene expression by directing modifications to histones that alter chromatin structure. In fission yeast, short interfering (si)RNAs produced via the RNA interference (RNAi) pathway direct modifications associated with heterochromatin formation. siRNAs associate with the RNAi effector protein Ago1, targeting the Ago1-containing RITS complex to homologous nascent transcripts. This promotes recruitment of the Clr4 H3K9 methyltransferase complex, CLRC, to cognate chromatin. However, how the RNAi and chromatin modification machineries are connected has remained poorly understood.

Stc1 is a small protein specifically required for RNAi-dependent heterochromatin assembly in fission yeast (1). Stc1 associates with both the RNAi effector Ago1, and the Clr4 complex CLRC. Moreover, tethering Stc1 to a euchromatic locus is sufficient to induce heterochromatin formation independently of RNAi, suggesting that Stc1 plays a key role in mediating the recruitment of chromatin modifiers in response to RNAi.

To understand its mode of action, we have performed a detailed structural and functional analysis of the Stc1 protein. Our analyses reveal that the conserved N-terminal region of Stc1 represents a novel tandem zinc finger domain, with similarities to common LIM domains, but distinguished by a lack of preferred relative orientation of the two zinc fingers. We demonstrate that this tandem zinc finger domain is involved in binding Ago1, while the non-conserved C-terminal region mediates association with CLRC, thereby elucidating the molecular basis for the coupling of RNAi to chromatin modification in fission yeast.

(1) Bayne et al., Cell 140: 666-777 (2010)

Argonaute and Trimmer generate Dicer-independent priRNAs and mature siRNAs to initiate heterochromatin formation

Mirela Marasovic, Manuel Zocco, Mario Halic

Gene Center, University of Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany

Presented by: Halic, Mario

RNA interference (RNAi) is a conserved mechanism in which small RNAs induce silencing of complementary targets. We have previously identified a class of Dicer-independent small RNAs, called primal small RNAs (priRNAs), in fission yeast. The mechanism by which Dicer-independent small RNAs are generated is not well understood in any species. Here we reconstitute final steps of priRNA and siRNA biogenesis in vitro. We identify 3'-5' exonuclease Trimmer and demonstrate that Argonaute, loaded with longer RNA precursors, recruits Trimmer to generate mature priRNAs and siRNAs. We show that in tri1Δ cells remaining longer siRNAs are not functional in guiding Argonaute to slice complementary targets and to maintain facultative heterochromatin at developmental genes. Furthermore, we demonstrate that exosome mediated degradation of antisense transcripts protects the genome from RNAi. In rrp6Δ cells heterochromatin is established at diverse genes in Trimmer/priRNA dependent way. Our results suggest that Argonaute association with RNA degradation products triggers RNAi in a process of transcriptome surveillance.

The RNA Polymerase II CTD code in fission yeast

Beate Schwer, Stewart Shuman

[a] *Dept. of Microbiology and Immunology, Weill Cornell Medical College, New York, NY 10065, USA*
[b] *Molecular Biology Program, Sloan-Kettering Institute, New York, NY 10065, USA*

Presented by: Schwer, Beate

The carboxyl-terminal domain (CTD) of the Rpb1 subunit of RNA polymerase II (Pol II) consists of tandem Y1S2P3T4S5P6S7 heptapeptide repeats. Dynamic remodeling of the structurally plastic CTD, especially via its serine phosphorylation pattern, conveys informational cues about the transcription apparatus – a CTD code – to a large ensemble of CTD-binding receptor proteins that orchestrate mRNA processing, regulate Pol II elongation, and modify chromatin.

The native heptad repeat array in fission yeast is relatively homogeneous compared to other taxa, making it an attractive model to decipher the informational rules that govern the CTD code in vivo. Our genetic dissection of what is essential for fission yeast CTD function provides novel insights to the CTD code, including: (i) structure-activity relations at essential residues Tyr1, Pro3, Ser5, and Pro6; (ii) parsing the minimal functional CTD coding unit as a decapeptide Y1S2P3T4S5P6S7Y1S2P3; and (iii) the distinctive roles of position-specific serine phosphorylations, dominated by a strict requirement for Ser5 during vegetative growth, while Ser2 and Ser7 are dispensable (singly and pairwise). The requirement for Ser5 (and Ser5-PO4) for vegetative growth can be bypassed by covalently tethering mRNA capping enzymes to the CTD, demonstrating that capping enzyme recruitment is a chief function of the Ser5-PO4 mark. We are pursuing genetic and biochemical studies to elucidate the impact of viable CTD variants lacking position-specific phosphorylations.

The fission yeast non-coding transcriptome

Sophie Atkinson[a,b], Samuel Marguerat[a,c], Jean-François Lemay[d], Francois Bachand[d], Jürg Bähler[a]

[a] *University College London, Department of Genetics, Evolution & Environment and UCL Cancer Institute, Darwin Building, Gower Street, London WC1E 6BT, UK*
[b] *CoMPLEX, University College London, London WC1E 6BT, UK*
[c] *Current address: MRC Clinical Sciences Centre, Imperial College London, Du Cane Road, London W12 0NN, UK*

[d] *Department of Biochemistry, RNA Group, Université de Sherbrooke, Sherbrooke, Quebec J1H 5N4, Canada*

Presented by: Atkinson, Sophie

Long non-coding RNAs (lncRNAs) are emerging as important regulators of gene expression, although it remains unclear to what extent they contribute to the information flow from genotype to phenotype. Using strand-specific RNA-sequencing, we identify thousands of unstable, or cryptic, lncRNAs in *S. pombe*. These transcripts are degraded by both overlapping and specific pathways involving the core exosome, the nuclear exosome, the cytoplasmic exonuclease Exo2, and the RNAi machinery. Such cryptic lncRNAs are transcribed both from intergenic regions and antisense to protein-coding genes, and are enriched at centromeric and sub-telomeric regions. We describe a group of antisense lncRNAs that are up-regulated in meiotic cells, and regulated by the exosome, the RNAi machinery and Exo2. We additionally uncover a distinct group of lncRNAs that are up-regulated in nitrogen-starved cells. In contrast to the meiotic lncRNAs, the lncRNAs up-regulated during nitrogen starvation arise from bidirectional transcription from mRNA promoters, and are predominantly regulated by the exosome. Importantly, our data implicate Exo2 in regulation of meiotic gene expression.

Uridylation-mediated RNA turnover

Sophie Fleurdépine[a], Luke Yates[b], Daniel Scott[a], Robert Gilbert[b], Chris Norbury[a]

[a] *Sir William Dunn School of Pathology, University of Oxford, UK*
[b] *Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, UK*

Presented by: Norbury, Chris

We previously detected the addition of non-templated UMP residues to the 3' ends of cytoplasmic, polyadenylated mRNAs in *S. pombe*, and showed was largely attributable to the uridyl transferase Cid1. Cid1-mediated mRNA uridylation acts upstream from Lsm1-7, and independently from deadenylation, to stimulate decapping and hence turnover of a wide variety of *S. pombe* mRNAs. In human cells the Cid1 orthologue ZCCHC11 acts in a similar way to target replication-dependent histone mRNAs for decapping and degradation following inhibition of DNA synthesis.

We have recently studied the relationship between the structure of Cid1 and its biochemical activity. Although Cid1 lacks a canonical RNA recognition motif, we found that it binds RNA substrates in a sequence-independent manner, but with high affinity, by a novel mechanism. The structure of Cid1-UTP, which we have determined to 3Å resolution, reveals three basic patches of amino acid side chains on the surface that are non-contiguous in the primary sequence, which together form a RNA-binding stripe across the surface of the enzyme. Mutation of any two of these patches greatly reduced RNA binding and hence RNA nucleotidyl transferase activity. The architecture of the active site, revealed by crystal structures of Cid1 in two Apo conformers and in complex with UTP, identifies a histidine residue (His336) crucial for the UTP selectivity of the enzyme, and conserved in ZCCHC6 and ZCCHC11. Remarkably, mutation of His336 to alanine converted Cid1 from a uridyl transferase to a poly(A) polymerase.

Strains lacking Cid1 lose most, but not all, mRNA uridylation, indicating the presence of a second mRNA uridyl transferase. Through a combination of circularised RACE and biochemical assays in vitro, we have determined that the residual uridylation is attributable to another member of the six-strong Cid1 family in *S. pombe*. These findings will guide further investigation of Cid1 orthologues in mammalian cells.

New targets and pathways involving the RNA methyltransferase Tgs1

Wen Tang[a], Marco Blanchette[b], Richard Dannebaum[c], Rachel Helston[a], Lili Pan[a], Peter Baumann[a]

[a] *HHMI, Stowers Institute for Medical Research, Kansas City, MO, USA*
[b] *Stowers Institute for Medical Research, Kansas City, MO, USA*
[c] *HHMI, Stowers Institute for Medical Research, Kansas City, MO, USA; University of Oregon, Eugene, OR, USA*

Presented by: Baumann, Peter

PolII transcripts are monomethyl guanosine (MMG) capped at their 5' end shortly after initiation of transcription. This modification stabilizes the RNA and in the case of mRNAs aids in their translation. On a few RNAs the MMG cap is subsequently hypermethylated by the enzyme Tgs1 to produce trimethyl guanosine caps. Until recently only a small handful of TMG-capped RNAs were known including the spliceosomal snRNAs and the telomerase RNA subunit. Using Illumina sequencing we have now identified a comprehensive set of TMG-capped RNAs in the fission yeast and have characterized the phenotypes associated with the loss of the methyl transferase responsible for this modification. We will present data relating to the role of Tgs1 in splicing, the regulation of gene expression and telomere maintenance.

Tf1 integration increases the expression of adjacent genes and improves resistance to environmental stress

Caroline Esnault[a], Gang Feng[a], Atreyi Chatterjee[b], Henry Levin[a]

[a] *Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health*
[b] *NIH, and present affiliation Presidency University, Kolkata*

Presented by: Levin, Henry

Transposable elements (TEs) are a substantial portion of eukaryotic genomes. The success of TEs is commonly thought to result from parasitic behavior. However, after discovering TEs, Barbara McClintock proposed that they reorganize the genome in response to stress and thus are beneficial. The LTR retrotransposon Tf1 of *Schizosaccharomyces pombe* integrates specifically into the promoters of pol II transcribed genes. Deep sequencing experiments revealed that stress-response promoters are preferred by Tf1. To determine what impact Tf1 integration has on the biology of *S. pombe* we measured how de novo insertions altered the expression of the adjacent genes. RT-PCR of 32 genes showed Tf1 insertions did not reduce the expression of the adjacent genes. Interestingly, Tf1 increased the expression of adjacent genes in 40% of the insertions. The mRNAs increased by Tf1 had the same 5' start sites as the native RNAs indicating that Tf1 functioned as a transcriptional enhancer. In addition, we found the Tf1 promoter was induced by heat shock and it was the expression of heat shock response promoters that were increased by adjacent inserts of Tf1. This indicates that Tf1 contains a heat shock response enhancer that stimulates the activity of promoters that also contain heat shock enhancers.

The integration of Tf1 into stress-response promoters together with its ability to increase the expression of these genes suggests that Tf1 may have evolved these mechanisms to benefit cells exposed to stress. To test this hypothesis we grew cultures containing approximately 50,000 insertions for 80 generations in toxic concentrations of CoCl2. Deep sequencing of integration sites revealed that growth in CoCl2 caused reproducible enrichment of cells with insertions next to 17 specific genes including a metal transporter, (zrt1), a cobalt resistance gene (cat1) and a cell cycle regulator (wee1). These results indicate that Tf1 insertion at a variety of sites increases resistance to CoCl2.

Developing fission yeast for chemical biology

Shigehiro Kawashima[a], Ai Takemoto[b], Paul Nurse[b], Tarun Kapoor[b]

[a] *Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan*
[b] *The Rockefeller University, New York, USA*

Presented by: Kawashima, Shigehiro

Chemical inhibitors help the analysis of dynamic cellular processes, particularly when probes are active in genetically tractable model systems such as yeast. Yeast genetics can also help identify novel chemical inhibitors and their targets, and provide a good starting point for developing new drugs, because fundamental cellular mechanisms are conserved between yeast and human. However, as yeast has robust multidrug resistance (MDR) mechanisms, its use for chemical biology has been limited. Using genomics and genetics approaches, we identified the key transcription factors and drug-efflux transporters responsible for MDR in *Schizosaccharomyces pombe* (fission yeast) and designed MDR-sup (for MDR suppressed) strain that is sensitive to a wide-range of chemical inhibitors. Using this strain and "chemical synthetic lethality screen" strategy, we have identified several potential first-in-class small-molecule inhibitors. Genetics-based target identification have revealed that these small-molecules target essential factors for chromosome segregation and ribosome biogenesis, processes that are conserved from fission yeast to human.

To inhibitors and beyond: PKA-regulated phenotypes in *S. pombe* for use in genetic and small molecule screens

Charles S. Hoffman, Ana Santos de Medeiros, Kyle Nelson, Alexander Magee, Liora Friedberg, Karolina Trocka

Boston College Biology Department, USA

Presented by: Hoffman, Charles

Research on the *S. pombe* glucose/cAMP signaling pathway has been facilitated by a PKA-repressed fbp1-ura4 reporter gene that allowed the identification of mutations that reduce PKA activity to promote glucose-insensitive transcription of the reporter, as well as suppressor screens and gene cloning. More recently, we have adapted this system to allow for small molecule screening of heterologously-expressed cyclic nucleotide phosphodiesterases that elevate PKA activity. Here, we describe two additional PKA-regulated phenotypes that permit the detection of either mutations or small molecules that elevate PDE activity to reduce PKA activity under conditions in which the fbp1-ura4 reporter is not well-suited for this purpose. First, elevated PKA activity leads to a rapid loss of viability in cells as they grow to saturation due to a defect in stationary phase entry. We have used this to identify mutant alleles of cAMP-specific PDEs that permit cGMP hydrolysis as such alleles confer resistance to the toxic effect of exogenous cGMP. These mutations demonstrate the importance of residues that position the substrate-binding residues in preventing PDE4 and PDE7 enzymes from hydrolyzing cGMP. Secondly, we have replaced the fbp1-ura4 reporter with an fbp1-GFP reporter and determined conditions under which small molecule PDE activators could be detected by their ability to enhance the GFP signal. While our focus has been on heterologously-expressed PDEs, these reporters and phenotypes could be used to study other components of the *S. pombe* cAMP pathway or other proteins from mammals or pathogens that can substitute for components of this pathway.

Artificial amplification of Sty1 activity in *S. pombe*, using a conditional chemical protein kinase modulator

Johanna J. Sjölander, Carlos Solano, Grötli Morten, Per Sunnerhagen

[a] *University of Gothenburg, Department of Chemistry and Molecular Biology, Box 462 40530 Gothenburg, Sweden*

Presented by: J. Sjölander, Johanna

A central problem when studying cellular responses to stress is to distinguish between responses caused by direct trauma to molecules (i.e. the cellular stress itself) and the result of regulated cellular signalling. As a tool to dissect this, we have recently identified a small molecule modulator which has the unusual ability to both repress and enhance the activity of the Sty1 pathway, depending on the stress applied to the cells. The compound does not directly target the Sty1 protein, but Wis1, the MAP kinase kinase directly upstream of Sty1. We have currently characterized the effect of the compound on activation of Sty1 depending on type of stress, concentration, and timing. The compound is a potent inhibitor of the basal activity in the absence of stress, whereas under e.g. oxidative stress, the activation is much higher. This amplification of the signal under oxidative stress is interesting, as it opens up the possibility to use this novel molecule to reach the same activity of the pathway under a much lower external stress level, and thus to study the effects of the pathway isolated from the impact of the stress. A long-term goal is to identify modified small molecules for enhancing the activity of the homologous human kinase, p38, based on the experience from the present compound. This may become relevant as adjuvant cancer therapy together with genotoxic treatment in certain settings, shifting the balance in the tumour towards apoptosis.

Integrated model of gene expression in response to stress

Dieter A. Wolf[a], McQuary Philip[a], Lev S. Tsimring[b]

[a] *Sanford-Burnham Medical Research Institute, USA*
[b] *University Of California San Diego, USA*

Presented by: Wolf, Dieter

Oxidative stress has been widely implicated in aging-related disorders, including neurodegenerative disorders, inflammatory diseases, and cancer. Using the fission yeast *Schizosaccharomyces pombe* as a model system, our project addresses the fundamental question of how cells adjust their gene expression program in response to oxidative stress with the primary objective of maintaining fitness. In fission yeast, as in other eukaryotes, OS triggers a signal transduction cascade that culminates in the activation of a transcriptional program characterized by downregulation of biosynthetic activities and upregulation of cell protective functions. Whereas this program is relatively well characterized at the level of mRNA abundance, much less is known about the coordination with posttranscriptional layers of gene expression control. We are exploring the overarching hypothesis that gene expression in response to OS is shaped by an integrated multi-layered program that precisely coordinates transcriptional and posttranscriptional mechanisms to maximize survival. Specifically, we are addressing a novel model of integrated control inspired by our preliminary studies which highlighted the limitations of predicting protein changes from changes in mRNA levels. Rather mathematical modeling and simulations suggest critical roles for control at the levels of mRNA and protein stability during stress that lead us to propose that: a) Increased synthesis of stress-induced proteins coincides with increased proteolytic clearance probably as a quality control mechanism. b) OS-triggered rapid translational shutdown leads to downregulation of OS-suppressed mRNAs but not proteins in order to liberate ribosome capacity for the efficient translation of OS-induced mRNAs. We are presently testing these predictions genetically and biochemically to arrive at a comprehensive framework of stress-regulated gene expression.

Expanding the *S. pombe* polarity network using a SILAC mass spectrometry approach

Tara Finegan, James Dodgson, Marco Geymonat, Rafael E. Carazo Salas

The Gurdon Institute, University of Cambridge, Cambridge, CB2 1QN, UK

Presented by: Finegan, Tara

Despite cellular polarity being key to countless, paramount aspects of cell function such as morphogenesis, growth and division and despite its dysregulation being implicated in diverse developmental abnormalities and tumourigenesis, we still possess a fragmentary mechanistic picture of how polarity is established and maintained by the polarity regulatory network even for the simplest of organisms.

SILAC labelling coupled with mass spectrometry is a powerful proteomics technique that facilitates the high-specificity identification of putative interactors from protein pull-down experiments. Differential SILAC labelling of bait and control samples allows the exclusion of proteins which bind non-specifically to the immobilized affinity ligand and removes the need for extensive purification steps, such as is found in the more conventional TAP-tagging, which allows more transient interactions to be identified.

We have developed and utilised a SILAC/GFP-pull down protocol to identify physical interactors of a number of known, GFP-labelled polarity factors in *S. pombe*, in order to identify novel interacting proteins involved in polarity and to search for new physical interactions between known polarity factors, to seek to obtain a more complete network picture of the polarity machinery. Here, we describe the implementation of the method and present a few candidate novel interactors and links we have identified.

Through this approach, and by combining the information obtained with known genetic relationships, localisation interdependencies and physical interaction data for the polarity machinery, we hope to obtain the most complete regulatory network picture of cell polarity for this organism.

Screening for overexpression-mediated bypass-of-essential-gene (O-BOE) suppressors in *Schizosaccharomyces pombe*

Hai-Tao Wang, Jun Li, Jing-Yi Ren, Wei-Tao Wang, Peng-Cheng Wu, Wen Hu, Li-Lin Du

National Institute of Biological Sciences, Beijing, China

Presented by: Wang, Hai-Tao

Essential genes are involved in biological processes key to survival and growth, but the inviability of their deletion mutants makes these genes difficult to study. To get insight into their functions, we here developed a mating-based strategy to screen overexpression-mediated bypass-of-essential-gene (O-BOE) suppressors in *S. pombe*. An O-BOE suppressor is a gene whose overexpression renders an essential gene dispensable. In our screening pipeline, the first step was generating an h- query strain with the chromosomal copy of an essential gene replaced by the natMX marker, but kept alive by an episomal plasmid expressing the essential gene and a counter-selectable marker gene. Then, a library consisting of h+ strains transformed with the ORFeome plasmids expressing *S. pombe* ORFs driven by the nmt1 promoter was crossed to the query strain. Among the cross progenies, we next selected the ones that retained the natMX marker but not the counter-selectable marker under nmt1 induction conditions. The enriched ORFs were identified by high throughput sequencing at last. In our pilot screens, the essential genes themselves were nearly always the most enriched ORFs, demonstrating the specificity of the screening procedure. Both previously reported and potentially novel O-BOE suppressors were also uncovered. As about one-fourth of fission yeast genes are essential, systematic identification of the O-BOE suppressors will likely reveal heretofore-unknown genetic connections between many genes and provide useful clues on their functions.

Quantitative single molecule microscopy reveals that CENP-A (Cnp1) deposition occurs during G2 in fission yeast

Ulrike Endesfelder[a], David Lando[b], Ernest D. Laue[b], Mike Heilemann[a]

[a] *Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University, Frankfurt, Germany*
[b] *Department of Biochemistry, University of Cambridge, Cambridge CB2 1EW, UK*

Presented by: Endesfelder, Ulrike

Fluorescence microscopy is a powerful tool in the life sciences. During the last two decades, a new set of technologies termed "super-resolution" microscopy was developed and revolutionized the field by allowing discriminating objects which are closer than about 200 nm in the imaging plane, overcoming diffraction-limited light microscopy. Today, molecular resolution in intact biological cells with fluorescence microscopy is possible.

Among the different techniques are some which employ photoswitchable fluorophores, a temporal confinement and single-molecule detection (e.g. (d)STORM (Rust et al. 2006; Heilemann et al. 2008) and PALM (Betzig et al. 2006). As single-molecule techniques, one can look at biomolecules one by one, thereby avoiding averaging over many molecules and discovering heterogeneities or subpopulations. Single-molecule registration allows counting molecules, analyzing their spatial distribution or co-localization with other molecules, or determining the composition of tiny biomolecular clusters. In summary, single-molecule super-resolution techniques provide both quantitative and super-resolved information.

We have recently applied quantitative single-molecule photo-activated localization microscopy (PALM) to studies of the fission yeast *Schizosaccharomyces pombe* kinetochore. We use PALM imaging to count single molecules, to investigate the levels and dynamics of the centromere-specific histone H3 variant CENP-A (Cnp1).

In our studies we were able to identify changes in the number of CENP-A (Cnp1) proteins at *S. pombe* centromeres during the cell cycle (Lando, Endesfelder et al. 2012) by PALM imaging, and for the first time demonstrated quantitative single-molecule based super-resolution microscopy of small, densely packed protein clusters.

We thus like to present this new localization-based toolbox for quantitative single-molecule imaging and how it has been applied to study CENP-A (Cnp1) proteins with respect to the cell cycle.

The genomic diversity and population history of *S. pombe*

Daniel Jeffares[a], Charalampos Rallis[a], Adrien Rieux[a], Theodora Sideri[a], Sandra Codlin[a], Francesc Xavier Marsellach Castellví[a], Rodrigo Pracana[a], Nikolas Maniatis[a], Winston Lau[a], Garrett Hellenthal[a], Francois Balloux[a], Jacqueline Hayles[b], Sendu Bala[c], Jared Simpson[c], Thomas Keane[c], Zamin Iqbal[d], Martin Převorovský[e], Leanne Bischof[f], William Brown[g], Tobias Mourier[h], Rafael Carazo Salas[i], Jonathan Lawson[i], Markus Ralser[i], Richard Durbin[d], Jürg Bähler[a]

[a] *Department of Genetics, Evolution & Environment, University College London, UK*
[b] *CRUK London Research Institute, London, UK;*
[c] *Wellcome Trust Sanger Institute, Hinxton, UK*
[d] *Wellcome Trust Centre for Human Genetics, Oxford, UK*
[e] *Charles University in Prague, Prague, Czech Republic*
[f] *CSIRO Mathematics, North Ryde, Australia*
[g] *University of Nottingham, UK*
[h] *University of Copenhagen, Denmark*
[i] *University of Cambridge, UK*

Presented by: Jeffares, Dan

Most of our knowledge of fission yeasts is derived from one isolate of *S. pombe* that was collected from French wine. Very little is known about the genetic or phenotypic diversity of the species as a whole. To advance our understanding of the diversity of *S. pombe* worldwide, we are characterising a collection of 161 natural isolates. These strains have been collected over the past 100 years from over 20 countries across the globe, mostly from cultivated fruits or various fermentations. Our expectation is that the analysis of these strains will begin to shed light on the diversity, evolutionary history and ecology of this (not so) well studied species, and it will also make a valuable resource to relate genotypes to complex phenotypes.

We sequenced the genomes of these strains at high coverage and identified 173,000 high-quality, single-nucleotide polymorphisms (SNPs) and ~14,000 insertion-deletion polymorphisms (indels). The species shows moderately high diversity ($\pi = 2.9 \times 10^{-3}$, compared to *S. cerevisiae* = 5.6×10^{-3}). Analyses of population structure indicate a recent human-associated dispersal for this species. Although we find significant correlation between geographic and genetic distances, we observe only weak evidence for population structure between continents. Consistent with this, modelling of mitochondrial evolutionary rates suggests that dispersal of these human-associated strains began at ~300BC.

We will also outline our analysis of selection in the genome, and initial work to describe phenotypic variation in these strains using both quantitative growth assays in over 40 conditions and quantitative cellular morphology measures.

The Rad3/ATR-γH2A-Brc1 genome protection module

Eva Mejia-Ramirez, Oliver Limbo, Paul Russell

Cell and Molecular Biology, The Scripps Research Institute, USA

Presented by: Russell, Paul

ATM (Tel1) and ATR (Rad3) checkpoint kinases phosphorylate an SQ motif at carboxyl-terminus of histone H2A (or H2AX) in multi-kilobase regions of chromatin flanking DNA lesions. Critical genome protection proteins such as mammalian Mdc1 and fission yeast Crb2 dock with phospho-H2A (γH2A) through their C-terminal paired BRCT domains. Whilst γH2A is recognized as a universal marker for double-strand breaks, there is increasing evidence that γH2A also recruits proteins to stalled and damaged replication forks. The best-characterized characterized example is fission yeast Brc1, which plays important but as yet poorly understood roles in protecting genome integrity during S-phase. Brc1 binding to γH2A is especially critical in mutants lacking Rqh1, which is a member of the RecQ-family of DNA helicases, loss of which can cause cancer predisposition and premature aging in humans. Here, we present our studies aimed at identifying additional genetic defects that create a critical requirement for the Rad3-γH2A-Brc1 pathway. One of these genetic interactions involves a common subunit of Replication Factor C (RFC) and Rad17-RFC complexes, which are ATP-fueled heteropentameric enzymes that clamp the DNA polymerase processivity factor PCNA and the Rad9-Hus1-Rad1 checkpoint factor, respectively, onto DNA. Our findings provide new insights about checkpoint signaling at stalled replication forks, and about the role of Brc1-coated chromatin at these forks.

Regulation of Mus81-Eme1 Holliday junction resolvase in response to DNA damage

Pierre-Marie Dehé[a], Stéphane Coulon[a], Sarah Scaglione[a], Paul Shanahan[b],Arato Takedachi[a], James A Wohlschlegel[c], John R. Yates 3rd[b], Bertrand Llorente[a], Paul Russell[b], Pierre-Henri Gaillard[a]

[a] *CNRS UMR7258, Cancer Research Center of Marseille, France*
[b] *The Scripps Research Institute, CA 92037, USA*
[c] *University of California Los Angeles, CA 90095, USA*

Presented by: Dehé, Pierre-Marie

Structure-specific DNA endonucleases have critical roles during DNA replication, repair and recombination, yet they also harbor the potential for causing genome instability. Controlling these enzymes may be essential to ensure efficient processing of ad hoc substrates and to prevent random, unscheduled processing of other DNA structures, but it is unknown whether structure-specific endonucleases are regulated in response to DNA damage. Here, we uncover DNA damage-induced activation of Mus81-Eme1 Holliday junction resolvase in fission yeast. This novel regulation requires both Cdc2CDK1 and Rad3ATR-dependent phosphorylations of Eme1. Mus81-Eme1 activation prevents gross chromosomal rearrangements in cells lacking the BLM-related DNA helicase Rqh1. We propose that linking Mus81-Eme1 DNA damaged-induced activation to cell cycle progression ensures efficient resolution of Holliday junctions that escape dissolution by Rqh1-TopIII while preventing unnecessary DNA cleavages.

The Chromatin Assembly Factor 1 promotes homologous recombination by counteracting Rqh1-dependent D-loop dissociation

Violena Pietrobon, Karine Fréon, Audrey Costes, Ismail Iraqui, Sarah Lambert

Institut Curie/CNRS, Paris, France

Presented by: Lambert, Sarah

The Chromatin Assembly Factor 1 (CAF-1) is a histone H3/H4 chaperon involved in replication-coupled nucleosome assembly and in chromatin restoration at sites of DNA repair. Homologous recombination (HR) is a ubiquitous DNA repair mechanism but is also responsible for chromosome rearrangement by promoting non allelic HR. Here, we report that CAF-1 promotes ectopic recombination while being dispensable for allelic recombination induced by replication arrest. Using an assay allowing the analysis of joint-molecules induced by a fork-barrier and the recombination products of their resolution, we show that CAF-1 stabilizes joint-molecules made by the recombinase Rad51. Recruitment of the main recombination mediator Rad52 is unaffected by the absence of CAF-1, excluded a role for CAF-1 in promoting early steps of HR process. In contrast, deletion of the anti-recombinase Rqh1 restores the instability of joint-molecules in the absence of CAF-1. Finally the ability of CAF-1 to stabilize early joint-molecules relies on its ability to interact with the replication factor PCNA. Altogether, our data support a model in which CAF-1 promotes histone deposition during D-loop extension, thus counteracting its dissociation by Rqh1. Thus, chromatinization of recombination intermediates by CAF-1 influences their resolution and recombination outcomes.

Meiotic crossover control – from DNA breakage to DNA repair

Kyle Fowler[a], Cristina Martín-Castellanos[b], Scott Keeney[c], Gerry Smith[a],

[a] *Fred Hutchinson Cancer Research Center, Seattle, WA, USA*
[b] *Instituto de Biología Funcional y Genómica, Salamanca, Spain*
[c] *Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Presented by: Smith, Gerry

Crossovers, important for meiotic homolog segregation, are initiated by DNA double-strand breaks (DSBs) made by Rec12 (Spo11 homolog) aided by a dozen other proteins. Meiosis-specific sister chromatid cohesin subunits Rec8 and Rec11 are loaded nearly uniformly across the genome and promote the binding of linear element proteins Rec10 uniformly with some enrichment at DSB hotspots and Rec25-Rec27-Mug20 with exceptionally high enrichment at hotspots [1]. Elimination of Rec25-Rec27-Mug20 eliminates nearly all DSBs at most hotspots. Thus, these three proteins are determinants of and required for genome-wide DSB hotspots, the first such proteins reported in any species.

DSBs are not made independently but interfere with each other over ~100 kb. We propose that hotspot sites are spatially clustered and exactly one DSB is made in each cluster. We find by a type of chromosome conformation capture ("Hi-C") analysis [2] that DNA from a Rec27-immunoprecipitate ligates preferentially to genomic DNA nearby (<100 kb) and dependent on Rec8, as predicted.

Rec12 attaches covalently to DSB ends and is clipped off by the endonuclease of the Mre11-Rad50-Nbs1(MRN)-Ctp1 complex [3,4]. "Deep sequencing" [5] of the resulting Rec12-oligo complexes reveals the distribution of all DSBs at nearly single-nucleotide resolution. Because there is very low background, this method provides the near absolute level of DSBs across the genome, including low-level DSBs in cold regions, which collectively are abundant.

We proposed that crossover invariance, nearly constant cM/kb in spite of DSB hotspots, results from partner choice for DSB repair – primarily with the sister at hotspots and with the homolog in DSB-cold regions [6]. Our analysis of Rec12-oligos supports this interpretation across the genome.

1. Fowler et al, Mol Cell 2013. 2. Lieberman-Aiden et al, Science 2009. 3. Milman et al, MCB 2009. 4. Rothenberg et al, PloS Genet 2009. 5. Pan et al, Cell 2011. 6. Hyppa & Smith, Cell 2010.

Stochastic hybrid modeling of DNA re-replication across the fission yeast genome

Maria-Anna Rapsomaniki[a], Manuel Ramirez[a], Patroula Nathanailidou[a], Stavros Taraviras[a], John Lygeros[b], Zoi Lygerou[a]

[a] *School of Medicine, University of Patras, 26504, Rio, Patras, Greece*
[b] *Automatic Control Laboratory, ETH-Zurich, ETL I 22, Physikstrasse 3, 8092 Zurich, Switzerland*

Presented by: Lygerou, Zoi

DNA replication initiates from hundreds of sites along the fission yeast genome, ensuring complete and accurate duplication of the genome during S-phase. DNA replication dynamics are complex and uncertain. There are many more putative origins than will be used in each cell in a population, while each origin is selected to fire in a small fraction of cells. The progression of DNA replication along the genome is therefore unique in every cell. We have previously developed a stochastic hybrid model of DNA replication which accurately captures the interplay between discrete dynamics associated with the firing of origins, the continuous progression of the replication forks along the genome, and stochastic events due to uncertainty in origin firing, and have used this model to simulate the replication process along the entire fission yeast genome. Aberrations in the control mechanisms regulating DNA replication lead to re-firing of origins in regions already replicated, resulting in DNA over-replication. We will present a stochastic hybrid model of DNA re-replication across the fission yeast genome. This model has been exploited to visualize how different regions along the entire genome respond to over-replication in different cells in a population and to capture and analyze cell-to-cell heterogeneity inherent in the re-replication process. Comparison to experimental data from rereplicating cells is used to validate model assumptions and test model predictions.

Clusters with active replication origins identified on single DNA molecules correspond to nuclear replication foci

Atanas Kaykov[a], Paul Nurse[b]

[a] *The Rockefeller University, New York, USA*
[b] *The Royal Society, London, UK*

Presented by: Kaykov, Atanas

DNA synthesis initiates at precise location along chromosomes called replication origins. Population studies established that each cell uses a subset of many potential origins to replicate the genome. Using DNA combing technique, we analyzed long single DNA molecules covering the entire genome and found that at the onset of S-phase origins fire at random positions along chromosomes. Later in S-phase newly fired origins tend to fire in the vicinity of already fired origins leading to clusters of active origins. The cluster size grows from 196 Kb to 350 Kb on average and the number of clusters per genome increases from 13 to 25 as cells progress from 20% to 50% of DNA synthesis. The position of individual active origins and the position of individual clusters vary among cells. We determined that origin selection between two consecutive S-phases is not epigenetically controlled. During the course of a single S-phase the rate of origin firing increases 5 fold and the fork speed increases by 1Kb/minute.
We imaged replication foci as locations within the nucleoplasm that incorporate BrdU. Replication foci number increases from 14 to 27 as cells progress from 20% to 50% of DNA synthesis and correlates with the number of active origin clusters per cell. In addition, we perturbed foci number and intensity and examined how this reflects on cluster number and origin number within clusters by comparing BrdU incorporation in wt and rad3Δ (ATR homologue) cells in the presence of hydroxyurea. We imaged an average of 22 foci per nucleus in wt cells and an average of 35 foci per nucleus in rad3Δ cells. Replication foci in rad3Δ are half intense when compared with wt foci. Next we examined single DNA molecules and found that compared to wt cells, rad3Δ mutant has on average 45% more clusters with each cluster containing 50% less active origins. We propose that the clusters of active origins mapped on single DNA molecules correspond to replication foci imaged at the cellular level.

Meiotic telomere clustering depends on the telocentrosome, a novel microtubule-organizing center formed at the telomere

Masashi Yoshida[a], Satoshi Katsuyama[a], Kazuki Tateho[a], Hiroto Nakamura[a], Junpei Miyoshi[a], Tatsunori Ohba[a], Hirotada Matsuhara[b],Futaba Miki[c], Koei Okazaki[c], Ayumu Yamamoto[a]

[a] *Department of Chemistry, Shizuoka University, Japan*
[b] *Graduate School of Science and Technology, Shizuoka University, Japan*
[c] *Kazusa DNA Research Institute, Japan*

Presented by: Yamamoto, Ayumu

During meiosis, telomeres cluster at the spindle pole body (SPB) and promote homologous chromosome pairing. Telomere clustering requires the interaction of telomeres with Sad1, an SPB component that belongs to SUN nuclear membrane proteins. However, how the Sad1-localized telomeres move and cluster remains elusive. We found that telomere clustering depends on microtubules and the microtubule motors, cytoplasmic dynein and kinesins. We also found that Kms1 and Kms2, which belong to KASH nuclear membrane proteins and form a complex with Sad1, become localized to the telomeres. Furthermore, the γ-tubulin complex (γ-TuC) is localized at the telomeres to form a novel microtubule-organizing center that we termed the "telocentrosome." Telocentrosome formation depends on the γ-TuC regulator Mto1 and on Kms1, and depletion of either Mto1 or Kms1 caused severe telomere clustering defects. In addition, the dynein light chain (DLC) contributes to telocentrosome formation in a dynein-independent manner, and simultaneous depletion of DLC and dynein also caused severe clustering defects. Thus, telomere clustering depends on the telocentrosome. We propose that telomere-localized SUN and KASH induce telocentrosome formation and that subsequent microtubule motor-dependent aggregation of telocentrosomes via the telocentrosome-nucleated microtubules causes telomere clustering.

SUMOylation regulates telomere length homeostasis by targeting the shelterin subunit Tpz1 in fission yeast

Keisuke Miyagawa, Hiroki Tsuji, Venny Santosa, Katsunori Tanaka

Department of Bioscience, School of Science and Technology, Kwansei Gakuin University, Japan

Presented by: Tanaka, Katsunori

Telomeres are nucleoprotein structures at the ends of linear chromosomes that are essential for preventing chromosomal end-to-end fusions and degradation. Telomere length homeostasis is important for telomere biology. In fission yeast, SUMOylation is required for the regulation of telomere length. SUMOylation, the covalent attachment of the polypeptides SUMO to target proteins, is pervasive mechanism for controlling cellular functions. However, how SUMOylation is crucial for the telomere length homeostasis is unknown. Here we show that SUMOylation of the shelterin subunit Tpz1 on Lys242 is required for the telomere length homeostasis. Mutation of the Tpz1 SUMOylation site lengthens telomeres and reduces the recruitment of Stn1-Ten1 complex onto telomeres. Tpz1 SUMOylation peaks in late S phase to G2 phase, in which Stn1-Ten1 complex is loaded onto telomeres. Taking these observations together, we thus establish the Tpz1 SUMOylation as a critical regulatory mechanism that ensures stable maintenance of telomeres in fission yeast.

Responding to stress – the fundamental roles of peroxiredoxins and thioredoxin in coordinating adaptation and survival responses to H_2O_2

Alison Day, Jonathon Brown, Sarah Taylor, Lewis Tomalin, Jonathan Rand, Brian Morgan, Elizabeth Veal

Institute for Cell and Molecular Biosciences, Newcastle University, UK

Presented by: Veal, Elizabeth

Peroxiredoxins (Prx) are extremely abundant thioredoxin peroxidases with important roles in protecting against oxidative stress, ageing and cancer. The thioredoxin peroxidase activity of eukaryotic 2-Cys Prx detoxifies H2O2 but is highly sensitive to inactivation by H2O2-induced hyperoxidation of a catalytic cysteine.

Counterintuitively, we have previously established that in *S. pombe* the single 2-Cys Prx, Tpx1, is vital for adaptive transcriptional responses to H2O2 due to essential roles in the H2O2-induced activation of the Sty1(Spc1) MAPK and the AP-1-like transcription factor Pap1. In trying to understand Tpx1's signalling functions, we have made the exciting discovery that the H2O2-induced inactivation of the thioredoxin peroxidase activity of Tpx1 is an important new mechanism to limit cell damage and prolong survival under acute stress conditions: In H2O2-treated cells, as the major substrate of thioredoxin(Trx1), Tpx1 promotes Trx1 oxidation, competitively inhibiting Trx1-mediated reduction of other substrates, including the methionine sulphoxide reductase, Mxr1. Consequently, oxidative inactivation of the thioredoxin peroxidase activity of Tpx1 is important to maintain active Mxr1, repair oxidative protein damage and maintain cell viability in cells exposed to toxic levels of H2O2. Based on these discoveries, we propose that eukaryotic Prx have evolved a vital role in responses to lower levels of H2O2 by mediating the H2O2-dependent regulation of thioredoxin activity. We will present exciting new data to support this hypothesis, revealing that Tpx1 also promotes the H2O2-induced oxidation of the thioredoxin-like protein Txl1(Trx3). We find that Txl1 directly reduces oxidised, active Pap1 and show that the Tpx1-mediated oxidation of both Txl1 and Trx1 is essential for Pap1 activation and a robust, transcriptional response to H2O2. The wider implications of this work for how cells regulate their growth and behaviour in response to H2O2 will be discussed.

Role of glucan synthase Bgs1 in the control of growth polarity

Mariona Ramos Vecino, J. Carlos García Cortes, Belén Moreno, Javier Munoz, Jose Ángel Clemente Ramos, Juan Carlos Ribas

Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas (CSIC)/Universidad de Salamanca, Salamanca, Spain

Presented by: Ramos Vecino, Mariona

The cell wall is a structure external to the plasma membrane. Its integrity is vital and it constitutes the exoskeleton that confers mechanical strength and osmotic resistance to the fungal cell. The main structural component of the *Schizosaccharomyces pombe* cell wall is the β(1,3)glucan. Fission yeast contains four essential putative β(1,3)glucan synthase (GS) subunits, Bgs1 to Bgs4. Bgs1 localizes to the contractile ring and septum, and it is responsible for the synthesis of a special linear β(1,3)glucan and the primary septum that this glucan generates. Besides, Bgs1 is also localized to the growing poles. The establishment of growth polarity is controlled by a complex termed "polarisome", which is at least comprised by the marker proteins Tea1 and Tea4, the membrane protein Mod5, Bud6, the formin For3 and the GTPase Cdc42. Bgs1 depletion produces cells with bifurcated ends. We are studying the role of Bgs1 in growth polarity and we found that bgs1+ shut-off in tea1Δ or tea4Δ mutants originates spherical cells. However, this effect is not produced by bgs1+ shut-off in mod5Δ, bud6Δ or for3Δ single, double or triple mutants. This suggests that both Bgs1 and Tea proteins cooperate in an essential function for polarity establishment and maintenance. We also found that Bgs1 absence produces Mod5-GFP, actin patches and lipid-rafts delocalization all around the plasma membrane. We were able to cause actin patches delocalization by using low concentrations of the actin depolymerizing drug Latrunculin B, and observed that actin patches delocalization in tea1Δ and tea4Δ cells produces the loss of polarity. It has been described that some cdc42 thermosensitive mutants and the pob1Δ or wsp1Δ deletion mutants present delocalized actin patches around the cell periphery. Combination of these mutations with the tea1Δ and tea4Δ mutations resulted in spherical cells. These results show that Bgs1 has a role in cell polarity by controlling the actin patches localization.

A novel regulatory role for the calcineurin inhibitor DSCR1 (Down syndrome candidate region 1) in p38 MAPK-mediated stress signalling

Reiko Sugiura, Nanae Umeda, Mari Higa, Yukiko Nota, Anna Ochiai, Yoshimi Kiyose, Ayako Kita

[a] *Kinki University, Japan*

Presented by: Sugiura, Reiko

Calcineurin (CN) is a highly conserved Ca2+/calmodulin-dependent protein phosphatase that plays a critical role in T-cell activation, cardiac development, and Ca2+ homeostasis through the activation of various transcription factors. We have been studying CN-mediated signalling and found that CN plays an important role in Cl--homeostasis and demonstrated that the Pmk1 MAPK signalling and CN play antagonistic roles in the Cl- homeostasis (Nature 1999, 2003 Mol. Bio. Cell 2006, 2007, 2009, 2010). We also identified the downstream transcription factor Prz1 that mediates CN-mediated Ca2+-signalling and have established an in vivo real-time monitoring system for CN activation (Mol. Bio. Cell 2006, JBC 2003, 2012).

Here, we identified the regulator of calcineurin Rcn1, which is highly similar to a new class of conserved endogenous calcineurin inhibitor RCAN1/DSCR1. The RCAN1 in humans was originally named DSCR1 (Down Syndrome Candidate Region 1), as it is encoded by a segment of chromosome 21 that is duplicated in Down syndrome patients. Although DSCR1 has been associated with successful adaptation to oxidative stress and calcium stress and with devastating diseases such as Alzheimer's and Down syndrome, no rationale for these findings has been tested. We demonstrated that Rcn1 binds and inhibits calcineurin in vivo. Surprisingly, however, we discovered that Rcn1 possesses target(s) other than calcineurin. Notably, we found that Rcn1 inhibits Spc1/Sty1 p38 MAPK signaling and regulates oxidative responses. Moreover, Rcn1 gene expression is stimulated upon oxidative stress and this induction is dependent on p38 MAPK, thus making a negative feedback loop that regulates SAPK. To our knowledge, this is the first demonstration that the DSCR1 homologue acts as a repressor of p38 signaling. These findings may shed light on the molecular basis of pathogenesis of diseases such as Alzheimer's and Down syndrome.

Fission yeast Cpc2 facilitates stress-activated MAP kinase-mediated stress response

Yusuke Tarumoto[a], Junko Kanoh[b], Toshihide Ueno[c], Hiroyuki Mano[c], Fuyuki Ishikawa[a]

[a] *Graduate School of Biostudies, Kyoto University, Japan*
[b] *Institute of Protein Research, Osaka University, Japan*
[c] *Division of Functional Genomics, Jichi Medical University, Japan*

Presented by: Tarumoto, Yusuke

Regulation of gene expression program is crucial for cells to rapidly adapt to environmental fluctuations. Stress-activated MAP kinase (SAPK) signaling links extracellular stress stimuli to the expression of stress-responsive genes. In fission yeast *Schizosaccharomyces pombe*, the SAPK Spc1 and the transcription factor Atf1 play a pivotal role in stress-induced transcriptional response. However, their precise mechanism remains incompletely understood.

We identified fission yeast Cpc2 as the factor required for adaptive phenomenon called cross-tolerance, in which pretreatment of mild dose of stress renders cells transient resistance to subsequent otherwise lethal dose of the same or different stress. Cpc2 is highly conserved among eukaryotes (mammalian homolog is called RACK1), and is thought to function in various physiological processes through interactions with signaling molecules. We found that recruitments of Spc1 to the promoter of stress-responsive genes were remarkably delayed in cpc2Δ cells, suggesting that Cpc2 is involved in SAPK-dependent transcriptional regulation. Consistently, microarray analysis revealed that cpc2 deletion caused a significant defect in the induction of SAPK-dependent stress-responsive genes. We are currently investigating how Cpc2 regulates the SAPK signaling.

Osmostress signaling by the Wis4-Win1 MAPKKK heteromer stabilized by the Mcs4 response regulator

Kaz Shiozaki[a,b], Susumu Morigasaki[a], Aminah Ikner[b], Hisashi Tatebe[a]

[a] *Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan*
[b] *Department of Microbiology and Molecular Genetics, University of California, Davis, CA 95616, USA*

Presented by: Shiozaki, Kaz

The Spc1 MAP kinase (MAPK) cascade in fission yeast is activated by two MAPK kinase kinases (MAPKKKs), Wis4 and Win1, in response to multiple forms of environmental stress. Interestingly, these MAPKKK paralogs do not appear to be redundant, and it was proposed that Win1, but not Wis4, is responsible for transmitting osmostress signals to the Wis1 MAPKK-Spc1 MAPK cascade [1,2]. However, we have found that both Wis4 and Win1 are required for osmostress signaling and that these MAPKKKs function as a heteromer. Intriguingly, only one of the MAPKKKs in the heteromer complex needs to be catalytically active, but disruption of the complex results in reduced MAPKKK-MAPKK interaction and consequently, compromised MAPK activation. Although some of mammalian MAPKKKs, such as B-Raf and C-Raf, are known to form a heteromer, the Wis4-Win1 association is the first example of a functional MAPKKK heteromer in fungal species.

Peroxide stress signals are sensed and transmitted to the Spc1 MAPK cascade by the phosphorelay module composed of the Mak sensor kinases, the Mpr1 histidine phosphotransferase and the Mcs4 response regulator [3,4]. It has been a conundrum that the mcs4 null mutant is defective in transmitting not only peroxide stress but also osmostress signals to Spc1 MAPK. We have discovered that Mcs4 is required for stable heteromer formation between Wis4 and Win1 MAPKKKs and that the destabilized MAPKKK heteromer in the mcs4 null mutant is responsible for compromised activation of Spc1 in response to osmostress. Such phosphorelay-independent function of Mcs4 is quite unexpected, because response regulator proteins normally act as terminal effectors of phosphorelay signaling in both bacteria and eukaryotes.

[1] Samejima et al., EMBO J. 16, 6162 (1997)
[2] Samejima et al., Mol. Biol. Cell 9, 2325 (1998)
[3] Nguyen et al., Mol. Biol. Cell 11, 1169 (2000)
[4] Buck et al., Mol. Biol. Cell 12, 407 (2001)

Growth regulation via opposite effects of TORC1 and TORC2 on amino acid homeostasis

Dana Laor[a], Adi Cohen[b], Martin Kupiec[a], Ronit Weisman[b]

[a] *Tel Aviv University, Israel*
[b] *Open University of Israel*

Presented by: Weisman, Ronit

Cell growth and proliferation are conserved and highly regulated processes. The target of rapamycin (TOR) is a key regulator of cellular growth in response to nutrition status. Fission yeast contains two conserved TOR complexes, TORC1 and TORC2. TORC1 is an essential complex, while TORC2 is required under stress conditions. Previously, we observed that deletion mutations in the TSC complex (Δtsc1 or Δtsc2) render cells highly sensitive to rapamycin on proline medium. Here we show that this rapamycin sensitive phenotype is mediated via TOR complex 1 (TORC1) and is suppressed by over expression of isp7+, a putative 2-oxoglutarate-Fe(II) dependent oxygenase, which acts to induce TORC1 activity. Functional and transcriptional analysis of isp7 mutant cells revealed that Isp7 is a master regulator of amino acid permease expression and amino acid uptake. Isp7, similar to TORC1 and opposite to TORC2, suppresses transcription of nitrogen-starvation induced permeases. Overexpression of Isp7 induced TORC1-dependnet phosphorylation towards the ribosomal protein S6 even under nitrogen starvation, conditions under which Rps6 is normally de-phsophorylated. Thus, high activity of Isp7 may mimic nitrogen or amino acid sufficiency. Remarkably, the transcription of isp7+ is downregulated by TORC1 and upregulated by TORC2, revealing an isp7+-dependent regulatory loops that involves both TORC1 and TORC2 and an elaborate mechanism to control amino acid homoeostasis.

The TOR kinases and the SAGA transcriptional co-activator coordinately control gene expression in response to nutrient availability

Thomas Laboucarié, Gwenda Lledo, Yves Romeo, Ghislaine Yagoubi, Dom Helmlinger

Macromolecular Biochemistry Research Center, CNRS UMR 5237, Montpellier, France

Presented by: Helmlinger, Dom

The regulation of gene expression plays a fundamental role in the ability of cells to respond to external changes. Although there are multiple levels of regulation, including transcription and translation, little is known about how these processes are synchronized. Using *S. pombe* as a model system, we are addressing this issue in the context of cell fate control by nutrient availability. We and others have established that the TOR kinases and the SAGA transcriptional co-activator complex are critical for *S. pombe* to respond to a change in nutrient levels and to decide whether to proliferate or differentiate. We have started to determine whether SAGA is directly regulated by the TOR pathway. Our preliminary genetic analyses show that SAGA functions downstream of the TOR pathway to control the expression of genes induced in response to nutrient starvation. In addition, we and others have recently discovered that one SAGA subunit, Tra1, directly associates with an HSP90 co-chaperone, the ASTRA complex. Work in *S. pombe* and in mammals has recently established that ASTRA is a novel, important regulator of TOR kinase activity. Therefore, we hypothesize that Tra1 plays a central role in nutrient sensing and, through its function in ASTRA, synchronizes the activity of the TOR pathway with the regulatory roles of SAGA at specific promoters. Mass spectrometry analysis of ASTRA purified from either wild-type or tra1 deletion mutant cells showed that Tra1 controls the association of the TOR kinases with ASTRA. This observation suggests that Tra1 plays a direct role within ASTRA to regulate the TOR kinases. We will discuss how these results contribute to our understanding of the control of transcription by signal transduction pathways.

Full TORC1 inhibition, reduce Wee1 levels and advance mitotic commitment in fission yeast and mammalian cells

Jane Atkin, Jennifer Ferguson, Claudia Wellbrock, Janni Petersen

University of Manchester, Michael Smith Building, Manchester M13 9PT, UK

Presented by: Petersen, Janni

The highly conserved protein kinase, Target Of Rapamycin (TOR) is a key regulator of cell growth and cell division. *S. pombe* contains two TOR proteins Tor1 and Tor2, which exist as part of at least two distinct protein complexes: TORC1 (mainly containing Tor2) and TORC2 (predominantly containing Tor1). It is widely established that rapamycin inhibits a subset of TOR activities by binding to the FRB domain adjacent to the kinase domain of TORC1 only. We previously demonstrated that rapamycin treated cells advance mitosis to reduce cell size however cells continue to proliferate. We now characterise an ATP-competitive Tor1/Tor2 inhibitor. In contrast to the mild impact of rapamycin on cell division, blocking the catalytic site of TOR kinases with this competitive inhibitor completely arrests growth. This growth arrest occurs without death or a specific arrest in the G1 phase of the cell cycle. Specific read-outs of both TORC1 and TORC2 activities establish that the activities of both complexes are inhibited. A screen for inhibitor-resistant mutants identified mutations in Tor2 (TORC1 – the essential complex), confirming the specificity of the ATP-competitor for TOR. Furthermore this shows that, unlike rapamycin, the competitive inhibitor fully inhibits TORC1. We have exploited this mutation to show that Wee1 levels are reduced and cell advance mitotic commitment as soon as TORC1 is completely inhibited. Growth arrest follows this acceleration of the cell cycle. Experiments in mammalian cells mirror these results from fission yeast as mTORC1 inhibition advances mitosis, as Wee1 is lost.

Identification of novel upstream regulators of fission yeast transcription factors by Ssynthetic dosage lethality

Kate Chatfield-Reed, Eun-Joo Kwon, Amy Laderoute, Gordon Chua

University of Calgary, Canada

Presented by: Chatfield-Reed, Kate

Mapping the interactions of transcription factors with upstream regulatory pathways is critical to enhancing our comprehension of transcriptional control and gene regulatory networks. These pathways involve regulatory proteins that affect the expression and activity of the transcription factor by altering its structural conformation, localization or rate of degradation. Approximately half of fission yeast transcription factors have fitness defects when overexpressed, likely due to their hyperactivation and the aberrant expression of their target genes. In contrast, the remaining transcription factors exhibit minor or no changes in colony size as assessed by synthetic genetic array (SGA) technology. These transcription factors are not toxic to the cell because their overexpression may not be sufficient for activation. If this is the case, then deletion backgrounds that sensitize the overexpression of these transcription factors may represent negative regulators. We are developing an SGA-based synthetic dosage lethality method to identify novel upstream regulators of fission yeast transcription factors. An *nmt1*-driven transcription factor gene is systematically overexpressed in every mutant of a regulator deletion miniarray by SGA, and the double mutants are assayed for decreased colony size. This miniarray contains over 250 Bioneer gene deletions of regulatory molecules including kinases, phosphatases, acetylases and ubiquitin ligases. We will present preliminary data on synthetic dosage lethality screens of the transcription factors Cbf11, Scr1, Toe1, Tos4 and Yox1. These screens can be used to unravel another level of control in the *S. pombe* gene regulatory network.

Quantitative analysis of fission yeast genome expression at population and single-cell levels

Samuel Marguerat[a,b], Anna Köferle[b], Tom Livermore[b], Jürg Bähler[b]

[a] *Since March 2013: New Group Leader at MRC Clinical Sciences Centre, Imperial College London, Du Cane Road, London W12 0NN, UK*
[b] *University College London, Department of Genetics, Evolution & Environment and UCL Cancer Institute, London WC1E 6BT, UK*

Presented by: Marguerat, Sam

Genome-wide studies have made important contributions to our understanding of the dynamics of eukaryotic transcriptomes and proteomes. However, absolute concentrations of individual transcripts and proteins remain largely unexplored on a global scale, and should provide unique insight into cell regulation. We have recently quantified the transcriptome and proteome of proliferating cells providing cellular concentrations for all RNAs and most proteins. Proteins are found in hundreds to millions of copies per cell, greatly exceeding mRNAs which are mainly present between 1 and 10 copies per cell on average [1]. Interestingly, transcriptional and translational outputs scale with cell size and growth rates at a genome-wide level. As a result, cells of different size and physiological states contain different numbers of mRNAs and proteins. How such remarkably coordinated regulation is achieved remains mysterious [2]. Quantitative genomics approaches now provide new, powerful tools to understand what controls the amounts of proteins and mRNAs that a given genome can produce. In addition, mRNA and protein numbers are also affected and controlled by natural cell-to-cell fluctuations due to the stochastic nature of gene expression. Knowing how variability in mRNA and protein numbers impacts on single-cells phenotypes is therefore important to understand how a cell functions. Here we present initial data on an innovative approach developed in order to measure global mRNA numbers in individual fission yeast cells. This analysis will bring us a step closer to understanding what regulates the quantitative outputs of single genomes, and to understanding how cell-to-cell variability and noise in gene expression translate into complex phenotypes such as cell-size.

1 Marguerat et al. (2012) Cell, 151:671-83
2 Marguerat & Bähler (2012) Trends Genet, 28:560-5

Global coordination of the *S. pombe* stress response by the non-coding transcriptome

Hui Sun Leong, Keren Dawson, Crispin Miller

Cancer Research UK, Applied Computational Biology and Bioinformatics Group, Paterson Institute for Cancer Research, UK

Presented by: Miller, Crispin

Approximately 40% of human genes are non-coding. While a subset have been assigned a function, the majority are currently uncharacterised, and the novelty of these loci means that the full mechanistic catalogue describing their potential modes of operation is still being assembled. The fission yeast genome also features functional non-coding RNAs, and the close conservation of core pathways with human cells, including the RNAi machinery, makes it a particularly useful model system with which to investigate the role of these transcripts.

Using a combination of strand specific deep sequencing of total RNA, quantitative protein mass spectrometry, and a systems' level integration of these data that includes a context-specific re-annotation of the genome, we have identified a global system of regulatory non coding RNAs that help coordinate the *S. pombe* stress response. In addition, we show that systematic alterations to the 5' and 3' UTRs of specific transcripts are used as an additional regulatory tool. These loci include convergent, overlapping, 3' adjacent gene-pairs.

Mind the gap: pervasive splicing in the fission yeast transcriptome

Danny Asher Bitton[a], Daniel Charlton Jeffares[a], Samuel Marguerat[a], Sandra Codlin[a], Graeme Christopher Smith[a], Yuan Chen[a], Sophie Atkinson[a], Valerie Wood[b], Jürg Bähler[a]

[a] *University College London, Department of Genetics, Evolution & Environment London, WC1E 6BT, UK*
[b] *PomBase, Department of Biochemistry, University of Cambridge, 80 Tennis Court Road Cambridge CB2 1GA, UK*

Presented by: Bitton, Danny

RNA-sequencing technology is now widely used for global gene expression profiling and detection of novel transcripts. A typical RNA-seq run results in the production of millions of reads, of which a considerable fraction typically cannot be mapped to the genome in question. To detect novel splicing events and to evaluate splicing noise, we developed an exhaustive search algorithm that utilises a large collection of unmapped reads originated from the transcriptomes of numerous wild-type and RNA processing mutant strains grown under different conditions. To make an exhaustive search of all possible splicing events, we generated an exon-junction sequence databases by partitioning the complete fission yeast genome according to all possible di-nucleotide splice donor and acceptor site combinations (GT..AG, CT..AG, TA..TT, etc.). Unmapped reads were systematically aligned to these junctions. Using this approach, we identified thousands of novel introns with high confidence (FDR <0.05), some of which were experimentally validated. Novel introns found within mRNAs, non-coding RNAs, UTRs and intergenic regions unearthed a much more pervasive splicing activity than previously appreciated. Moreover, interrogation of lariat reads from the transcriptome of a de-branching enzyme mutant enabled mapping of the exact branch-points in numerous introns. Finally, a search for alternative splice variants suggested that exon-skipping events are likely to represent erroneously spliced transcripts that are actively degraded by the exosome. Taken together, this approach uncovered a much larger complexity of RNA processing than expected in this relatively simple eukaryotic model. We discuss how these findings could shape our current view of RNA processing and splicing in multi-cellular, more complex systems.

Chromodomain Helicase DNA binding protein 1 (CHD1) regularly spaces nucleosomes over coding regions in *S. pombe*

Punit Prasad[a], Julia Pointner[b], Jenna Persson[a]

[a] *Dept. of Bioscience and Nutrition, NOVUM, Karolinska Institutet, Stockholm, Sweden*
[b] *Adolf-Butenandt-Institut, University of Munich, Schillerstr., Munich, Germany*

Presented by: Prasad, Punit

Genome-wide nucleosome mapping has revolutionized our understanding of the general architecture of eukaryotic chromatin organization in vivo. Several studies have revealed a conventional nucleosome positioning pattern with nucleosome free region (NFR) before transcription start site (TSS) followed by regularly spaced nucleosomal arrays over coding regions. Such chromatin architecture is manipulated by SNF2 family of DNA translocases called chromatin remodeling enzymes for various DNA dependent processes. These enzymes change chromatin states by nucleosome assembly, spacing, sliding, eviction and dimer displacement. Therefore we investigated the role of chromatin remodelers in global nucleosome organization in *S. pombe*. We identified Hrp1 and Hrp3, two orthologs of chromodomain helicase DNA binding proteins 1 (CHD1) subfamily of remodelers to be involved in generating TSS-aligned nucleosomal arrays over the coding regions in *S. pombe*. We also give direct evidence by in vitro assays that Hrp1 and Hrp3 space nucleosomes in redundant manner. The consequences of having impaired nucleosome arrays at coding regions are increased cryptic antisense transcription with subtle changes in overall gene expression. Taken together, our study demonstrates that CHD1 enzymes are the sole chromatin remodeling ATPases involved in generating regularly spaced nucleosomal arrays.

Ergothioneine biosynthesis pathway in *S. pombe* revealed by metabolomic analysis

Tomáš Pluskal, Mitsuhiro Yanagida

G0 Cell Unit, Okinawa Institute of Science and Technology Graduate University, Japan

Presented by: Pluskal, Tomáš

Ergothioneine is a low-molecular weight thiol compound synthesized by relatively few species of bacteria and fungi, which can accumulate in the tissues of plants and animals up to millimolar levels. Since its discovery over a century ago, numerous publications have described its antioxidative and protective properties, although the true purpose of ergothioneine in vivo is still debated. The research of ergothioneine has accelerated following the recent discovery of an ergothioneine-specific transporter OCTN1 in higher organisms including humans. The biosynthetic pathways of ergothioneine have so far been described in *Mycobacterium smegmatis* and *Neurospora crassa*. We have established the method of metabolome analysis in the fission yeast (Pluskal et al., 2010). Among the several hundred of observed metabolites, we also identified ergothioneine and described its accumulation under glucose starvation (Pluskal et al., 2011). The fission yeast, unlike the budding yeast, is one of the few eukaryotic species that can synthesize this compound, although vegetative cells normally contain only trace amounts. In the present study, we discovered the genes forming the two-step biosynthetic pathway of ergothioneine in *S. pombe*. We described the accumulation of intracellular ergothioneine in quiescent cells and characterized its contribution to the antioxidative protection systems in this organism by treating ergothioneine-deficient mutant strain with oxidative agents. We anticipate this research will establish *S. pombe* as an excellent model organism for the study of the ergothioneine function in higher organisms.

Pluskal et al. (2010) Metabolic profiling of the fission yeast S. pombe: quantification of compounds under different temperatures and genetic perturbation. Mol BioSyst 6:182

Pluskal et al. (2011) Specific biomarkers for stochastic division patterns and starvation-induced quiescence under limited glucose levels in fission yeast. FEBS J 278:1299

Condensin complex negates an inhibitory effect of gene transcription on mitotic chromosome segregation

Takashi Sutani, Katsuhiko Shirahige

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan

Presented by: Sutani, Takashi

Condensin is a highly conserved protein complex and required for mitotic chromosome condensation. A defect in condensin causes impaired chromosome segregation, leading to aneuploidy and lethality. Though it has been investigated intensively how condensin acts on DNA in vitro, it still remains unanswered what function condensin execute in vivo. Here we report a binding profile of fission yeast condensin on metaphase chromosomes, which was obtained by ChIP-seq technology. The data indicates that condensin accumulated at several hundred of sites on chromosome arms, besides known binding sites including centromeres and rDNA loci. Detailed analysis revealed that condensin binding sites were located at genes expressed in mitotic cells. The binding was dependent on transcription, and transcription inhibition led to dissociation of condensin. Interestingly, transient inhibition of transcription rescued impaired chromosome segregation phenotype of condensin mutants. This data suggests that gene transcription on mitotic chromosomes is harmful to faithful chromosome segregation, and one of condensin's functions is to negate the inhibitory effect of transcription. Condensin is known to promote renaturation of ssDNA strands in vitro. Consistently, condensin-bound DNA purified in ChIP experiments was found to contain ssDNA structure. We also discovered that condensin brought about exclusion of RNApol II from gene bodies of mitotically expressed genes. Based on these results, we currently hypothesize that condensin removes melted DNA structure associated with transcription, promotes nucleosome re-assembly, and thereby facilitates chromosome condensation.

Protein kinases required for the proper segregation of chromosomes during meiosis.

Juro Gregan[a,b], Lubos Cipak[b], Ines Kovacikova[b], Naina Phadnis[c], Gerald Smith[c]

[a] *Comenius University in Bratislava, Slovakia*
[b] *University of Vienna, Austria*
[c] *Fred Hutchinson Cancer Research Center, Seattle, USA*

Presented by: Gregan, Juro

Reversible protein phosphorylation has been established as the major regulatory mechanism in the cell. Genome-wide surveys of protein kinases and phosphatases have been instrumental in characterizing novel mechanisms regulating various processes, including mitosis and meiosis. Our aim is to systematically analyze the role of *Schizosaccharomyces pombe* protein kinases in meiotic chromosome segregation. We identified a novel function of Spo4, the fission yeast ortholog of Dbf4-dependent Cdc7 kinase, in regulating the length of anaphase II spindles. *S. pombe* produces linear asci in which the order of spores reflects the descent of nuclei from the two meiotic divisions. Interestingly, we observed that the abnormal elongation of anaphase II spindles in the spo4-mutant cells pushed sister nuclei apart and thus destroyed the linear order of nuclei in the ascus such that the two spores at one end of the ascus contained non-sister nuclei. The fact that the spo4-mutant phenotype can be partially suppressed by inhibiting Cdc2-as suggests that dysregulation of the activity of this cyclin-dependent kinase may cause abnormal elongation of anaphase II spindles in spo4-mutant cells. We further found that the fission yeast casein kinase 1 (CK1) delta/epsilon isoforms Hhp1 and Hhp2 are novel proteins required for normal levels of meiotic recombination and proper segregation of homologous chromosomes during meiosis I. Our observation that Hhp1 and Hhp2 co-purify with the cohesin complex during meiosis suggests that meiotic cohesin may be the relevant CK1 target.

Haploinsufficiency of the sec7 guanine nucleotide exchange factor gea1 impairs septation in fission yeast

Melanie Styers, Alan Eckler, Caroline Wilder, Antonio Castanon, Veronica Ferris, Rachael Lamere, Benjamin Perrin, Ross Pearlman, Blaise White, Clifton Byrd

Dept. of Biology; Birmingham-Southern College; Birmingham, AL, USA

Presented by: Styers, Melanie

Membrane trafficking is essential to eukaryotic life and is controlled by a complex network of proteins that regulate movement of proteins and lipids between organelles. The GBF1/GEA family of Guanine Nucleotide Exchange Factors (GEFs) regulates trafficking between the endoplasmic reticulum and Golgi by catalyzing the exchange of GDP for GTP on ADP Ribosylation Factors (Arfs). Activated Arfs recruit coat protein complex 1 (COP-I) to form vesicles that ferry cargo between these organelles. To further explore the function of the GBF1/GEA family, we have characterized a fission yeast mutant lacking one copy of the essential gene gea1 (gea1+/-), the Schizosaccharomyces pombe ortholog of GBF1. The haploinsufficient gea1+/- strain was shown to be sensitive to the GBF1 inhibitor brefeldin A (BFA) and was rescued from BFA sensitivity by gea1p overexpression. No overt defects in localization of arf1p or arf6p were observed, but the fission yeast homolog of the COP-I cargo sac1 was mislocalized, consistent with impaired COP-I trafficking. Although Golgi morphology appeared normal, a slight increase in vacuolar size was observed in the gea1+/- mutant strain. Importantly, gea1+/- cells exhibited dramatic cytokinesis-related defects, including disorganized contractile rings, an increased septation index, and alterations in septum morphology. Septation defects appear to result from altered secretion of enzymes required for septum dynamics, as decreased secretion of eng1p, a beta-glucanase required for septum breakdown, was observed in gea1+/- cells, and overexpression of eng1p suppressed the increased septation phenotype. These observations implicate gea1 in regulation of septum breakdown and establish S. pombe as a model system to explore GBF1/GEA function in cytokinesis.

Regulation of nuclear envelope dynamics via APC/C is necessary for the progression of semi-open mitosis in *Schizosaccharomyces japonicus*

Keita Aoki[a], Yuh Shiwa[b], Hiraku Takada[c], Hirofumi Yoshikawa[d], Hironori Niki[a]

[a] *Microbial Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, Japan*
[b] *Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture*
[c] *Department of Bioscience, Tokyo University of Agriculture, Japan*
[d] *Department of Bioscience, Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture, Japan*

Presented by: Aoki, Keita

Eukaryotic cells have two types of mitosis: the open mitosis and the closed mitosis. However, the mechanism and the biological significance of the difference remain to be fully understood. Here, to elucidate those, we focus on 'semi-open mitosis' of Sz. japonicus, in which the nuclear envelope is partially broken during anaphase, because the semi-open mitosis includes features of the both types of mitosis: open and closed mitosis.

So far, we found that the nuclear envelope was less broken down during anaphase in APC/cyclosome mutants, nevertheless spindle microtubules were fully extended. It was seemed that the amount of nuclear envelope was excess so that the semi-open mitosis was compromised in APC/C mutants. To understand the phenotype of APC/C mutants, we obtained an oar2 mutation as a suppressor of apc2-361. The Oar2 is a homologue of a ketoacyl reductase that functions in the fatty acid elongation cycle. And interestingly, Oar2 contains two putative destruction boxes, and the amount of Oar2 was increased in APC/C mutants. When the destruction boxes were mutated, the amount of Oar2 was abnormally increased or stable in a cell. So, it was seemed that Oar2 was degraded in metaphase to anaphase dependent on APC/C. Further, to know how apc2-361 is suppressed by the oar2 mutation, mitotic durations and dynamics of nuclear envelope were eagerly examined. As a result, the excess amount of the nuclear envelope in apc2-361 during anaphase was decreased by addition of the oar2 mutation so that the semi-open mitosis was restored. These observations may suggest that restriction of supply of the fatty acids is necessary for the progression of the semi-open mitosis. Therefore, we propose that the types of mitosis are determined by the level of fatty acids.

Medial assembly of actomyosin ring in the absence of Mid1p

Evelyn Yaqiong Tao[a], Balasubramanian Mohan[a,b]

[a] *Department of Biological Sciences, National University of Singaproe*
[b] *Temasek Life Sciences Laboratory, Singapore*

Presented by: Tao, Evelyn Yaqiong

The Anillin-like protein Mid1p has been shown to be crucial for the recruitment of precursor nodes to the middle of a cell before they assemble into an actomyosin ring in the fission yeast Schizosaccharomyces pombe. In this study, we demonstrate that the medial ring can be assembled in the absence of Mid1p through an artificial system, in which GFP tagged cytokinetic protein (Rng2p, Myo2p, or Cdc12p) were individually prematurely targeted to the medial nodes, through the strong binding between GFP and its antibody fragment Gab. When Gab is fused to the node protein Cdr2p (which localizes independent of Mid1p), it brings a GFP tagged ring protein to the medial region in the form of nodes, which subsequently recruits other cytokinetic partners. These non-canonical interphase nodes, in the absence of Mid1p, stay at the medial cortex through G2 and early mitosis, and later assemble into a contractile ring upon activation of the Septation Initiation Network (SIN). These findings suggest that medial ring assembly can proceed in the absence of Mid1p if ring proteins are targeted to medial nodes through synthetic approaches, although Mid1p is required for ring assembly in metaphase.

Schizosaccharomyces pombe DJ-1 and *Saccharomyces cerevisiae* Hsp31 are glyoxalase III, revealing an evolutionarily conserved function shared by members of two distant subfamilies of the DJ-1 superfamily

Ying Huang[a], Zikang Wang[a], Qiaoqiao Zhao, Yang Su[a]

[a] *Nanjing Normal University, China*

Presented by: Huang, Ying

Methylglyoxal (MG), known as reactive carbonyl species (RCS), is a toxic metabolite synthesized in living systems primarily as a byproduct of glycolysis. Mechanisms of MG detoxicification include the glutathione (GSH)-dependent pathway consisting of glyoxalase I and glyoxalase II, and GSH-independent pathway catalyzed by glyoxalase III. DJ-1 and Hsp31 proteins belong to two different subfamilies of the DJ-1 superfamily, including the Parkinson's disease-associated DJ-1 protein (HsDJ-1) and the Escherichia coli heat shock protein Hsp31 (EcHsp31), and are the most likely candidates for glyoxalase III. We performed a systematic survey of homologs of these proteins in fungi. DJ-1 homologs have a very limited distribution in fungi, whereas Hsp31 homologs are widely distributed among different fungal groups. Phylogenetic analysis of these sequences revealed that fungal DJ-1 and Hsp31 homologs form two distinct monophyletic groups, and that Hsp31 homologs comprise four subgroups. We show that both Schizosaccharomyces pombe DJ-1 (SpDJ-1) and Saccharomyces cerevisiae Hsp31 (ScHsp31) have glyoxalase III activity in vitro. This glyoxalase activity requires a predicted catalytic triad Glu16-Cys109-His128 in SpDJ-1. We further show that disruption of SpDJ-1 renders stationary-phase but not log-phase cells more sensitive to MG in wild-type and glyoxalase I gene deletion (ΔSpglo1) backgrounds, albeit to a much lesser extent than deletion of Spglo1. The enhanced MG sensitivity of ΔSpDJ-1ΔSpglo1 cells could be rescued by overexpression of HsDJ-1, EcHsp31 and ScHsp31. Finally, we show that like ScHsp31, SpDJ-1 is significantly up-regulated during stationary phase. Our results suggest that fungal DJ-1 and Hsp31 homologs encode glyoxalase III, which may play a role in protecting cells from MG toxicity in stationary phase. Our results also support the view that glyoxalase III activity likely evolved through convergent evolution.

TORC2 dysfunction in a fission yeast model for neurodegeneration

Michael Bond[a], Sara Mole[a,b]

[a] *MRC Laboratory for Molecular Cell Biology,University College of London, London WC1E 6BT, UK*
[b] *Institute of Child Health and Department of Genetics, Evolution and Environment, University College of London, London WC1E 6BT, UK*

Presented by: Bond, Michael

There are many highly conserved genes involved in inherited neurodegeneration that perform basic eukaryotic cellular functions. Due to their fundamental roles in eukaryotic cell biology, they are ideally suited to study in simple model systems. The neuronal ceroid lipofucinoses (NCLs) represent one such group of conditions. NCLs are characterised by accumulation of autofluorescent lysosomal material, with symptoms including dementia, seizures and blindness, eventually leading to a premature death. Juvenile CLN3 disease is the most common form of NCL. CLN3 is a lysosome and Golgi resident membrane protein, and has been associated with a number of cellular processes; including lysosomal function, trafficking, cytoskeletal organisation and cell polarity. The function of CLN3 is still unknown.

Fission yeast contains a single non-essential orthologue of CLN3 (btn1), which evidence suggests shares the same function as CLN3. Here we present data linking btn1 to target of rapamycin (TOR) signalling, specifically signalling through TORC2. These data indicate that TORC2 dysfunction is linked to a number of the pleiotropic effects associated with loss of btn1. Given the overlapping roles of btn1/CLN3 and TORC2 these data could indicate that TORC2 dysfunction underlies a number of the pathological changes associated with CLN3 disease, providing new avenues for therapeutic development.

The fission yeast MCM-BP, Mcb1, regulates MCM function during pre-replicative complex formation in DNA replication

Venny Santosa, Sabrina Martha, Noriaki Hirose, Katsunori Tanaka

Kwansei Gakuin University, Japan

Presented by: Santosa, Venny

The MCM complex is a famous helicase essential for chromosome DNA replication. The identification of a novel MCM-binding protein (MCM-BP) in most eukaryotes has led to numerous hypothetical functions for the protein in its relationship to the MCM complex. However, the mechanisms of MCM-BP function and association with MCM complexes are still poorly understood; in addition, the functional role of MCM-BP remains controversial and may vary between model organisms. The present study aims to elucidate the nature and biological function of the MCM-BP ortholog, Mcb1, in fission yeast. The Mcb1 protein continuously interacts with MCM proteins during the cell cycle in vivo and can interact with any individual MCM subunit in vitro. To understand the detailed characteristics of mcb1+, two temperature-sensitive mcb1 gene mutants (mcb1ts) were isolated. The mcb1ts mutants displayed synthetic defects with many S-phase-related gene mutants and their phenotype were suppressed by a mcm5+ multi-copy plasmid. Moreover, CDK modulation by Cig2 repression or Rum1 overproduction suppressed the mcb1ts mutants, suggesting the role of Mcb1 in pre-RC assembly during DNA replication. These data are consistent with the observation that Mcm7 loading onto replication origins is reduced and S-phase progression is delayed in mcb1ts mutants. Furthermore, the mcb1ts mutation led to the redistribution of MCM subunits to the cytoplasm, and this redistribution was dependent on an active nuclear export system by Crm1 protein. These results strongly suggest that Mcb1 promotes efficient pre-RC formation during DNA replication by MCM complex regulation.

A chaperone-assisted degradation pathway targets kinetochore proteins to ensure genome stability

Franziska Kriegenburg[a], Visnja Jakopec[b], Sofie V. Nielsen[a], Assen Roguev[c], Nevan Krogan[c], Colin Gordon[d], Ursula Fleig[b], Rasmus Hartmann-Petersen[a]

[a] *Department of Biology, University of Copenhagen, Denmark*
[b] *Heinrich-Heine University, Düsseldorf, Germany*
[c] *Department of Pharmacology, UCSF, USA*
[d] *MRC Human Genetics Unit, Edinburgh, UK*

Presented by: Hartmann-Petersen, Rasmus

In nature cells are regularly exposed to various stress conditions that may lead to protein misfolding. To cope with this challenge, cells have developed a protein quality control system where molecular chaperones selectively target structurally perturbed proteins for degradation via the ubiquitin-proteasome pathway. In higher eukaryotes the co-chaperone BAG-1 plays an important role in this system. However, no studies of BAG-1 have yet been performed in a genetically more tractable model organism. Here we characterize the BAG-1 orthologues, Bag101 and Bag102, in the fission yeast Schizosaccharomyces pombe. We show that both Bag101 and Bag102 interact with 26S proteasomes and Hsp70. By systematic epistasis mapping we identify the conserved kinetochore component Spc7 (Spc105/Blinkin) as a target for a protein quality control system that also involves, Bag102, the 26S proteasome, E2 ubiquitin conjugating-enzyme Ubc4 and E3 ubiquitin-ligases Ubr11 and San1. Accordingly, the defects in chromosome segregation connected with mutation of spc7 are alleviated by mutation of components in this pathway. In addition, we isolated a cDNA encoding a dominant negative version of the deubiquitylating enzyme Ubp3 as a suppressor of the spc7-23 mutant, suggesting that the proteasome associated Ubp3 is required for this degradation system. Finally, our data suggest that the identified pathway is also involved in quality control of other kinetochore components and therefore likely to be a common degradation mechanism to ensure kinetochore integrity.

The telomere bouquet controls SUN-domain protein accumulation and centrosome activity during meiosis

Alfonso Fernandez-Alvarez, Cecile Bez, Julia Promisel Cooper

Telomere Biology Lab. London Research Institute (CRUK). 44 Lincoln's Inn Fields, London WC2A 3LY, UK

Presented by: Fernandez-Alvarez, Alfonso

The telomere bouquet, a conserved meiotic prophase structure, promotes homolog pairing and, at least in fission yeast, meiotic spindle formation. In this bouquet, all the telomeres form a tight cluster at the spindle pole body (SPB, the centrosome equivalent). How association between the bouquet and SPB during prophase could control spindle formation later in meiosis is still unknown.

Bouquet formation is driven by movements of Sad1, a conserved SUN-domain inner nuclear membrane (NM) protein that not only contacts meiotic telomeres, but also binds an outer NM protein that contacts the SPB. In examining how the SPB and underlying NM differ in the presence and absence of the bouquet, we have observed that normal bouquet formation leads to enhanced levels of Sad1 at the SPB during prophase. In bouquet-deficient cells with spindle defects, this rise in Sad1 level is absent, while it is present in those bouquet-deficient cells that do develop bipolar spindles. We are now assessing various strategies for artificially providing enhanced Sad1 levels at the SPB in the absence of the bouquet in order to investigate whether Sad1 concentration is a relevant aspect of bouquet function. Notably, the levels of two core SPB components show no alteration in the absence of the bouquet. Indeed, our data suggest that the SPB is able to duplicate in the absence of the bouquet, but fails to separate and insert properly into the NM; recruitment of the gamma tubulin complex to the SPB is also compromised in a bouquet-defective setting. Taken together, these data suggest that the telomere bouquet ensures levels of Sad1 at the SPB to accomplish its NM insertion and spindle formation. Current studies aim to test this idea that Sad1 is a key signal linking the early occurrence of the bouquet with spindle formation in late stages of meiosis.

Interchangeable roles of the telomeres and centromeres in promoting meiotic spindle formation

Alex Fennell, Alfonso Fernandez-Alvarez, Julie Cooper

Cancer Research UK, London Research Institute, UK

Presented by: Fennell, Alex

Meiosis is a dynamic and regulated process involving significant nuclear reorganisation and striking nuclear movements before the onset of two sequential divisions. Recent evidence in fission yeast has indicated the importance of the telomere bouquet in controlling meiotic spindle pole body (SPB) function, spindle formation and chromosome segregation. We will present results that highlight a surprising level of interchangeability between telomeres and centromeres in conferring successful nuclear division.

We find that in the absence of the telomere bouquet, contacts between chromatin and the fluctuating SPB during meiotic prophase are capable of rescuing SPB function and spindle formation. Strikingly, in almost all cases in which we observe such rescue, the chromatin that 'accidentally' contacts the SPB is centromeric chromatin. Moreover, when we artificially maintain centromeres at the SPB throughout meiotic prophase in a bouquet-deficient setting, in cells both with and without horsetail nuclear movement, we observe almost complete rescue of the spindle defect, suggesting an active role of the centromeres in controlling bipolar spindle formation.

Interestingly, disruption of heterochromatin in a bouquet-deficient setting using both clr4Δ bqt1Δ and dcr1Δ bqt1Δ double mutants does not prevent spindle rescue upon contact between chromatin and the SPB during meiotic prophase. This suggests a common function of the telomeres and centromeres that is independent of their heterochromatic nature.

We will present our plans to further characterise these intriguing observations especially with regards to a potential role of specialised chromatin in controlling mitotic spindle formation.

Role of nuclear movement in chromosome pairing

Mariola Chacón[a], Petrina Delivani[a], Iva M Tolić-Nørrelykke[a]

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

Presented by: Chacón, Mariola

Pairing of homologous chromosomes is a crucial step in meiosis. In fission yeast, proper pairing requires oscillatory nuclear movement, which is driven by microtubules emanating from the spindle pole body (SPB). Is the nuclear movement a stringency factor to dissolve unwanted connections between chromosomes? Here we use live-cell microscopy of cells with labeled Ade3 loci and the SPB, at a high time resolution. We examine the relation of the loci association and dissociation to nuclear movement. We measured the position of the SPB and the distance between homologous loci during the whole meiotic prophase. To specifically elucidate the role of the nuclear movement in the pairing process, we depolymerized microtubules to arrest the nuclear oscillations. Our results indicate: (i) a correlation between the loci distance and the SPB position. When the SPB is at the cell poles, the distance between the loci is smaller than when the SPB is at the cell center. This correlation disappears in the last five oscillations. (ii) The first pairing occurs after the first nuclear oscillation, when the SPB moves away from the cell center. We determined that the first pairing event is typically shorter than 20 seconds. As shown before, the average distance between homologous loci decreased as the oscillations proceeded. (iii) Arresting the oscillations when the loci were paired caused a constant pairing of the loci during the arrest and after the oscillations restarted. Stopping the oscillations when the loci were unpaired led to maintenance of the distance during the arrest and normal pairing and unpairing of loci was restored after the oscillations restarted. Taken together, our results show that the stretching and relaxation of the nucleus promote chromosome pairing and unpairing, respectively, and suggest that nuclear movement is necessary to avoid excessive chromosome associations.

Fission yeast nucleolar protein Dnt1 regulates G2/M transition and cytokinesis through downregulating Wee1 kinase

Zhi-yong Yu, Yamei Wang, Quan-wen Jin

State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen 361102, Fujian, China

Presented by: Jin, Quan-wen

Cytokinesis involves temporally and spatially coordinated action of the cell cycle and cytoskeletal and membrane systems to achieve separation of daughter cells. The septation initiation network (SIN) and mitotic exit network (MEN) signaling pathways regulate cytokinesis and mitotic exit in the yeasts Schizosaccharomyces pombe and Saccharomyces cerevisiae, respectively. Previously, we have shown that nucleolar protein Dnt1 in fission yeast negatively regulates SIN pathway in a manner independent of Cdc14-family phosphatase, Clp1/Flp1, but the detailed mechanism on how Dnt1 modulates SIN pathway has remained elusive. This is in contrast to its budding yeast relative Net1/Cfi1, which regulates the homologous signaling pathway MEN through sequestering the Cdc14 phosphatase in the nucleolus before mitotic exit. In this study, we made several observations indicating that dnt1+ positively regulates the G2/M transition during cell cycle. By conducting epistasis analyses measuring the cell length at division of double mutants between dnt1D and genes involved in G2/M control, we found a link between dnt1+ and wee1+. We further pinpointed that elevated protein level of mitotic inhibitor Wee1 kinase and thus attenuated Cdk1 activity is responsible for the rescuing effect of dnt1D on SIN mutants. Finally, our data also suggested that Dnt1 modulates Wee1 activity in parallel with SCF-mediated Wee1 degradation. Therefore, our study revealed an unexpected missing link between the nucleolar protein Dnt1 and the SIN signaling pathway, which is mediated by Cdk1 regulator Wee1 kinase. Our data also defined a novel mode of Wee1/Cdk1 regulation important for integrating signals controlling SIN pathway in fission yeast.

Phosphorylation of a conserved residue in the ATP-binding pocket reduces TOR kinase activity

Lenka Halova[a], Wei Du[a], Duncan Smith[b], Janni Petersen[a]

[a] *University of Manchester, C.4223 Michael Smith building, Faculty of Life Sciences, Oxford Road, Manchester M13 9PT, UK*
[b] *Biological Mass Spectrometry, Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Manchester, M20 4BX, UK*

Presented by: Halova, Lenka

TOR (Target of Rapamycin) kinases regulate cell growth and division in response to changes in the environment. S. pombe contains two Tor kinases: Tor1, found predominantly in TORC2 (TOR complex 2), and Tor2, a main component of TORC1. Mammalian cells contain only one TOR kinase (mTOR), which is present in both complexes. We find that a conserved Ser/Thr residue within the Tor1, Tor2 and mTOR ATP-binding pocket is phosphorylated in vivo. When mutated to a non-phosphorylatable Ala, the kinase activity of the mutant protein increased. This was seen both in an in vitro kinase assay and in vivo as an increased phosphorylation of the TORC2/mTORC substrates Gad8/AKT. Therefore, phosphorylation of this conserved residue within the ATP-binding pocket inhibits TOR kinase activity, possibly by changing the affinity for ATP. Furthermore, this novel mode of TOR kinase inhibition appears to be generally conserved.

Absence of the Indy protein homolog prolongs the life span in the presence of high glucose levels in *Schizosaccharomyces pombe*

Cagatay Tarhan[a], Ayse Malci[a], Aysegul Topal-Sarikaya[b]

[a] *Istanbul University, Faculty of Science, Department of Moleculer Biology and Genetics, Turkey*
[b] *Medical School of Yeni Yuzyil University, Department of Molecular Biology and Genetics, Turkey*

Presented by: Tarhan, Cagatay

Mutations in the Indy gene in Drosophila melanogaster have demonstrated that a reduction in normal INDY activity is associated with extension of life span. It has been proposed that a decrease in INDY might extend life span especially by creating a metabolic state that mimics calorie restriction (CR). But it is not yet understood how an alteration in the level of expression of INDY could result in life span extension. To investigate the cellular response to the absence of INDY, first, we searched for the INDY protein homologs in S.pombe. According to the BLAST results, we found INDY like protein which is predicted to be a Na+/dicarboxylate and Na+tricarboxylate transporter (PONDY). Then we evaluated the life span, glucose consumption and intracellular oxidation levels in the PONDY mutant of S.pombe. The PONDY mutant didn't exhibit a prolonged lifespan according to its parental strain but surprisingly it displayed remarkable resistance to the increased levels of glucose and showed an increase in lifespan in the presence of elevated glucose levels. We also evaluated the effect of different glucose concentrations on the expression levels of the PONDY gene. Results may contribute to the characterization of the PONDY gene, glucose resistance mechanisms and aging process in S.pombe.

Actomyosin ring assembly and function in *Schizosaccharomyces japonicus*

Ying Gu[a], Candice Yam[a], Snezhka Oliferenko[a]

Temasek Life Sciences Laboratory, National University of Singapore

Presented by: Gu, Ying

Many eukaryotic cells divide using a cortical actomyosin-based contractile ring. In the fission yeast Schizosaccharomyces pombe, the anillin-like protein Mid1 promotes medial assembly of the actomyosin ring components early in mitosis. Here I show that in a related organism, Schizosaccharomyces japonicus, the myosin II complex together with some myosin-interacting proteins form nodes at the equatorial cortex throughout the interphase. These nodes coalesce into a ring structure following the mitotic exit, in a septation initiation network-dependent manner. Surprisingly, although Mid1 is required for myosin recruitment to the medial cortex in interphase, it is dispensable for assembly of properly positioned actomyosin rings at the end of mitosis. I will further discuss molecular requirements of actomyosin assembly and function in the two sister organisms.

Polypeptone induces dramatic cell lysis in *ura4* mutants of fission yeast

Yuzy Matsuo, Kouhei Nishino, Makoto Kawamukai

Life and Environmental Science, Shimane University, Japan

Presented by: Kawamukai, Makoto

Polypeptone is widely excluded from *Schizosaccharomyces pomb* growth medium. However, the reasons why polypeptone should be avoided have not been documented. Polypeptone dramatically induced cell lysis in the *ura4*deletion mutant when cells approached the stationary growth phase, and this phenotype was suppressed by supplementation of uracil. To determine the specificity of this cell lysis phenotype, we created deletion mutants of other genes involved in de novo biosynthesis of uridine monophosphate (*ura1*,*ura2*, *ura3*, and *ura5*). Cell lysis was not observed in these gene deletion mutants. In addition, concomitant disruption of *ura1*, *ura2*, *ura3*, or *ura5* in the *ura4* deletion mutant suppressed cell lysis, indicating that cell lysis induced by polypeptone is specific to the *ura4* deletion mutant. Furthermore, cell lysis was also suppressed when the gene involved in coenzyme Q biosynthesis was deleted. This is likely because Ura3 requires coenzyme Q for its activity. The *ura4* deletion mutant was sensitive to zymolyase, which mainly degrades (1,3)-beta-D glucan, when grown in the presence of polypeptone, and cell lysis was suppressed by the osmotic stabiliser, sorbitol. Finally, the induction of cell lysis in the *ura4* deletion mutant was due to the accumulation of orotidine-5-monophosphate. Cell wall integrity was dramatically impaired in the *ura4* deletion mutant when grown in the presence of polypeptone. Because *ura4* is widely used as a selection marker in *S. pombe*, caution needs to be taken when evaluating phenotypes of *ura4*mutants.

The AGC kinase Gad8 down regulates Tor1 activity through phosphorylation of the ATP binding site

Wei Du, Lenka Hálová, Janni Petersen

University of Manchester, C.4255 Michael Smith building, Faculty of Life Sciences, Oxford Road, Manchester M13 9PT, UK

Presented by: Du, Wei

Target of Rapamycin (TOR) signaling pathways mediate cell metabolism, growth and cell cycle progression in response to a variety of environmental settings. Schizosaccharomyces pombe have two TOR kinases Tor1 and Tor2, which are the main components of TORC2 and TORC1 respectively. We have identified a conserved phosphorylation site within the ATP binding pocket of TOR kinases. An alanine mutation to block phosphorylation increase TOR activity. Here we show that the AGC kinase Gad8 (an AKT homologue) phosphorylates this novel site. Consistently, inactive gad8KD mutant fails to phosphorylate Tor1, which increases Tor1 kinase activity. Nitrogen starvation of fission yeast inhibits TOR signalling to arrest cell cycle progression in G1 phase and promote sexual differentiation. We show that nitrogen starvation, stimulates Ksg1 dependent activation of Gad8. This enhanced Gad8 activity in turn reduces Tor1 kinase activity. Importantly, Gad8 dependent phosphorylation of Tor1 is essential for the decrease in TORC2 signalling that promotes sexual differentiation. Thus, environmentally controlled fine-tuning of TORC2 signalling can be achieved through changes in the phosphorylation status of the Tor1 ATP binding site to promote rapid, physiologically significant, changes in TOR signalling.

Sterol-rich membrane domains and the balance of membrane traffic

Shinichi Nishimura[a], Masato Tokukura[a], Junko Ochi[a], Minoru Yoshida[b], Hideaki Kakeya[a]

[a] *Graduate School of Pharmaceutical Sciences, Kyoto University, Japan*
[b] *Chemical Genetics Laboratory, RIKEN, Japan*

Presented by: Nishimura, Shinichi

Cell membranes serve as a physical barrier that defines the boundary and segregates the interior into distinct compartments that perform specialized functions. Cell membranes consist of lipids, proteins and glycans. Among lipids of many species, sterols are unique in that they are major regulators of membrane fluidity and contribute to the formation of specific membrane microdomains. In addition, sterols are medically important molecules since they are often targeted by antibiotics and toxins. Many studies using artificial membranes have been carried out to gain insights into the molecular mode of action of sterol-targeting molecules. However, how cellular membrane domains are created for and recognized by the antibiotics largely remains unknown.

Fission yeast is an excellent model organism for studying relationships between membrane domains and sterol-targeting antibiotics because of its highly polarized distribution of sterol-rich domains in the plasma membrane. Recently we found that when fission yeast cells were treated with a microbial metabolite that was reported to inhibit protein farnesyl transferase, cellular binding and/or the action of filipin, theonellamides and amphotericin B, all of which are ergosterol-binders, was abolished. Cdc42 activity was attenuated and exocytosis was inhibited after the compound treatment. In this presentation, we will discuss the relationships between sterol-rich membrane domains and the balance of membrane traffic.

Replication stress triggers the nuclear accumulation of the Rho1p exchange factor Rgf1p in fission yeast

Sofía Muñoz[a], Elvira Manjón[a], Patricia García[a], Per Sunnerhagen[b], Yolanda Sánchez[a]

[a] *Instituto de Biología Funcional y Genómica, CSIC/Universidad de Salamanca and Departamento de Microbiología y Genética, Universidad de Salamanca, Spain*
[b] *Department of Chemistry and Molecular Biology, Lundberg Laboratory, University of Gothenburg, PO Box 462, S-405 30, Gothenburg, Sweden*

Presented by: Muñoz, Sofía

Guanine nucleotide exchange factors (GEFs) control many aspects of cell morphogenesis by turning on Rho-GTPases. Our lab study polarized growth in fission yeast and is focused on understanding the role of Rho1p and its GEFs - Rgf1p, Rgf2p and Rgf3p- in cell morphogenesis.

Rgf1p is a multi-domain protein involved in different macromolecular processes. Rgf1p localises to cortical sites at the cell ends, from where this protein activates the β-GS subunit Bgs4p and signals upstream from the Pck2p-Pmk1p MAPK signaling pathway. Moreover, Rgf1p is also necessary for the establishment of bipolar growth also called NETO (New End Take Off). As a first step toward analyzing the regulation of Rgf1p, we study the localization of mutated versions of the Rgf1p-GFP. Unexpectedly, we found that mutants in the Rgf1p DEP domain localized to the nucleus and that this domain was nearby a canonical nuclear localization signal (NLS).

Here we report that Rgf1p is re-localized to the cell nucleus during stalled replication caused by hydroxyurea (HU) and that the protein is required for survival in the presence of the drug. Import to the nucleus is mediated by the NLS sequence at the N-terminus of Rgf1p, while release to the cytoplasm requires two leucine-rich nuclear export sequences (NES) at the C-terminus. Moreover, the Rgf1p nuclear accumulation during replication arrest depends on the 14-3-3 protein chaperone Rad24p and on the DNA replication checkpoint kinase Cds1p. Both proteins control the nuclear accumulation of Rgf1p by inhibition of its nuclear export. In a regular cell cycle, Rgf1p enters the nucleus transiently in S phase. Our results suggest that Rgf1p might play a role during S phase, but whereas its absence during a normal cell cycle could be bypassed at the expense of lengthening the G2-phase, the protein becomes essential when replication is compromized. Thus, Rgf1p/Rho1p signaling is coupled to the S phase checkpoint to protect cells from DNA replication damage.

Inferring the cell polarity network by high-content microscopy screening

James Dodgson, Anatole Chessel, Rafael Carazo Salas

The Gurdon Institute, University of Cambridge, UK

Presented by: Dodgson, James

The key question of how cell polarity is regulated has mainly been investigated at the level of individual polarity-regulating factors, leaving a gap in our understanding of how all polarity factors co-operate as a single, coordinated system. Due to its genetic tractability, highly ordered polarized growth pattern and many characterized polarity factors, fission yeast provides an ideal model system to create a complete polarity network for the first time.
For this purpose we generated a collection of approximately forty GFP-tagged polarity factor strains that we combinationally crossed against strains deleted for each corresponding polarity factor, yielding a matrix of around 2,500 strains. High-throughput acquired cell images for each strain are then analyzed using computer vision methods to generate a high-content quantitative dataset, from which the precise localization pattern of each polarity factor within the cell is extracted. Further information regarding the growth status of the cell ends or the existence of the septa is provided by the marker RFP-Bgs4. Using that information, we are in the course of determining the distribution of the different polarity factors at each type of growth zone found in the fission yeast cells through the cell cycle, as well as obtaining information about their localization interdependencies. This will allow us to gain unprecedented quantitative information on the regulation and function of the polarity machinery as well as on the structure of the cell polarity regulatory network as a whole.

Control of Sty1 MAPK activity through stabilisation of the Pyp2 MAPK phosphatase

Katarzyna Kowalczyk[a], Sonya Hartmuth[a], David Perera[b], Peter Stansfield[a], Janni Petersen[a]

[a] *University of Manchester, UK*
[b] *University of Cambridge, UK*

Presented by: Kowalczyk, Katarzyna

In all eukaryotes tight control of mitogen-activated protein kinase (MAPK) activity plays an important role in modulating intracellular signalling in response to changing environments. The fission yeast MAPK Sty1 (also known as Spc1 or Phh1) is highly activated in response to a variety of external stresses. To avoid segregation of damaged organelles or chromosomes, strong Sty1 activation transiently blocks mitosis and cell division until such stresses have been dealt with. MAPK phosphatases dephosphorylate Sty1 to reduce kinase activity. Therefore, tight control of MAPK phosphatases is central for stress adaptation and for cell division to resume. In contrast to Pyp1, the fission yeast Pyp2 MAPK phosphatase is under environmental control. Pyp2 has a unique sequence (the linker region) between the catalytic domain and the amino-terminal MAPK binding site. Here we show that the Pyp2 linker region is a destabilisation domain. Furthermore, the linker region is highly phosphorylated to increase Pyp2 protein stability and this phosphorylation is Sty1 dependent. Our data suggests that Sty1 activation promotes Pyp2 phosphorylation to increase the stability of the phosphatase. This MAPK-dependent Pyp2 stabilisation allows cells to attenuate MAPK signalling and resume cell division, once stresses have been dealt with.

Response to oxidative stress by arsenic and antimonite metalloids is dependent on Cmk2

Marta Sanchez-Marinas[a], David Gimenez-Zaragoza[b], Rosa Aligue[b]

[a] *University of Barcelona, Spain.* [b] *University of Barcelona, IDIBAPS, Spain*

Presented by: Aligue, Rosa

In S. pombe, the Sty1 SAPK pathway, responds to multiple types of stress (reviewed in Iker and Shiozaki. 2005 and, Perez and Cansado. 2010). How Sty1 MAPK can orchestrate a specific response to particular stress is an issue far from be resolved. Thus, to find the elements responsible for this specificity is subject of numerous studies.

We aimed to characterize the role of the Cmk2 kinase, previously described as a component of the SAPK pathway in response to oxidative stress (Sanchez-Piris et al. 2002, Alemany et al. 2002). The results obtained demonstrate a specific role of Cmk2 in response to certain oxidants, resulting particularly relevant to stress by arsenite.

The role of Cmk2 in resistance to arsenite is dependent of Sty1 MAPK. However, the Cmk2 kinase function is independent of the transcription factors Pap1 and Zip1 previously involved in this response (Rodriguez-Gabriel and Russell, 2005).

The absence of Cmk2 sensitizes cells to the presence of arsenite causing a decrease in cell viability and an increase of activity of pathways involved in response to this metalloid (Sty1, Zip1 and Pap1). By contrast, Cmk2 overexpression desensitizes cells to arsenite conferring resistance and preventing the activation of Sty1 by arsenite. Therefore, we hypothesized that Cmk2 kinase may have a function in regulating the intracellular arsenite levels. Apparently, we have ruled out a relationship with the putative aquaglyceroporins SPAC977.17, homologue of budding yeast Fps1 channel and mammalian aquaglyceroporins involved in arsennic uptake (Torsen et al. 2006, Liu et al, 2002b).

However, Cmk2 could regulate intracellular levels of arsenic modulating hexose transporters (Liu et al, 2004), either stimulating an ABC type transporter as Pmd1 (Nishi et al, 1992; Liu et al, 2002a), or by facilitating the glutathione export to bind extracellular arsenic and therefore preventing their entry (Thorsen et al, 2012).

A novel protein, Spm1, assures the integrity of spindle and kinetochore structures

Masamitsu Sato[a], Ryo Kariyazono[b], Kunio Arai[a], Hayato Hirai[a], Masayuki Yamamoto[b]

[a] *Center for Advanced Biomedical Sciences/TWIns, Waseda University, Japan*
[b] *Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan*

Presented by: Sato, Masamitsu

We aimed for collective isolation of mutants defective in various aspects of microtubule organisation, such as spindle assembly and cytoplasmic microtubule formation. A chemical random mutagenesis was performed to the strain harbouring a mini-chromosome and expressing GFP-tubulin (GFP-Atb2) with markers for SPBs (Sfi1-CFP) and the nuclear envelope (Nup40-mCherry). ~2,500 candidate colonies that showed temperature sensitivity with frequent loss of mini-chromosome were isolated and then subjected to the visual screen through fluorescence microscopy. Collective live-cell observation at the restrictive temperature led to classify ~2,000 mutants into 13 categories according phenotype.

We focused on one of the mutants that exhibited spindle midzone defects, spm1. In the mutant, the central region of the spindle became fragile in early mitosis and the spindle frequently collapsed, leading to chromosome missegregation. Spm1-GFP localised to the SPB-kinetochore interface during interphase, but dispersed at mitotic onset. Interestingly, the kinetochore protein Mis6 was delocalised in the spm1 mutant, indicating that Spm1 plays an essential role also in kinetochore assembly. We discuss the mechanism how Spm1 maintains the integrity of the spindle and kinetochores.

Actin stabiliser induces formation of motile thick actin bundles in *S. pombe*

Jun Kashiwazaki, Issei Mabuchi

Dept. of Life Sci., Fac. of Sci., Gakushuin Univ., Japan

Presented by: Kashiwazaki, Jun

Actin cytoskeleton has been known to be responsible for various cellular activities, such as cell morphogenesis, polarised growth, and cytokinesis. In the fission yeast Schizosaccharomyces pombe, actin filaments form three major structures; patches, cables, and the contractile ring. During interphase, actin cables extend from the growing ends toward the cell equator. Although it has been reported that the actin cables are required for polarised growth of the cell, their detailed behaviour has not been clear. Recently, we found that anomalous thick actin bundles were formed in interphase cells by treatment with an actin stabiliser. Interestingly, these bundles moved along long axis of the cell. In this study, we tried to elucidate the mechanism of this movement and behaviour of actin cables during interphase. To identify factors which are involved in formation and/or movement of the actin bundles, we treated various mutant cells with the actin stabiliser. We found that tropomyosin is required for the thick actin bundle formation and that type V myosins are required for the rapid movement of the actin bundles. There are two type V myosins (Myo52/ Myo4, Myo51/ Myo5) in S. pombe. Myo51-GFP was detected on the actin bundles as an array of motile dots. These dots moved on the bundle toward the direction opposite to the bundle movement suggesting that the leading end of actin bundles was the pointed end. Myo52-GFP moved more rapidly on the actin bundles but less frequently. We propose that fission yeast has two distinct mechanisms to pull actin cables for proper organisation of them during interphase. In addition, this system seems to be good to study the dynamic actin cable behaviour.

Fission yeast symmetrical nuclear division requires SPB-kinetochore interaction

Stefania Castagnetti[a], Bojan Božič[b], Saša Svetina[c]

[a] *Biosciences, University of Exeter, Exeter, UK*
[b] *Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*
[c] *Institute of Biophysics, Faculty of Medicine, University of Ljubljana and Jožef Stefan Institute, Ljubljana, Slovenia*

Presented by: Svetina, Saša

The nucleus of the unicellular fission yeast (Schizosaccharomyces pombe) divides within an intact nuclear envelope (NE), undergoing closed mitosis. Early in this process the duplicated spindle pole body (SPB) moves to the nuclear side of the NE to allow the polymerization of an intranuclear microtubule spindle. Spindle microtubules push apart the SPBs causing nuclear shape changes from sphere to dumbbell to the final two spherical daughter nuclei connected by a thin tube. We combine theoretical and experimental evidence to show that proper NE shape transformations occur due to the binding of segregated chromosomes to their respective SPBs. NE is a double membrane bilayer in a continuum with the endoplasmic reticulum which suggests that it is possible to predict its shape by determining the minimum of its bending energy at a fixed value of the membrane lateral tension. The corresponding calculations performed for different microtubule lengths under the condition of fixed nuclear volume predict that the equatorially symmetrical dumbbell shape does not correspond to such a minimum. The obtained minimal shape is asymmetrical, a lemon-like main body with a single thin protrusion. Such shapes have been previously observed in fission yeast mutants which undergo spindle elongation in the absence of an associated SPB. By using a mutant in the Ndc80 complex, which causes loss of SPB-kinetochore interaction, we show that SPBs alone are not sufficient for maintenance of proper symmetrical nuclear shapes, suggesting that symmetrical shapes result from SPB-kinetochore interaction. Taking into consideration that chromosomes attached to SPBs affect the position of the surrounding NE, the model shows that the symmetrical dumbbell nuclear shapes become stable at sufficiently large chromosome sizes.

The role of protein kinases in meiotic chromosome segregation in fission yeast

Ines Kovacikova[a,b], Silvia Polakova[a], Zsigmond Benko[a], Lubos Cipak[a,c], Lijuan Zhang[a], Cornelia Rumpf[a], Eva Miadokova[b], Juraj Gregan[a,b]

[a] *Max F. Perutz Laboratories; Department of Chromosome Biology; University of Vienna, Vienna, Austria*
[b] *Department of Genetics; Comenius University; Bratislava, Slovak Republic*
[c] *Cancer Research Institute; Slovak Academy of Sciences; Bratislava, Slovak Republic*

Presented by: Kovacikova, Ines

The protein phosphorylation is known to be a major regulatory mechanism in the cell. There are more than one hundred protein kinases encoded by Schizosaccharomyces pombe genome. Some protein kinases are already known to be involved in meiotic chromosome segregation such as Hhp2, Bub1 and Ark1. In order to identify protein kinases involved in chromosome segregation during meiosis, we screened a collection of haploid knockout mutants. We showed that Mph1, a member of the Mps1 family of spindle assembly checkpoint kinases, is required to prevent meiosis I homolog non-disjunction. Next, we provided evidence for a novel function of Spo4, the fission yeast ortholog of Dbf4-dependent Cdc7 kinase, in regulating the length of anaphase II spindles. In the absence of Spo4, abnormally elongated anaphase II spindles, frequently overlap and thus destroy the linear order of nuclei in the ascus. We observed that the spo4Δ mutant phenotype can be partially suppressed by inhibiting Cdc2-as suggesting that dysregulation of the activity of this cyclin-dependent kinase may cause abnormal elongation of anaphase II spindles in spo4Δ mutant cells.

Wee1 and Cdc25 constitute the nuclear:cytoplasmic ratio that regulate cell size

Daniel Keifenheim[a], Nick Rhind[a]

Department of Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, MA 01655, USA

Presented by: Keifenheim, Daniel

The coordination between cell growth and division is a highly regulated process that is intimately linked to the cell cycle. Despite a wealth of knowledge about cell growth and division, little is known about direct mechanisms that control cell size. The nuclear:cytoplasmic ratio is a popular model for how cell regulate cell size that is supported by many observations where changes in nuclear content changes the size of the cell at division. In this model the nuclear factor inhibits mitosis and the cytoplasmic factor induces mitosis. When the cell is small, the nuclear factor inhibits mitosis, but when the cell increases to a sufficient size, the cytoplasmic factor overcomes the inhibition of the nuclear factor and triggers mitosis. Our results show that Wee1 and Cdc25 have the characteristics necessary to constitute the nuclear:cytoplasmic ratio by conveying antagonizing activity. Wee1 is expressed at a constant level during G2 and inhibits mitosis by inhibiting Cdc2 therefore has the characteristics necessary to be the nuclear factor. Cdc25 increases in concentration as the cell increases in size and activates Cdc2 to trigger mitosis. The observation that Cdc25 concentration increase with cell size is unusual since most protein in the cell have a constant concentration. Additionally, experiments with short pulses of cycloheximide have shown the Cdc25 is inherently unstable protein that it quickly returns to a size dependent equilibrium in the cell suggesting that Cdc25 concentration is dependent on size and not time. Furthermore, transcript levels mirror protein concentration suggesting that transcriptional or post transcriptional regulation play a part in regulating Cdc25 protein concentration. We are currently trying to elucidate the mechanism of how Cdc25 is expressed in a size dependent manner.

Analysis of growth polarity transition in fission yeast

Takayuki Koyano[a], Kazunori Kume[a], Masaki Mizunuma[a], Takashi Toda[b], Dai Hirata[a]

[a] *Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Japan*
[b] *Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3LY, UK*

Presented by: Koyano, Takayuki

How nuclear cell-cycle events are connected to cell polarization is a fundamental subject in cell biology. The regulation of cell polarity is coupled with the cell cycle, suggesting that the coordinated regulatory pathway exists in eukaryotes. Fission yeast changes the growth polarity during the cell cycle. At the beginning of G2, growth polarity changes dynamically from monopolar to bipolar, termed new end take off (NETO). For NETO to take place, two requirements, completion of DNA replication and attainment of critical cell size, have to be fulfilled. Although numerous molecules required for NETO have been identified, the signaling pathway(s) regulating NETO remains elusive. Recently, our group showed that the checkpoint kinase Cds1 is required for not only cell-cycle arrest but also NETO delay in the DNA polymerase-alpha mutant pol1/mon7, which was isolated as one of the temperature-sensitive mutants with monopolar growing manner. The microtubule plus-end tracking proteins (+TIPs) CLIP-170 homologue Tip1 and kinesin Tea2 are responsible for the NETO delay, which is accompanied by a reduction in microtubule dynamics at the cell tip. Further, NETO delay requires activation of calcineurin (CN), which is carried out by Cds1, resulting in Tip1 deposphorylation. Thus, our study established a critical link between calcineurin and checkpoint-dependent cell morphogenesis.

To understand the mechanism of NETO further more, we performed systematically screening for the protein kinase(s) related to NETO by using both G1-arrested cdc10 and pol1 mutants. From this screening, we identified several kinases as the new NETO regulator. We will discuss the functional relationship between the identified kinase and the Cds1-CN-TIPs (CCT) pathway.

Why is exogenous M-factor pheromone unable to recover mating defect in M-factor-less mutant?

Taisuke Seike, Taro Nakamura, Chikashi Shimoda

Department of Biology, Graduate School of Science, Osaka City University, Japan

Presented by: Seike, Taisuke

Mating pheromone signaling is essential for conjugation between haploid cells of P-type and of M-type in S. pombe. A peptide pheromone, M-factor, produced by M-cells is recognized by the receptor of P-cells. Here, we report the significance of pheromone-induced cell adhesion in oriented growth and subsequent cell fusion.

An M-factor-less mutant, in which the M-factor-encoding genes are deleted, is completely sterile. In liquid culture, sexual agglutination was not observed in the mutant, but agglutinability could be recovered by exogenously added synthetic M-factor (>10 nM), which stimulated expression of the P-type-specific cell adhesion protein, Map4. Exogenous M-factor (even at 1 μM), however, failed to recover the cell fusion defect in the M-factor-less mutant. When M-factor-less cells were mixed with wild-type P- and M-cells, marked cell aggregates were formed. Notably, M-factor-less mutant cells were also incorporated into these aggregates. In this mixed culture, P-cells conjugated preferentially with wild-type M-cells secreting M-factor, and rarely with M-factor-less M-cells. To address the question of why exogenous M-factor fails to rescue mating defect in M-factor-less mutant, we performed live-imaging analysis of conjugating cells in liquid culture. Our observations revealed that polarized cell growth did not occur before cell adhesion, and commenced from the contact region of opposite mating-type cells. We speculate that a pair of cells of the opposite mating types first adheres and the locally-concentrated M-factor pheromone at the contact site establishes the cell polarity. Directed cell extension starts from the contact region, and then the cross wall is dissolved. Our proposed mechanism may explain the reason why the pheromone-less mutant cells are not chosen by the opposite mating-type cells as a mating partner. This study may shed light on understanding of the pheromone-mediated polarity establishment in fission yeast grown in liquid medium.

In vitro contraction of cytokinetic ring depends on myosin II but not on actin dynamics

Mithilesh Mishra[a], Jun Kashiwazaki[b], Tomoko Takagi[b], Ramanujam Srinivasan[c], Yinyi Huang[c], Mohan Balasubramanian[d], Issei Mabuchi[b]

[a] Temasek Life Sciences Laboratory, The National University of Singapore, 1 Research Link, Singapore 117604
[b] Department of Life Sciences, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo, 171-8588, Japan.
[c] Mechanobiology Institute, The National University of Singapore
[d] Temasek Life Sciences Laboratory, The National University of Singapore, 1 Research Link, Singapore 117604. & Mechanobiology Institute, The National University of Singapore

Presented by: Mishra, Mithilesh

Cytokinesis in many eukaryotes involves the contraction of an actomyosin-based contractile ring (CR)1,2. However the detailed mechanism of CR contraction is not fully understood. Here, we establish for the first time an experimental system to study contraction of the CR to completion in vitro. We show that CR of permeabilised fission yeast cells undergo rapid contraction in an adenosine triphosphate (ATP) and myosin-II dependent manner in the absence of other cytoplasmic constituents. Surprisingly, neither actin polymerisation nor its disassembly is required for contraction of CR although addition of exogenous actin cross-linking proteins block CR contraction. Using CRs generated from fission yeast cytokinesis mutants, we show that not all proteins required for assembly of the ring are required for its contraction in vitro. Our work provides the beginnings in the definition of a minimal contraction-competent cytokinetic ring apparatus.

Sin1 is a conserved substrate-binding subunit of the Target Of Rapamycin (TOR) protein kinase

Toshiya Yonekura[a], Shinichi Murayama[a], Hisashi Tatebe[a], Saori Kataoka[b], Kyoko Furuita[b], Chojiro Kojima[b], Kaz Shiozaki[a]

[a] *Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan*
[b] *Institute for Protein Research, Osaka University, Japan*

Presented by: Yonekura, Toshiya

TOR (Target Of Rapamycin) is an evolutionarily conserved Ser/Thr protein kinase that integrates extracellular signals and controls proliferation and metabolism in eukaryotes. TOR kinase forms two distinct protein complexes referred to as TOR complex 1 (TORC1) and 2 (TORC2). These two complexes regulate discrete sets of downstream events, thorough phosphorylation of specific substrates. In human cells, TORC1 phosphorylates S6K1 and promotes protein synthesis and cell growth. On the other hand, TORC2 regulates cell survival and physiology by phosphorylating Akt, SGK and PKC. It is likely that such substrate specificities are determined by distinct subunit compositions of TORC1 and TORC2.

The TORC2-Gad8 pathway in Schizosaccharomyces pombe is highly homologous to the human TORC2-Akt/SGK pathway. We detected physical interaction between Gad8 and one of the conserved TORC2 subunits, Sin1. Importantly, its CRIM (Conserved Region In the Middle) domain is required and sufficient for interaction with Gad8 kinase. We also found that the CRIM domain of human Sin1 (hSin1) interacts with the TORC2 substrates such as Akt, SGK and PKC but not the TORC1 substrate S6K1. Furthermore, we successfully determined the NMR-structure of the S. pombe Sin1 CRIM domain with a characteristic acidic loop structure. Mutations to this acidic loop abrogate the interaction between Sin1 and TORC2 substrates in both fission yeast and human. These studies demonatrate that Sin1 is a conserved substrate-binding subunit of TORC2 and that the Sin1 CRIM domain is a crucial determinant of the TORC2 substrate specificity.

Role of palmitoylation in Rho2-dependent signaling in fission yeast

Marisa Madrid[a], Alejandro Franco[a], Laura Sánchez-Mir[a], Rebeca Martín-García[a], Jero Vicente-Soler[a], Teresa Soto[a], Mariano Gacto[a], Pilar Pérez[b], José Cansado[a]

[a] *Yeast Physiology Group, Department of Genetics and Microbiology, Faculty of Biology University of Murcia, 30071 Murcia, Spain*
[b] *Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas, Departamento de Microbiología y Genética, Universidad de Salamanca 37007, Salamanca, Spain*

Presented by: Madrid, Marisa

The cell integrity pathway (CIP), whose central element is the MAPK Pmk1, regulates in S. pombe processes like cell wall construction and maintenance during stress, vacuole fusion, cytokinesis, morphogenesis, and ionic homeostasis. Rho2, one of the six Rho GTPases found in S. pombe proteome, performs two fundamental tasks in fission yeast biology as modulator of the cell wall integrity and morphogenesis, and as an upstream regulator of the CIP. Like most small GTPases, Rho2 shows a CAAX box at its C-terminus, and it has been shown that in vivo farnesylation of cysteine-197 within this motif determines its plasma membrane binding and full function. In this study we demonstrate that, in addition to prenylation, Rho2 is also palmitoylated in vivo at cysteine-196. Whereas wild type Rho2 located mostly at the plasma membrane, this pattern changed drastically in the unpalmitoylated mutant, which localized mainly to endomembranes. Thus, we investigated the biological significance of Rho2 lipid modifications (prenylation and/or palmitoylation) in the context of the activity of the CIP. Indeed, in vivo palmitoylation at Cys-196 and plasma membrane binding are crucial for Rho2 control of morphogenesis and signaling to the CIP both during vegetative growth and in response to stress. Characterization of mutants expressing Rho2 chimeras where its natural CAAX box was substituted by the C-terminal end from Rho1 (which lacks palmitoylable cysteine residues and instead harbors a C-terminal polybasic sequence), or a palmitoylatable version of this tail, showed that the presence of C-terminal polybasic sequence prevented the in vivo palmitoylation and function of geranylgeranylated, but not farnesylated, Rho2. These observations strongly suggest that the co-existence of polybasic motif plus palmitoylable cysteine residue/s is restricted to specific Rho GTPases from higher eukaryotic organisms and might be attained late during evolution.

Isolation of a new spliceosome mutant with cell cycle defects by a novel conditional screening in *S. pombe*

Felix Reyes, Juan Jimenez, Victor A. Tallada

Centro Andaluz de Biologia del Desarrollo, Universidad Pablo de Olavide/CSIC, Ctra. Utrera Km1, 41013, Seville, Spain

Presented by: Reyes, Felix

Conditional screenings have so far identified hundreds of mutants in many biological processes that allow the study of loss-of-function under the restrictive condition, but also the propagation and genetic crosses of these strains under the permissive condition. Although drug sensitivity mutants have been isolated, the most spread condition used is temperature.

We have searched for formamide sensitive mutants, a novel conditionality in S. pombe, that display cell cycle defects. Among others, we have identified one mutation in the conserved spliceosome component cwf15, that allows growth in normal conditions but it blocks cell cycle progression in the presence of formamide. This mutation (cwf15.32Fs) consists of 13 nucleotides direct duplication that generates a premature stop codon and thus a C-terminal truncated protein.

Cwf15 (Complexed with cdc5 protein 15) is an essential and evolutionary conserved splicing factor from yeast to humans. It has been shown to physically interact to other spliceosome components but no further characterization has been done.

Here we characterize cwf15.32Fs cell cycle defects and test for genetic interactions with other splicing mutants.

Elucidation of Mid1 interactions and regulatory mechanisms contributing to stable cortical localization

Marian Testori[a], Ashley DeWitt[a], Jennifer Jakubowski[a], Brandon Nader[a], David Kovar[b], Dawn Clifford Hart[a]

[a] *Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI 49401, USA*
[b] *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA*

Presented by: Clifford Hart, Dawn

The anillin-related protein Mid1 serves as a central organizer of cytokinesis nodes by anchoring the protein structures to the cell membrane then recruiting proteins to complete assembly of cytokinesis nodes and the contractile ring. In the absence of Mid1, nodes fail to assemble and contractile rings are randomly placed. To stably bridge cytokinesis nodes with the cell cortex, Mid1 directly interacts with lipids and Cdr2 kinase at the cell membrane. Recently, the C-terminal Pleckstrin Homology (PH) domain of Mid1 was shown to directly bind lipids and stabilize Mid1 in cytokinesis nodes. We now report that the PH domain of Mid1 directly associates with F-actin in vitro. Through spontaneous actin polymerization assays, we find that the Mid1 PH domain inhibits F-actin polymerization in a dose-dependent manner. These results suggest that association of the PH domain with F-actin could provide an additional mechanism to stably maintain Mid1 in cytokinesis nodes. While a more complete model for Mid1's arrival at the cell cortex is emerging, Mid1 dissociation from the cell cortex is not well studied. Given that localization of Mid1 to the cell cortex is phosphorylation dependent, we hypothesized that Mid1 departure from the cell cortex may be similarly controlled. Interestingly, Mid1 dissociates from the cell cortex prior to contractile ring constriction. Since ring constriction requires activation of the Septation Initiation Network (SIN), we tested whether the most downstream SIN signaling protein, Sid2 kinase, targets Mid1. Indeed we find that Sid2 directly phosphorylates Mid1. Phenotypic analyses of phosphosite mutants reveal defects in Mid1 nuclear accumulation following septation. Instead, Mid1 appears to localize as cortical nodes, suggesting Mid1 might remain associated with the membrane in the absence of Sid2 phosphorylation. Taken together, these results provide two additional mechanisms that may contribute to Mid1 stability at the cell cortex.

Fission yeast Nod1 is a component of cortical nodes involved in cell size control and division site placement.

Isabelle Jourdain[a], Elspeth A. Brzezińska[b], Takashi Toda[c]

[a] *The Biotechnology Centre of Oslo, Norway*
[b] *Beatson Institute for Cancer Research, UK*
[c] *Cancer Research UK, LRI, London, UK*

Presented by: Jourdain, Isabelle

Most cells enter mitosis once they have reached a defined size. In the fission yeast Schizosaccharomyces pombe, mitotic entry is orchestrated by a geometry-sensing mechanism that involves the Cdk1/Cdc2-inhibiting Wee1 kinase. The factors upstream of Wee1 gather together in interphase to form a characteristic medial and cortical belt of nodes. Nodes are also considered to be precursors of the cytokinesis contractile actomyosin ring (CAR). Here we describe a new component of the interphase nodes and cytokinesis rings, which we named Nod1. Consistent with its role in cell size control at division, nod1Δ cells were elongated and epistatic with regulators of Wee1. Through biochemical and localisation studies, we placed Nod1 in a complex with the Rho-guanine nucleotide exchange factor Gef2. Nod1 and Gef2 mutually recruited each other in nodes and Nod1 also assembles Gef2 in rings. Like gef2 delta, nod1 delta cells showed a mild displacement of their division plane and this phenotype was severely exacerbated when the parallel Polo kinase pathway was also compromised. We conclude that Nod1 specifies the division site by localising Gef2 to the mitotic cell middle. Previous work showed that Gef2 in turn anchors factors that control the spatio-temporal recruitment of the actin nucleation machinery. It is believed that the actin filaments originated from the nodes pull nodes together into a single contractile ring. Surprisingly however, we found that node proteins could form pre-ring helical filaments in a cdc12-112 mutant in which nucleation of the actin ring is impaired. Furthermore, the deletion of either nod1 or gef2 created an un-expected situation where different ring components were recruited sequentially rather than simultaneously. At later stages of cytokinesis, these various rings appeared inter-fitted rather than merged. This study brings a new slant to the understanding of CAR assembly and function.

Dual role of Ras1 in coordinating meiotic differentiation in fission yeast

Emma Kelsall[a], Abel Vertesy[b], Gabriele Schreiber[b], Shubi Randhawa[a], Edda Klipp[b], Kayoko Tanaka[a]

[a] *University of Leicester, Department of Biochemistry, UK*
[b] *Berlin Humboldt University, Theorectical Biophysics, Germany*

Presented by: Kelsall, Emma

Fission yeast mating pheromone triggers the RAS-MAPK signaling pathway essential for meiotic differentiation. It also induces dramatic morphological change of the cells leading to mating, a cell fusion event between cells of opposite mating types. The system is ideal to dissect the mechanisms by which the RAS-MAPK signal activation is regulated and highlight basic regulatory concepts of RAS protein signalling.

We aim to evaluate the role of Ras1 in coordinating both MAPKSpk1 activation due to pheromone signalling and activation of the Cdc42 pathway responsible for actin reorganisation. We established a condition to induce highly synchronous mating of fission yeast cells and we also established an assay system to monitor the MAPK activation status with an anti-phospho ERK monoclonal antibody. In addition, the changes in cellular morphology during the time course of meiotic differentiation were monitored. These tools allow us to carry out quantitative measurement of the MAPKSpk1 signalling status in cells harboring various mutations in the signalling components.

We confirmed that a constitutively active mutation of the MEKByr1 induces constitutive activation of the MAPKSpk1 as we expect. Strikingly however, with a canonical oncogenic Ras mutation (ras1.val17), although the MAPKSpk1 activation occurs acutely, it is rapidly down-regulated therefore identifying the role of an unidentified modulator of the pathway at the level of the MEKKByr2 or MEKByr1. Further assessment of MAPKSpk1 activation in various mutants of the pathway allows us to conclude that Ras1 plays a key role in activation of both a morphological response and MAPKSpk1 activation and correct coordination of these two pathways is essential for successful meiotic differentiation.

Nuclear size control in fission yeast

Kazunori Kume, Paul Nurse

Cell Cycle Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Field, London, WC2A 3LY, UK

Presented by: Kume, Kazunori

Fission yeast cells maintain a nuclear size that is proportional to the total cell size of the cell leading to a constant ratio between nuclear volume and cell volume (the N/C ratio). It has previously been shown that nuclear size is proportional to cell size throughout the cell cycle, where a constant N/C ratio of 0.08 is maintained1. This ratio is not directly influenced by the nuclear DNA content but increased ploidy increases cell size which in turn increases nuclear size1. Furthermore, studies with multi-nucleated cells that have unevenly distributed nuclei revealed that the volume of each nucleus is proportional to its surrounding cytoplasm1. These results suggest the existence of a mechanism that coordinates nuclear and cellular volume. However, the cellular factors that determine nuclear volume and the mechanism by which they regulate nuclear size are unknown. To identify genes involved in nuclear size control, we carried out a genome-wide visual screen of haploid gene deletion mutants for strains with altered nuclear size. Screening approximately 3,000 gene deletion strains identified 10 strains that have a significantly higher N/C ratio when compared with wild-type cells. These genes are categorized into several processes such as nuclear transport, RNA processing, transcription and ribosome biogenesis.

1) F. Neumann and P. Nurse (2007) J. Cell Biol., 179(4), 593-600.

Dissecting the cell size monitoring system in fission yeast by quantitative analysis of the Pom1 concentration gradient

Olivier Hachet, Micha Hersch, Sascha Dalessi, Sven Bergmann, Sophie Martin

[a] *UNIL, Lausanne, Switzerland*

Presented by: Hachet, Olivier

Cell division in fission yeast is tightly coordinated with cell growth and occurs at a constant cell length. This relies in part on a geometric cell size monitoring system based on cortical concentration gradients of the DYRK kinase Pom1 emanating from cell tips. Pom1 modulates CDK1 (Cdc2) activity by negatively regulating the Cdr2 kinase localized at the cell middle; this inhibition is thought to be alleviated by cell elongation. However, the mechanistic nature of the Pom1 gradient readout remains to be described. We have shown that the formation of Pom1 gradients involves a cycle of dephosphorylation and autophosphorylation: Pom1 is locally dephosphorylated at cells tips by the Tea4-Dis2 complex, revealing a lipid-binding domain that mediates Pom1 binding to the plasma membrane. As Pom1 diffuses along the membrane, Pom1 autophophorylates at multiple sites to promote its release into the cytoplasm.

We have developed an Image J plugin for quantitative analysis of the Pom1 gradient. Using this tool we revealed a correlation between cell length and the size of the central domain with low Pom1 signal and high Cdr2 signal. We used two approaches to experimentally manipulate this medial cell zone: first we make use of a chemical genetic approach based on an analogue sensitive allele of Pom1. Using suboptimal doses of the inhibitor, we can manipulate the shape of the gradient and study its impact on mitotic commitment and Cdr2 localization. Second, by generating a Pom1 allele series with increasing numbers of auto-phosphorylation sites mutated, we show a progressive spreading of the Pom1 gradient and a progressive increase in cell size at division according to the number of mutated sites. This indicates that the multiple autophosphorylation sites impact on the gradient shape. Together, these approaches combined with quantitative analysis provide new tools to dissect the Pom1 gradient readout responsible for the control of mitotic entry.

APC/C-mediated proteolysis of Ams2 in G1 ensures the coupling of histone gene expression to DNA replication in fission yeast

Hiro Yamano[a], Michelle Trickey[a], Kazuyuki Fujimitsu[a]

Cell Cycle Control Group, UCL Cancer Institute, University College London, London, WC1E 6BT, UK

Presented by: Yamano, Hiro

The anaphase-promoting complex/cyclosome (APC/C) is a multi-subunit cullin RING E3 ubiquitin ligase that functions to regulate mitosis and G1 as well as meiosis. In order to understand novel roles of the APC/C in cells, we have carried out a genome wide screen for fission yeast APC/C substrates using a cell-free destruction assay reconstituted in Xenopus egg extracts. We have identified Ams2 as a new APC/C substrate, which is a GATA containing transcription factor responsible for core histone gene expression and for CENP-A loading at centromeres. Ubiquitylation and destruction of Ams2 in G1 is dependent upon a co-activator Cdh1/Ste9 and the KEN box in the C-terminus of Ams2. Ams2 levels are cell cycle regulated and after S phase it is degraded by the SCFpof3 ubiquitin ligase as histone transcription and deposition are tightly regulated with the DNA replication cycle in order to maintain genetic integrity. However the regulation of Ams2 in G1 or meiosis remains unclear. We show how APC/C-dependent Ams2 destruction in G1 is required for DNA replication and meiosis. We also find that stabilization of Ams2 sensitizes cells to the anti-microtubule drug thiabendazole and a histone deacetylase (HDAC) inhibitor Tricostatin A, when a HDAC gene hst4 is deleted, suggesting that histone acetylation together with Ams2 stability ensures the coupling of mitosis to DNA replication. Furthermore, in meiosis, the failure of the APC/C-mediated destruction of Ams2 is deleterious and pre-meiotic DNA replication is barely completed. These data suggest that Ams2 destruction via both the APC/C and the SCF ubiquitin ligases underlies the coordination of histone expression and DNA replication.

Modulation of fission yeast drug sensitivity by environmental and intracellular potassium ion concentrations

John Alao, Andrea Weber, Per Sunnerhagen

University of Gothenburg, Sweden

Presented by: Alao, John

The concentration of potassium and sodium ions has been shown to modulate drug sensitivity in Schizosaccharomyces pombe (S. pombe). It has previously been proposed, that external and internal ion concentrations affect membrane polarity and thus the import of polar drugs. However, the precise range of drugs whose activity is modulated by environmental potassium ion concentrations remains unclear. In the current study, we investigated the effect of environmental potassium concentrations on a wide range of structurally dissimilar drug classes. Our studies demonstrate that potassium chloride concentrations as low as 0.06M effectively suppress the sensitivity of S. pombe to a wide range of drugs independently of their polarity. This effect occurs independently of the Sty1-regulated stress response pathway and is due to ionic but not osmotic stress. Furthermore, the suppression of drug sensitivity occurs via the inhibition of drug import. The degree to which potassium ion concentrations suppressed sensitivity varied between different drug types. Interestingly, sensitivity to the antibiotics bleomycin and phleomycin was reduced by up to 40 times. In contrast, potassium enhanced sensitivity to the antifungal agents amphotericin B and nystatin. Our studies suggest that modulating environmental or intracellular potassium concentrations may suppress drug resistance in pathogenic fungi. Environments with high potassium ion concentrations may also allow S. pombe to compete more effectively with antimicrobial producing organisms in its natural environment.

Regulation of ribonucleotide reductase: Impacts for genome stability

Helena B.N. Nielsen, Olaf Nielsen, Christian Holmberg

Department of Biology, University of Copenhagen, Ole Maaløes vej 5, 2200 Copenhagen N, Denmark

Presented by: Nielsen, Helena

In all living cells, synthesis of the DNA building blocks, deoxy-ribonucleotides (dNTPs), is tightly regulated to ensure a precise DNA replication to maintain genomic stability. Ribonucleotide reductase (RNR) is the enzyme responsible for reducing ribonucleotides to deoxy forms.

RNR is a heterotetramer consisting of two R1 subunits and two R2 subunits, both of which are essential. The activity of RNR is strictly regulated to control the dNTP pool, both by allosteric feedback control and transcriptional and translational controls. Four inhibitory proteins of RNR have also been identified in yeast: Spd1 in fission yeast, and Sml1, Hug1, and Dif1 in budding yeast. Experiments from our group have shown that both a too high and a too low dNTP pool leads to increased mutation rates, hence regulation of RNR is very important in order to maintain genomic stability.

No human inhibitory proteins have yet been identified to regulate the human RNR. We work on regulation of human RNR using a fission yeast strain that depend solely on the human genes of R1 and R2 for dNTP synthesis. This strain grows like wild-type fission yeast and contains normal dNTP levels. It is, however, hypersensitive towards Hydroxyurea (HU). Also we find that an h90 strain with the human RNR genes can mate, but cannot form spores, suggesting a role for RNR during meiosis. We will use this strain in a functional screen, where we will take advantage of the powerful yeast genetics to isolate clones from a human cDNA library that can reduce the activity of RNR. Or in other words, we aim to isolate clones that exert Spd1-like function.

A systems analysis of microtubule regulation by +TIP proteins in fission yeast

Ignacio Flor-Parra, Pin-Joe Ko, Kally Pan, Fred Chang

Microbiology and Immunology, Columbia University, New York, NY, USA

Presented by: Flor-Parra, Ignacio

The dynamic behaviors of microtubules are regulated by a large number of +TIP proteins at their plus ends. S. pombe is an attractive model organism for studying the MT cytoskeleton in vivo, as the MT cytoskeleton is relatively simple and amenable for quantitative analysis of MT dynamics. In contrast to the large number of diverse +TIPs identified in mammalian cells, only eight +TIPs are known in fission yeast: Mal3 (EB1), Tip1 (CLIP170), Alp14 (XMAP215), Tea2 (kinesin-7), Klp2 (kinesin-14) and Klp5/6 (kinesin-8) and Tea1 and Tea 4 (cell polarity regulators). Although the +TIPs have been studied individually, still little is known about how all these proteins work collectively together at the plus end in vivo. Here, we are pursuing a quantitative systems approach to study all of these eight +TIPs as a group, in order to understand how they localize and function in MT regulation in vivo. All single null mutants are viable, allowing us to study true null mutants. Protein localization is assayed using functional GFP fusions expressed at near endogenous levels. We have analyzed all the single and double mutant combinations for their effects on MT dynamics. We have also analyzed the effects of each gene on localization of all the other +TIPs. Our current results suggest that the +TIPs can be divided into three modules: an EB1 group, an XMAP215 group, and a Kinesin-18 group. MT growth is strictly dependent on non-overlapping functions of EB1 and XMAP215. Using quantitative fluorescence intensity measurements, we find that each MT plus end is coated with 50-150 molecules of each +TIP, or about 1000 +TIP molecules total. These studies promise to provide a framework for studying the in vivo functions of +TIP proteins.

Comparative analysis of two mitotic kinesins Cut7 & Klp9

Liang Ji, Anne Paoletti, I-Mei Yu, Carlos Kikuti, Anne Houdusse, Phong Tran

Institut Curie, UMR144 CNRS, Paris 75005, France

Presented by: Ji, Liang

Kinesins are microtubule-based motor proteins, classified into different families by their homology and functions. They contribute to various cellular processes such as transporting cargo along microtubules, regulating microtubule end dynamics, and crosslinking and sliding microtubules to organize the mitotic spindle. Among the nine kinesins in fission yeast, kinesin-5 Cut7 and kinesin-6 Klp9 play seemingly similar role in spindle elongation. Both Cut7 and Klp9 are microtubule plus-end directed homotetramers which crosslink and slide antiparallel microtubule apart. However, they function in distinct spatiotemporal manner. Cut7 localizes to the early mitotic spindle and is essential for bipolar spindle formation (Hagan and Yanagida, 1992 Nature). In contrast, Klp9 localizes to the anaphase spindle midzone and is important for spindle elongation (Fu et al, 2009 Dev Cell). We hypothesize that these differences between Cut7 and Klp9 lie in their different head-tail structures, which tune the respective kinesin to different microtubule organization.

To test this hypothesis, we created head-tail chimeras of Cut7 and Klp9 called 7H9T1 (Cut7 head-Klp9 tail) and 9H7T1 (Klp9 head-Cut7 tail). Both chimera can localize to the spindle and elongate the anaphase spindle in vivo. However, the chimeras cannot fully compensate for Cut7 or Klp9. Surprisingly, 7H9T1 localization was similar to Cut7 and 9H7T1 localization was similar to Klp9. This suggests distinct head properties may regulate Cut7 and Klp9 localization and function independently of the tail. On going in vitro analysis with purified motors will clarify the distinct head-tail properties and functions of Cut7 and klp9.

A chromatin switch regulates DNA double-strand break repair pathway choice in fission yeast

Chen-Chun Pai[a], Rachel Deegan[a], Sandra Codlin[b], Jürg Bähler[b], Robin Allshire[c], Csenge Gal[d], Simon Whitehall[d], Timothy Humphrey[a]

[a] *Gray Institute for Radiation Oncology & Biology University of Oxford, Department of Oncology, ORCRB, Old Road Campus, Oxford, OX3 7DQ, UK*
[b] *University College London, Department of Genetics, Evolution and Environment, Darwin Building, Gower Street, London, WC1E 6BT, UK*
[c] *Institute of Cell Biology, School of Biological Sciences, The University of Edinburgh, Swann Building, Mayfield Road, Edinburgh EH9 3JR, UK*
[d] *Institute for Cell and Molecular Biosciences, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK*

Presented by: Pai, Chen-Chun

DNA double strand break (DSB) repair is a highly regulated process performed by the competitive non-homologous end joining (NHEJ) or homologous recombination (HR) pathways. We have investigated the role of the SETD2 tumour suppressor homologue, set2+, a histone H3K36 methyltransferase, in DSB repair pathway choice in fission yeast. Deleting set2+, or disrupting its methyltransferase acitivity, resulted in increased resistance to low levels of ionizing radiation (IR) or bleomycin. This corresponded with significantly elevated levels of homologous recombination (HR) compared to wildtype. Consistent with a competitive relationship with HR, set2Δ was epistatic with the NHEJ mutants ku70Δ or lig4Δ which behaved similarly. Further, a role for Set2 methyltransferase activity in protecting DSB ends and promoting NHEJ was identified. Surprisingly, an unmodifiable H3K36R mutant exhibited increased sensitivity to bleomycin and IR, suggesting an additional role for H3K36 modification in DSB repair. Accordingly, we found set2Δ exhibited increased Gcn5-dependent H3K36 acetylation. Moreover, deleting gcn5 resulted in increased Set2-dependent H3K36 methylation, significantly reduced HR and increased NHEJ. Together, these findings define a role for an H3K36 chromatin switch in regulating DSB repair pathway choice in fission yeast.

Mechanism of Pom1-dependent cell size regulation of mitotic onset and division plane positioning in fission yeast

Sergio A. Rincon[a], Payal Bhatia[b], Olivier Hachet[b], Laetitia Basterra[b],Claudia Bicho[c], Mercé Vendrell-Guzman[a], Kenneth E. Sawin[c], Anne Paoletti[a], Sophie Martin[b]

[a] *Institut Curie, CNRS UMR144, Paris, France*
[b] *Department of Fundamental Microbiology, UNIL, Lausanne, Switzerland*
[c] *Welcome Trust Center for Cell Biology, University of Edinburgh, UK*

Presented by: Rincon, Sergio

Cell size homeostasis is important for a variety of cellular functions. A first molecular model of cell size control has been established in fission yeast. It relies on cell length sensing by a spatial gradient of the DYRK kinase Pom1 emanating from the cell tip cortex. Pom1 gradient influences the activity of a Wee1 regulatory pathway for mitotic entry controlled by the SAD1-like kinase Cdr2 assembled into cortical nodes at the cell middle. This mechanism also contributes to geometrical sensing of the cell middle and medial division by Mid1/Anillin. However, how Pom1 regulates Cdr2 remains elusive. Here we show that Pom1 inhibits both Cdr2 kinase activity and assembly into nodes. Inhibition of the kinase activity may involve the phosphorylation of a C-terminal auto-inhibitory tail of Cdr2. Pom1 prevents node assembly by modulating Cdr2 association with membranes and clustering. This relies both on the phosphorylation of a basic lipid-binding motif, which works in conjunction with a C-terminal KA1 domain to anchor Cdr2 to the plasma membrane, and on the inhibition of Mid1-dependent clustering of Cdr2 mediated by its N-terminus. This latter effect could result indirectly from Pom1-dependent inhibition of Cdr2 kinase activity. We finally propose that a differential sensitivity to Pom1 of Cdr2 kinase activity and Cdr2 assembly into nodes may allow Cdr2 node assembly at the medial cortex in short cells to monitor cell length and predefine the division plane before Cdr2 activation triggering mitotic entry.

Pot1 phosphorylation regulates telomere functions

Yuan Zhao, Vitaliy Kuznetsov, Julie Cooper

Telomere Biology Laboratory, London Research Institute, UK

Presented by: Zhao, Yuan

The telomere is a conserved nucleoprotein structure at the ends of eukaryotic chromosomes. It is essential for maintenance of genomic stability: on the one hand, it suppresses DNA damage response and protects the natural chromosome ends from repair activities; on the other hand, it recruits telomerase, the specialized reverse transcriptase, to counteract the end-replication problem. The telomeric G-strand ssDNA-binding protein Pot1 plays a crucial role in both of these functions. In fission yeast S. pombe, inhibition of Pot1 induces rampant 5' resection and loss of telomere signal in a single cell cycle.

We have recently found that fission yeast Pot1 is phosphorylated within its N-terminal OB-fold by the DDK kinase, a conserved master cell cycle regulator, in late S/G2 phase. Phosphorylation was visualized using 2D gels of C-terminally tagged Pot1 with and without mutations in putative phosphorylation sites, revealing the relevant serines/threonines. Excitingly, this phosphorylation appears to be crucial for several aspects of telomere regulation, including telomere length control, checkpoint inhibition, and ssDNA generation. Our data point to a model in which cell cycle-regulated Pot1 phosphorylation coordinates telomere replication and protection at different cell cycle phases.

Chaperone-assisted degradation of misfolded proteins requires multiple E3s

Søs Mathiassen[a], Ida Larsen[a], Christian Madsen[b], Michael Nielsen[b], Franziska Kriegenburg[a], Rasmus Hartmann-Petersen[a]

[a] *University of Copenhagen, Department of Biology, Denmark*
[b] *University of Copenhagen, NNF Center for Protein Research, Denmark*

Presented by: Mathiassen, Søs

Various stress conditions may cause partial denaturation of cell proteins. Such proteins are either shielded from aggregation and refolded to the native state by molecular chaperones, or they are targeted for degradation via the ubiquitin-proteasome system (UPS). Understanding the degradation pathways for misfolded proteins is important since faults in this system may lead to a build-up of toxic species which in turn may cause diseases, including neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. Analyzing the misfolded model protein, Sdj1, in fission yeast, we found that Sdj1 is rapidly degraded via the UPS. Proteomic analyses of Sdj1 interaction partners showed strong binding to Hsp70-type chaperones and ribosomal components. Accordingly, Sdj1 degradation requires Hsp70-type chaperones and the ribosome-associated E3 ubiquitin ligase Ltn1. Further analyzes also revealed involvement of BAG domain co-chaperones, the E2 conjugating enzyme Ubc4 and the E3 Ufd4. When perturbing elements of this pathway Sdj1 accumulates in large cytosolic aggregates. In conclusion, our data suggest that multiple ubiquitin-protein ligases are involved in clearing cells of misfolded proteins.

Characterization of polyamine synthase and transporters in fission yeast

Aslıhan Örs Gevrekci[a], Ismail Gungor[a]

Bilecik Seyh Edebali Universitesi, Bilecik, Turkey

Presented by: Örs Gevrekci, Aslıhan

Spermine and spermidine are the two types of polyamines, which are known to interact with the DNA and, when externally applied to cells, they lead to modifications in the transcriptome (Chattopadhyay et al., 2009). There is also evidence that polyamines are involved in the cell cycle control (Igarashi and Kashiwagi, 2010) and stress response (Gill and Tuteja, 2010). Polyamine level in the cells is controlled by a combined activity of polyamine synthases and polyamine transporters. So far only one spermine family transporter has been identified in S.pombe, which is caf5+. This gene was believed to be involved in the stress response of the cells upon caffeine treatment (Benko et al., 2004). Bioinformatic tools revealed 7 more genes in S. pombe that showed sequence similarity to spermine and spermidine family transporters (1 spermine and 6 spermidine family transporters) and 1 gene to spermidine synthase.
In our research we are trying to characterize these hypothetical polyamine transporters and synthases, and reveal their potential roles in the stress response and cell cycle progression. To this end, we created deletion mutants of these genes. The mutants were tested for growth rate, cell size, sporulation efficiency as well as sensitivity to a variety of DNA damaging agents and osmotic stress. The mutants showed different phenotypes such as delayed cell division and sensitivity to DNA damaging agents as well as environmental stress. Our analysis will be the first experimental characterization of these genes in S. pombe.

Regulation of zearalenone-induced oxidative stress process in the fission yeast *Schizosaccharomyces pombe*

Mike Nora[a], Papp Gabor[a], Gazdag Zoltan[a], Mate Gabor[a], Turmer Katalin[b],Czibulya Zsuzsanna[c], Kunsagi-Mate Sandor[c], Pesti Miklos[a], Vagvolgyi Csaba[d], Certik Milan[e]

[a] *Department of General and Environmental Microbiology, Faculty of Sciences, University of Pécs, Pécs, Hungary*
[b] *Department of Biophysics, Faculty of Medicine, University of Pécs, Pécs, Hungary*
[c] *Dept of General and Physical Chemistry, Faculty of Sciences, Univ. of Pécs, Hungary*
[d] *Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*
[e] *Department of Biochemical Technology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia*

Presented by: Miklos, Pesti

Zearalenone (ZEA) is one of the most widely disseminated oestrogenic mycotoxins found in agricultural products. In this study the ZEA-induced accumulation of reactive oxygen species (ROS) and the regulation of the specific activities of antioxidant enzymes were investigated in the single cell eukaryotic organism Schizosaccharomyces pombe. In comparison with the untreated cells, 500 µM ZEA treatment caused 66% decrease in the concentration of the glutathione (GSH); this depletion of GSH initiated a 1.8- and 2.0-fold accumulation of superoxide anion and hydrogen peroxide, respectively, but did not increase the concentration of hydroxyl radicals; ROS-induced adaptation processes at cell and molecular level via the activation of Pap1 transcription factor resulted in significantly increased specific activities of superoxide dismutases, catalase, glutathione reductase and glutathione S-transferase, as well as decreased activity of glutathione peroxidase, glucose-6-phosphate dehydrogenase and Mn/superoxide dismutase. This treatment altered substancially the sterol composition of cell by inducing decreased concentration of ergosterol, squalene and 24-methylene-24,25-hydrolanosterol, as well as elevated the number of fragmented nuclei. In contrasz to GSH patulin interaction no detectable molecular interference between ZEA and GSH was observed. The adverse non-oestrogenic effects of ZEA may be exerted mainly through the accumulation of ROS and quantitative modification of plasma membrane composition.

Chk1 controls homologous recombination at dysfunctional telomere in *S. pombe*

Ahmed Habib, Kenta Masuda, Masaru Ueno

Graduate School of Advanced Sciences of Matter, Molecular Biotechnology Department, Hiroshima University, Japan

Presented by: Habib, Ahmed

Chk1 is an effector kinase of the G2 DNA damage checkpoint. Previous studies reveal several substrates of Chk1. S. pombe Chk1 phosphorylates Wee1 and Cdc25 and arrest the cell cycle at G2/M transition. Identification of additional targets is important to understand the novel role of Chk1. S. pombe RecQ helicase Rqh1 is involved in the several steps in homologous recombination (HR). Pot1 binds to single-stranded telomere overhang. Deletion of pot1 causes rapid telomere loss and chromosome circularization, which is mediated by single-strand annealing (1). We have reported that the double mutant between pot1 and rqh1-hd (helicase dead) maintains chromosome ends by HR, but is sensitive to microtubule drug thiabendazole (TBZ) (2). Recombination intermediates are accumulated near telomeres in the pot1 rqh1-hd double mutant even in M phase, which could disturb chromosome segregation (2). To understand the molecular details of the sensitivities to TBZ of the pot1 rqh1-hd double mutant, we screened mutant that suppresses the TBZ sensitivities of the double mutant.

Cell length of pot1 rqh1-hd double mutant is elongated in chk1 dependent manner, suggesting that DNA damage checkpoint is activated. Interestingly, we found that the TBZ sensitivities of the double mutant can be suppressed by deletion of chk1 or mutation of kinase domain of Chk1. We also found that deletion of chk1 or mutation of kinase domain of Chk1 suppresses the accumulation of recombination intermediates at chromosome ends in the double mutant. These results suggest that Chk1 controls homologous recombination activity by phosphorylation of unknown target.

Based on these previous data, we are now designing a new model for screening of novel Chk1 inhibitors as an anti-cancer therapy.

(1) Wang X, Baumann P. Mol. Cell. 2008. 463-73.
(2) Takahashi K. et. al. Mol. Cell. Biol. 2011. 495-506.

The effects of *Astragalus chrysochlorus* crude extract on cancer model of *Schizosaccharomyces pombe* cells

Semian Karaer Uzuner[a], Cagatay Tarhan[a], Sule Ari[a,b]

[a] *Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Turkey*

[ab] *Research and Application Center for Biotechnology and Genetic Engineering, 34134 Vezneciler, Istanbul, Turkey*

Presented by: Ari, Sule

We used a cancer model of Schizosaccharomyces pombe to investigate the inhibitor effect of Astragalus chrysochlorus extracts. Due to the rich content of saponines, flavonoids, polysaccharides and biological activities related to their secondary metabolites, Astragalus roots from various species have been used in the traditional Chinese medicine for the treatment of nephritis, diabetes, leukemia, and cancer. We showed before that the extracts inhibited the proliferation of HeLa cells. Thus we tested the extracts on cancer model of S.pombe. While the growth of the parental strain was stimulated in the presence of crude extracts, the growth of cancer model was inhibited. To investigate whether the growth retardation of cancer model was DNA damage dependent, we examined the expression level of the rad17 gene which encodes a RFC related checkpoint protein and found no significant increase. Therefore, presently we are studying on other cellular parameters which can shed more light on growth inhibition mechanism.

The Ndc80 internal loop interacts with Dis1/TOG and Alp7/TACC-Alp14/TOG to ensure proper kinetochore-spindle attachment

Ngang Heok Tang, Kuo-Shun Hsu, Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London WC2A 3LY, UK

Presented by: Tang, Ngang Heok

The kinetochore-spindle microtubule attachment lies at the heart of proper chromosome segregation. The Ndc80/Hec1 complex (Ndc80/Hec1, Nuf2, Spc24 and Spc25), which is found in the outer kinetochore, has been shown to directly bind to microtubules through the unstructured N-terminal tail and following calponin-homology (CH) domain situated in Ndc80. Hence the Ndc80 complex is deemed to be a major player in microtubule-kinetochore attachment. However, the Ndc80 complex binds only the microtubule lattice, not their plus ends, which are critical for microtubule dynamics and stable association with the kinetochore. Therefore, our understanding of the mechanisms by which this complex establishes proper bipolar attachment remains largely elusive.

Here, by using two novel ndc80 mutants (ndc80-21 and ndc80-NH12), we show that the Ndc80 internal loop plays critical roles in establishing stable bipolar attachment to microtubules. We show that this loop binds the microtubule plus-end tracking proteins Dis1/TOG and Alp7/TACC-Alp14/TOG. In the ndc80-21 mutant, the Dis1 protein is delocalised from the kinetochores and causes frequent spindle collapse. Mitotic progression is halted in the middle of early mitosis due to the activation of the spindle assembly checkpoint. Intriguingly, in the ndc80-NH12 mutant, which contains a different mutation in the same internal loop region, Dis1 localises normally to the kinetochores and overall mitotic spindle morphology appears not defective. Nonetheless, in this mutant, an enhanced chromosome mis-segregation is observed later during anaphase A. We have found that this is largely due to mis-localisation of the Alp7-Alp14 complex from the kinetochores. Our work, therefore, establishes that besides the N-terminal domains of Ndc80 that directly bind to the microtubule, the third motif, the internal loop indirectly interacts with the plus end of microtubules through Dis1 and Alp7-Alp14 and ensures proper kinetochore-spindle attachment.

The regulatory mechanism of phospholipid balance by lipid binding protein

Cuifang Li[a], Ayako Kita[b], Yuuka Hashimoto[b], Misako Ihara[b], Ayaka Kato[b], Naoya Ogura[b], Reiko Sugiura[b]

[a] *1) Laboratory of Molecular Pharmacogenomics, School of Pharmaceutical Sciences, Kinki University, Higashi-Osaka, Japan*
2) Research Fellow of the Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo, Japan
[b] *Laboratory of Molecular Pharmacogenomics, School of Pharmaceutical Sciences, Kinki University, Higashi-Osaka, Japan*

Presented by: Li, Cuifang

Phosphatidylinositol-4,5-bisphosphate (PI4,5P2) is important for a variety of cellular processes as a precursor for second messengers and by regulating the cytoskeleton, membrane trafficking events, and signal transduction in eukaryotic cells.

We have previously screened for mutations that display sensitivity to the calcineurin inhibitor FK506 and high temperature, and isolated the its3+ gene that encodes a phosphatidylinositol-4-phosphate 5-kinase (PI4P5K) in fission yeast. The its3-1 mutant cells exhibited extremely low levels of PI4,5P2, and displayed cytokinesis defects. (J. Biol. Chem., Zhang et al., 2000, J. Biol. Chem., Lu et al., 2005).

We also found that overexpression of Its3 causes cell death. In order to gain insights into the regulatory mechanisms of the PI4,5P2 signaling, we screened for genes that can suppress the PI4,5P2-induced lethality. As a result, we identified a novel gene slp2+, encoding a phospholipid-binding protein which contains two pleckstrin homology (PH) domains. We then proved that the N-terminal PH domain was sufficient to suppress the cell death induced by Its3 overproduction (OP). In addition, we analyzed the phospholipid levels in Its3 OP cells and found that the balance between PI4P and PI4,5P2 was broken. However, when Slp2 was overexpressed in Its3 OP cells, PI4P and PI4,5P2 displayed a normal balance as that in wild-type cells, indicating that Slp2 possibly functions as an important regulator for the balance between PI4P and PI4,5P2. Here, we discuss the regulatory mechanism of phospholipid balance by lipid binding protein.

The dual roles of the tubulin folding pathway components in microtubule formation and homeostasis

Risa Mori, Takashi Toda

Cancer Research UK London Research Institute, UK

Presented by: Mori, Risa

Supplying a proper amount of correctly folded α/β-tubulin heterodimers is critical to ensure proper microtubule assembly and dynamics. Formation of assembly-competent heterodimers is remarkably elaborate at the molecular level, in which the α- and β-tubulins are separately processed in a chaperone-dependent manner. This sequential step is performed by the evolutionarily conserved tubulin folding cofactor pathway, comprising of a specific set of regulatory proteins - cofactors A to E. We have found that, as well as its canonical roles in tubulin folding, Tbc1/cofactor C has another role as a GAP in regulating a highly conserved small GTPase, Alp41/Arl2. Interestingly, the expression of either GDP- or GTP- bound Alp41 resulted in microtubule destabilisation and eventual loss, suggesting that the continuous cycling between these forms is important for the maintenance of microtubules. Alp41 interacts with Alp1/cofactor D, specifically when in the GDP bound form. Intriguingly, Alp1, but not any of the other components, co-localises with microtubules when in excess, subsequently leading to depolymerisation and cell lethality. In addition, remarkably the GDP-bound form of Alp41, but not the GTP-bound form, was capable of sequestering Alp1 from microtubules. We present a model of the final stages of the tubulin cofactor pathway, where we propose a dual role for both Tbc1 and Alp1 in opposing regulations of the microtubule.

A role for AMPKα in the response to nitrogen stress

Elizabeth Davie, Janni Petersen

University of Manchester, Michael Smith building, Faculty of Life Sciences, Oxford Road, Manchester M13 9PT, UK

Presented by: Davie, Elizabeth

Cell growth and cell cycle progression are tightly co-ordinated, allowing cells to adjust their size to the demands of proliferation in varying nutritional environments. This co-ordination is regulated by Target of Rapamycin (TOR) signalling. In fission yeast, a shift from rich to poor nitrogen environment – nitrogen stress – results in TORC1 down-regulation and advanced mitotic onset at a reduced cell size. It is not yet fully understood how cells sense this environmental stress.

Here, we show that subjecting cells to a nitrogen-specific stress reduces cellular ATP levels. Consistently, we demonstrate that the AMP-activated protein kinase (AMPK) alpha subunit, Ssp2, is required for cells to respond to nitrogen stress. Our data suggests that activated Ssp2 can signal to down-regulate TORC1 to control cell size at division.

Sid2 phospho-regulation of the anillin-related scaffolding protein Mid1 in fission yeast cytokinesis

Ashley K. DeWitt[a], Jennifer Phelan[a], Brandon Nader[a], Kathleen L. Gould[b], Dawn M. Clifford Hart[a]

[a] *Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI, USA*
[b] *Howard Hughes Medical Institute, Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA*

Presented by: DeWitt, Ashley

The anillin-related protein Mid1 plays a critical role in organizing the early steps of contractile ring formation and functions as a scaffold to bridge the cell cortex with the contractile ring. Cells lacking mid1 form off-centered, highly disorganized ring structures and exhibit severe cytokinesis defects. Coincident with its cortical accumulation, Mid1 becomes hyper-phosphorylated. Mid1 contains several RXXS motifs, which fit the phosphorylation consensus sequence for Sid2 kinase. Sid2 is the most downstream kinase in the Septation Initiation Network signaling cascade, which signals from the spindle pole body to trigger constriction of the contractile ring. To identify specific sites of phosphorylation, a combination of site-directed mutagenesis (serine to non-phosphorylatable alanine) with mass spectroscopy and two-dimensional phosphopeptide mapping were used. Both methods identified multiple Sid2 phosphorylation sites within Mid1. To analyze the significance of Sid2 phosphorylation in vivo, phospho-site mutants were generated at the endogenous mid1 locus and examined for cell division defects. As expected, Mid1-GFP is detected in the contractile ring during pro-metaphase followed by nuclear localization when the majority of cells display a visible septum. In contrast, Mid1-GFP in phospho-site mutants is detected in the contractile ring during pro-metaphase but is not visible in the nucleus following septation. Instead, Mid1 appears to localize as nodes, suggesting Mid1 might remain associated with the membrane in the absence of Sid2 phosphorylation. Given that Mid1 departure from the contractile ring coincides with Sid2 relocalization to the division site, Sid2 may temporally regulate the interaction of Mid1 with the membrane or other contractile ring components.

Environmental regulation of the Pom1 dependent cell-size homeostasis system in fission yeast

Manasi Kelkar, Sophie Martin

Department of Fundamental Microbiology, University of Lausanne, Switzerland

Presented by: Kelkar, Manasi

A fundamental challenge for a cell is to achieve a balanced growth in face of a fluctuating environment. Cell size homeostasis mechanisms serve to buffer these variations by coupling cell growth to cell division. In fission yeast, Schizosaccharomyces pombe, nutrient limitation causes cells to divide at a shorter cell size. Recent work has shown that these cells possess a geometric cell-size sensing mechanism, wherein a kinase Pom1 serves to couple cell length with mitotic entry. Pom1 is localized to the cell ends through microtubule-deposited Tea1-Tea4 complex, from where it forms plasma membrane associated concentration gradients towards the cell middle. There, it negatively regulates its substrate, a mitotic inducer Cdr2. In short cells, Pom1 gradients overlap with Cdr2 and inhibit it, preventing precocious mitosis, whereas in longer cells this overlap reduces, Cdr2 gets activated, and cells divide. Here we find that upon glucose depletion, Pom1 dramatically delocalizes from the cell ends and spreads around the entire cell cortex. This phenomenon is regulated by the glucose sensing cAMP-PKA pathway, as Pom1 does not spread in pka1Δ cells. Conversely, overexpression of Pka1 leads to partial spreading of Pom1, even under glucose rich conditions. We show that Pka1 alters microtubule stability, potentially via regulation of the conserved kinesin-8 family proteins: Klp5/6 even prior to the onset of glucose starvation. This regulation primes microtubules for de-polymerization and Tea4 spreading to the cell sides thereby mediating Pom1 delocalization under low glucose. Wild-type cells divide at a shorter cell length in low glucose, dependent on MAPK-signaling, as MAPK mutants become longer, rather than shorter in low glucose. By contrast, pom1Δ cells hyper-adapt and become excessively short. Pom1 delocalization to inhibit Cdr2, may thus represent a buffering mechanism to reset the balance for CDK1 activation and antagonize excessive cell shortening.

Involvement of the multi-pass membrane protein Prm1p in cell fusion and cell integrity during mating

María Ángeles Curto[a], Mohammad Reza Sharifmoghadam[b], Eduardo Calpena[a], Nagore De León[a], Marta Hoya[a], Janet Leatherwood[c], Henar Valdivieso[a]

[a] *Departamento de Microbiología y Genética/IBFG, Universidad de Salamanca/CSIC, Salamanca, Spain*
[b] *Department of Molecular Genetics and Microbiology, Stony Brook University, New York, USA*
Faculty of Veterinary Medicine, University of Zabol, Iran
[c] *Department of Molecular Genetics and Microbiology, Stony Brook University, New York, USA*

Presented by: Curto, María Ángeles

Cell fusion requires the reorganization of cellular envelopes, which is a challenging process that can produce cell lysis. The involvement of Schizosaccharomyces pombe Prm1p in cell fusion and its relationship with other fusion-promoting proteins has been addressed. prm1Δ cells fail to fuse due to an abnormal organization of plasma membrane and cell wall at the area of cell-cell contact. Although this phenotype is similar to that described for cells devoid of the claudin-like Dni proteins, prm1+ and the dni+ genes act in different pathways. A significant number of prm1Δ, dni2Δ, and fus1Δ zygotes lyse during mating. For prm1Δ and fus1Δ, lysis is maximal in the presence of high calcium concentrations at low temperature. In prm1Δ, zygote lysis does not correlate with an abnormal actin distribution at the shmoo tip, and Prm1p is normally localized in fus1Δ zygotes, suggesting that prm1+ and fus1+ act in different pathways. Reducing calcium concentration or deleting dni2+ does not enhance zygote lysis in prm1Δ, showing that the roles of budding and fission yeast Prm1 proteins are not equivalent. Our results show that multiple functional pathways and environmental conditions are required to ensure cell fusion and cell integrity during mating, and that the role of each of those cues varies with the biology of different organisms.

The Cfr1/Csr1p complex regulate cell wall synthesis in *Schizosaccharomyces pom*be

Marta Hoya[a], Sandra Moro[a], Mohammad Reza Sharifmoghadam[b], Nagore de León[a], M.-Ángeles Curto[a], Cristina Doncel[a], M.-Henar Valdivieso[a]

[a] *Departamento de Microbiología y Genética/IBFG, USAL/CSIC, Salamanca, Spain*
[b] *Faculty of Veterinary Medicine, University of Zabol, Iran*

Presented by: Hoya, Marta

One of the most attractive areas of Biology is the knowledge of how organisms generate and maintain their shape. In fungi, the cell wall is an essential morphogenetic element, since it is the last organelle responsible for the maintenance of the cellular shape. Our objective is to study how the biosynthesis of β-glucan, an essential component of the cell wall, is regulated by the mechanisms of vesicular transport. In this work we have addressed the study of the regulation of the synthesis of this polymer by the Cfr1/Csr1p complex. Cfr1p and Csr1p share similarity with the exomer proteins from S. cerevisiae. The term exomer refers to a set of proteins that form a vesicle coat required for the transport of certain proteins from the trans-Golgi network to the plasma membrane. The exomer is formed by a scaffold protein, Chs5p, to which Chs6p and its homologues (Bch1p, Bud7p and Bch2p, termed generically "ChAPs") anchor. The ChAPs are responsible for cargo specificity.

S. pombe Cfr1p shares similarity with Chs5p and Csr1p is the only protein similar to the ChAPs. Previous studies have shown that Cfr1p localizes at the Golgi and is required for cell fusion during mating, a function that shares with Chs5p. We have cloned csr1+ and have found that Cfr1p and Csr1p form a complex at the Golgi and that the localization of each protein in this organelle depends on each other. Additionally, cfr1Δ and csr1Δ mutants are sensitive to compounds that interfere with cell wall synthesis, interact genetically with mutants in the β(1,3)glucan synthase, and have a reduced level of β-glucan in their cell wall. Our goal is to study the role that these proteins might play in vesicular transport, and to determine how they regulate cell wall synthesis and morphogenesis.

PBF complex regulates directly Ace2p-target genes in *Schizosaccharomyces pombe*

M. Belén Suárez[a], María Luisa Alonso-Núñez[a], Javier Encinar del Dedo[a], Christopher J McInerny[b], Francisco Del Rey[a], Carlos R Vázquez de Aldana[a]

[a] *Instituto de Biología Funcional y Genómica (IBFG), CSIC/Univ. of Salamanca, Spain*
[b] *Division of Biochemistry and Molecular Biology, Faculty of Biomedical an Life Sciences, University of Glasgow, UK*

Presented by: Suárez, Belén

Yeast division cycle is completed with the activation of a cell separation program that involves dissolution of the septum assemblied during cytokinesis between the two daughter cells, allowing them to become independent entities. Gene expression of hydrolytic enzymes (eng1+ and agn1+) responsible for septum dissolution is periodically activated at the end of the cell cycle by the specific transcription factor Ace2p (Alonso-Núñez et al., 2005). ace2+ expression is, in turn, periodically activated during mitosis by the transcriptional complex PBF ("PCB Binding Factor"). This complex consists of at least two forkhead-like proteins Sep1p, Fkh2p and a MADS box-like protein Mbx1p, although more recently it has been shown that other proteins participating in different events at the end of the cell cycle, as the kinase Plo1p, the phosphatase Clp1p and the anillin-like protein Mid1p, are also components of PBF (McInerny, JC 2011).
In our laboratory, we have found that Ace2p-dependent genes show in their upstream promoter region sites for Ace2p binding but also PCB sites ("Pombe Cell-Cycle Box") for PBF complex binding. Chromatin immunoprecipitation analyses (ChIP-qPCR) showed that at least Fkh2p and Sep1 bind in vivo to the eng1+ promoter. ChIP analyses through the cell cycle also revealed that Ace2p binding was coincident with maximum level of eng1+ mRNA, while Fkh2p was found to bind later, when mRNA level of this gene is low. Additionally, RT-qPCR assays in ace2Δ, fkh2Δ, sep1Δ, and mbx1Δ mutants show that expression of the genes under the control of Ace2p is affected in a different way by PBF components, indicating that transcriptional regulation is more complex than initially expected.

Alonso-Nuñez ML, et al. 2005. Mol Biol Cell.16: 2003-17.
McInerny, JC. 2011. Cell Cycle 10: 1184-1185.

Msd2, a novel SPB protein required for mitotic spindle anchoring to the spindle pole

Masashi Yukawa, Chiho Ikebe, Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3LY, UK

Presented by: Yukawa, Masashi

In most cells, the microtubule organizing center (MTOC) nucleates microtubules and orchestrates microtubule assembly. The minus ends of nucleated microtubules are anchored to the MTOC, thereby ensuring accurate chromosome segregation. In contrast to our knowledge of microtubule assembly, little is known about the molecules that anchor spindle microtubules to the MTOC during mitosis. In fission yeast, we have previously reported that Msd1 (mitotic-spindle disanchored) acts as a mitotic-spindle anchoring protein through an interaction with the γ-tubulin complex (γ-TuC) protein, Alp4/GCP2. In this study, we show that a novel protein, Msd2 also anchors mitotic spindle microtubules to the SPB. The msd2 deletion mutant cells showed the same phenotype as the msd1 mutant (i.e. the protruding spindle and the mini-chromosome loss) and the msd1msd2 double mutant did not show additive defects. In addition, Msd1 and Msd2 localised to mitotic SPBs in an interdependent manner. A model for the molecular functions of these two proteins in spindle anchoring will be discussed.

A genetic screen to identify components of the nutrient sensing pathway

David Cobley, Janni Petersen

University of Manchester, Faculty of Life Sciences, Michael Smith Building C.4223, Oxford Road, Manchester M13 9PT, UK

Presented by: Cobley, David

Cell division is a highly regulated process which is dependent upon a number of criteria including the availability of nutrients. The ability of eukaryotic cells to couple the two important processes of growth and division is key to maintaining a regular cell size. The target of rapamycin (TOR) pathway is a key player in nutrient sensing but knowledge of how cells respond to nutrients remains limited. Here we describe a genetic screen which was employed to elucidate the mechanisms involved in nutrient sensing upstream of TOR. When cells are grown in good nitrogen source (glutamate) and transferred into a poor nitrogen source (proline), there is a reduction in TOR signaling which results in an advanced commitment to mitosis and thus a reduced cell length at division. As a result, cell size can be used as an indirect readout of TOR signaling. This knowledge was exploited and used to screen for mutants that were unable to advance mitosis. We have isolated and characterized a mutant which is defective in its ability to sense nutrients and reduce cell size. In addition to this phenotype, this mutant also harbours a vacuolar size defect. As TOR complexes localize to the vacuole it is possible that this phenotype is involved in the inability to sense nutrients. Our findings agree with the current literature which demonstrates that TORC1 (the main nutrient sensing complex) localizes to vacuoles (lysosomes in mammalian cells), implicating vacuoles in the nutrient sensing response.

A novel, non-gatekeeper, mutations enhances protein kinase sensitivity to inhibition by ATP analogues and generates an approach for mitotic studies in fission yeast.

Ye Dee Tay, Avinash Patel, Iain Hagan

Paterson Institute for Cancer research, University of Manchester, UK

Presented by: Tay, Ye Dee

The chemical genetic strategy in which mutational enlargement of the ATP binding site sensitizes of a protein kinase to bulky ATP analogues has proven to be an elegant tool for the generation of conditional, analogue-sensitive (as), kinase alleles in a variety of model organisms. plo1.as kinase alleles that were generated using canonical gatekeeper mutations did not display the high sensitivity to ATP analogues that is exhibited upon identical mutation of human PLK1. The crystal structure of human PLK1 bound to AMP-PNP revealed a potential contribution of a bulky phenylalanine residue (F183) at the bottom of the ATP binding pocket of PLK1 kinase towards the affinity of the ATP analogue. Plo1 has a methionine residue (M170) in the corresponding position. We therefore introduced the M170F mutation in plo1.as3 and found that Plo1.as8 was highly sensitive to ATP analogue. S. pombe Wee1 and Orb5 kinases also harbor a methionine residue at the equivalent position. Wee1.as8 and Orb5.as8 strains displayed sensitivity to 3BrB-PP1 that was markedly enhanced over their respective as1 counterpart. Kinome alignment showed that between 5-9% of kinases in key model organisms have methionine residues in the F183-equivalent of human PLK1. We therefore propose that this structural modification may have broad application for enhancing the sensitivity of as alleles that are not readily inhibited by ATP analogue. Wee1.as8 inhibition by 3BrB-PP1 suppressed cdc25.22 lethality at 36°C. In liquid culture wee1.as8 cdc25.22 cells arrested efficiently at the G2/M boundary after 4.25hrs at 36°C. Subsequent analogue inhibition of Wee1.as8 prompted a highly synchronous mitosis. Because cells remain at 36°C throughout this synthetic rescue, the wee1.as8 cdc25.22 strain offers a powerful approach with which to study the consequences of temperature dependent inactivation of mitotic or DNA replication molecules in a highly synchronous mitosis/replication.

S. pombe Mzt1/MOZART1 is an essential component of the γ-tubulin complex.

Hirohisa Masuda, Risa Mori, Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, 44Lincoln's Inn Fields, London WC2A 3LY, UK

Presented by: Masuda, Hirohisa

Mozart1 is a novel component of the γ-tubulin complex (γ-TuC), which was identified by a genome-wide screening of protein complexes in human cells (Hutchins et al., Science 2010, 328, 593-599). RNAi experiments imply that Mozart1 functions in assembly of a bipolar spindle and recruitment of the γ-TuC to the centrosome, but its precise roles remain to be established. Orthologs of Mozart1 are found in the genomes of a wide range of eukaryotes including Arabidopsis, Drosophila, and Schizosaccharomyces, suggesting that the function of this protein is conserved through evolution. We show here that the S. pombe MOZART1 ortholog (Mzt1) is essential for growth, and interacts with the γ-TuC. Mzt1 colocalises with the γ-TuC to all the MTOCs, including the spindle pole body (SPB) throughout the cell cycle, to the eMTOC, a site for cytoplasmic microtubule formation upon mitotic exit, and to the iMTOC, a site for cytoplasmic microtubule assembly during interphase. We propose that Mzt1 is an essential component of the γ-TuC, which is required for formation of interphase cytoplasmic arrays of microtubules and for mitotic spindle assembly and functions.

The clathrin light chain is essential for cell wall synthesis and viability in *Schizosaccharomyces pombe*

Nagore De León[a], Mohammad Reza Sharifmoghadam[b], Marta Hoya[a], M.-Ángeles Curto[a], Cristina Doncel[a], M.-Henar Valdivieso[a]

[a] *Dept. Microbiología y Genética/Instituto de Biología Funcional y Genética (USAL/CSIC), Spain*
[b] *Faculty of Veterinary Medicine, University of Zabol, Iran*

Presented by: De León, Nagore

The regulation of cell wall synthesis by the clathrin light chain has been addressed. Schizosaccharomyces pombe clc1Δ mutant was inviable in the absence of osmotic stabilization, while in other organisms as Saccharomyces cerevisiae and Dictyostelium discoideum clc1Δ mutants are viable in all the conditions tested. Why this difference is? When grown in sorbitol-supplemented medium clc1Δ cells grew slowly, formed aggregates, and had strong defects in morphology. Additionally, clc1Δ cells exhibited an altered cell wall composition. A mutant that allowed modulating the amount of Clc1p was created to analyze in more detail the dependence of cell wall synthesis on clathrin. A 40% reduction in the amount of Clc1p did not affect acid phosphatase secretion and bulk lipid internalization. Under these conditions, β(1,3)glucan synthase activity and cell wall synthesis were reduced. Also, the delivery of glucan synthases to the cell surface, and the secretion of the Eng1p glucanase were defective. These results suggest that the defects in the cell wall observed in the conditional mutant were due to a defective secretion of enzymes involved in the synthesis/remodeling of this structure, rather than to their endocytosis. Our results show that a reduction in the amount of clathrin that has minor effects on general vesicle trafficking has a strong impact on cell wall synthesis and perhaps, this is one of the main reasons why clc1 gen deletion is lethal in S. pombe.

New calmodulin functions in *Schizosaccharomyces pombe*

Tula del Carmen Yance Chavez, David Gimenez Zaragoza, Rosa Aligué

Departament de Biologia Cellular, Immunologia i Neurociències, Universitat de Barcelona, Institut d'investigacions Biomèdiques August Pi i Sunyer, C/Casanova 143, 08036 Barcelona, Catalunya, Spain

Presented by: Yance Chavez, Tula del Carmen

Calmodulin is a small, highly conserved and ubiquitous protein, of 184 aminoacids and 16 kDa weight. Calmodulin (CaM) is essential for proliferation in Schizosaccharomyces pombe (Takeda et al. 1984). CaM has two globular domains connected by a helix alpha; each globular domain has two binding sites for Ca2+ this structure is denominated "helix-loop-helix" or "EF Hand " , therefore it has four binding sites for Ca2+(Babu et al. 1985). The Ca2+/CaM complex binds in a reversible way and induce a conformational change on CaM promoting its interaction with a large number of proteins. The ability of CaM to bind Ca2+ and the interaction with its targets defines CaM as important regulator in Ca2+signal transduction.

Calmodulin is implicated in different cellular processes in fission yeast among them, cell proliferation (Rasmussen et al, 1990), membrane traffic and cytokinesis (Moser et al. 1997; Sugiura, et al. 2002), sporulation (Itadani et al, 2010) and signal transduction (Flory et al. 2002).

In order to have further knowledge of CaM functions during cell proliferation, we have looked for new calmodulin-binding proteins through affinity chromatography and S. pombe cells extracts. Eluted proteins were analyzed by mass spectrometry, and the result of the CaM-BP will be presented.

Role of Cmk1 kinase in calcium signaling

Maria Eugenia Cisneros-Barroso, Alba Gómez-Hierro, Rosa Aligué

Departament de Biologia Cellular, Immunologia I Neurociències, Universitat de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer, C/ Casanova 143, 08036 Barcelona, Catalunya, Spain

Presented by: Cisneros-Barroso, Maria Eugenia

Cmk1 was identified in Schizosaccharomyces pombe as a member of the CaM-dependent protein kinase cascade (Rasmussen, 2000). The cmk1 gene encodes a 335-amino acid protein with significant homology to mammalian CaMK-I, including a conserved sequence for phosphorylation by CaM kinase kinase (CaMKK). Cmk1 activity is dependent on calcium and calmodulin and it increases when threonine 192 is mutated to aspartic acid (Cmk1-T192D) indicating that it can be activated by a CaMKK as in mammalian cells (Rasmussen, 2000). It was also described that the overexpression of the Cmk1T192D causes cell cycle arrest (Rasmussen, 2000).

We were focused in unraveling the function of Cmk1 by studying the growth of the viable Δcmk1 under various stress conditions. Unexpectedly, cmk1 deletion showed a high tolerance to chronic exposure of calcium. Cmk1 mRNA and protein levels are increased in response to calcium. We have also found that Cmk1 was phosphorylated after calcium treatment. These results confirmed a role of Cmk1 in calcium signaling.

The first to explain the increase of calcium tolerance of cells lacking cmk1 was to analyze the calcium intake. Therefore we measured intracellular free calcium levels using Cameleon as a Ca2+ sensor. Although the level of calcium was modified in Δcmk1 cells, calcium intake was not impaired. Currently we are analyzing the activation of Pmk1 and Prz1 pathways (activated by Ca2+) in Δcmk1 cells.

The fission yeast Rho GAP, Rga7, ensures proper cytokinesis independently of its GAP-catalytic activity

Rebeca Martin, Pedro M Coll, Nuria Pujol, Pilar Pérez

Instituto de Biología Funcional y Genómica, CSIC/ Universidad de Salamanca, Salamanca, Spain

Presented by: Martin, Rebeca

Rho GTPases are involved in the regulation of critical biological responses in eukaryotic cells such as cellular morphogenesis and cytokinesis. Rho GTPases cycle between an active and inactive state and are negatively regulated by GAPs (GTPase activating proteins) that increase the hydrolysis of GTP. Rga7 is one of the nine GAPs present in fission yeast. We have found that Rga7 is a GAP for Rho2-GTPase which acts upstream of Pmk1, a MAPK involved in the regulation of the cell integrity and cell separation. Besides its GAP domain, Rga7 has an F-BAR domain at the N-terminal region. F-BAR domain proteins act as linker between the cell cortex and the cytoskeleton and are involved in membrane binding and tubulation. Indeed, Rga7 localizes to the membrane of the cell division site and the cell tips in an F-BAR domain- dependent manner.

Mutant cells lacking Rga7 are wider than wild type and have cytokinesis defects with higher percentage of cells having one or more septa in the population, asymmetric or aberrant septa and a percentage (13%) of dead cells. Fission yeast cytokinesis requires coordinated contraction of an actomyosin ring and synthesis of the septum. Our findings suggest that Rga7 function during cytokinesis is to ensure proper actomyosin ring tension and ring integrity during contraction as well as proper septum formation and cell separation. These Rga7 roles in cytokinesis and cell integrity seem to be independent of its GAP catalytic activity, while the F-BAR domain is necessary for these functions.

Rga6 is a Cdc42 GAP that collaborates with Rga4 in the maintenance of fission yeast shape

María Teresa Revilla, María Antonia Villar-Tajadura, Rebeca Martín-García, Beatriz Santos, Pilar Pérez

Instituto de Biología Funcional y Genómica, CSIC/ Universidad de Salamanca, Salamanca, Spain

Presented by: Revilla, María Teresa

Rho GTPases are negatively regulated by GTPase-activating proteins (GAPs) that play important roles in the specificity of Rho functions. Fission yeast Cdc42 is essential for the maintenance of the cell dimensions and cylindrical morphology and is negatively regulated by the GAP Rga4. This study establishes that the protein Rga6 is a Rho GAP that regulates Cdc42 and Rho2 and collaborates with Rga4 in the maintenance of fission yeast shape. Rga6 localizes to plasma membrane but is excluded from the growing areas. Cells lacking Rga6, as cells lacking Rga4, are viable but slightly broader and shorter than wild type cells. Overexpression of rga6+ also promotes a morphological phenotype very similar to that caused by Rga4 overexpression. Cells lacking simultaneously Rga4 and Rga6 are rounded and active Cdc42 is observed all around the membrane. These additive effect suggest that both GAPs collaborate in the negatively regulation of Cdc42 and the restriction of the fission yeast growth to the tip area.

Cdc42 regulates different steps of fission yeast mating

Miguel Estravís[a], Sergio A. Rincón[b], Maria Henar Valdivieso[a], Pilar Pérez[a]

[a] *Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas / Universidad de Salamanca, Spain*
[b] *Institut Curi, Paris, France*

Presented by: Estravís, Miguel

Fission yeast conjugation takes place in conditions of starvation and involves morphological changes that, in response to external signaling, lead to the formation of "shmoos", cell fusion, and formation of zygotes. Schizosaccharomyces pombe Cdc42 GTPase is a key regulator of cell morphogenesis during vegetative growth and also has functions during mating in the pheromone signal transduction and the establishment of the growth pattern that leads to the "shmoo" formation. Here we analyze the role of Cdc42 in cell fusion during mating using the cdc42-L160S thermosensitive mutant strain. While most cdc42 related mutant strains are sterile, cdc42-L160S cells can activate the pheromone pathway and mate at low efficiency. Nuclear accumulation of Ste11 transcription factor is low, cells have defects in polarity establishment, and mating-specific cell adhesion is impaired. Besides these defects, which may be related to an impaired pheromone signal transduction, cdc42-L160S mutant strain also shows cell fusion defects after the mating partners contact, as well as defects in the widening of the conjugation bridge once the cell fusion occurs. These late defects are not caused by mislocalization of the exocyst, Fus1 formin or Dni1, a tetraspan protein required for correct membrane organization and cell wall remodelling during mating. By contrast, the septin ring appears disorganized and that might cause the lack of expansion of the conjugation bridge.This suggests for the first time in fission yeast that septins are regulated by Cdc42 in a critical manner to allow proper accomplishment of the mating process.

Physiological analysis of photoresponse of *Schizosaccharomyces japonicus*

Sho Okamoto, Kanji Furuya, Shingo Nozaki, Keita Aoki, Hironori Niki

Microbial Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, 1111 Yata, Mishima, Shizuoka 411-8540, Japan

Presented by: Okamoto, Sho

Daily changing light affects organisms, not only in plants and animals but also in fungi and bacteria. Each organism has different ways to response to light. Well-known photoreceptors, phytochromes and cryptochromes are involved in photosynthesis and photoperiodism. In fungi, blue light affects development and metabolism through a transcription factor, white collar (WC) complex including flavin. There are two subunits of the WC complex, WC-1 and WC-2 which are found in *Neurospora crassa*. Orthologs of WC-1 and WC-2 are widely conserved in many filamentous fungi. However, these orthologs are not found in yeast except a few species including*Schizosaccharomyces japonicus*, which is dimorphic yeast. Dimorphic yeast cells can transit to hyphal cells according to environmental growth conditions. We found that hyphal cells of *Sz. japonicus* response to blue light. In response to blue light, the cell division cycles of hyphal cells are synchronously activated. We analyzed this photoresponse to various light cycles. As a result, 1: light for one minute is effective, 2: three hours of darkness is necessary for cycling the photoresponse, 3: activation of synchronous cell division starts 20 hours after the light stimulus, 4: after more than 14 hours of continuous light is applied, darkness can also stimulate the response. These results indicate that the photoresponse of *Sz. japonicus* is regulated temporally and each hyphal cell synchronously starts and ends cytokinesis. We show that circadian clock is not involved in this response. However, other type biological clock might be responsible for the photoresponse in order to carry out timely synchronization of cytokinesis in hyphal cells.

Role of Adf1 in the contractile ring formation and contraction in fission yeast

Ei-ichi Uyeda, Jun Kashiwazaki, Issei Mabuchi

Department of Life Science, Graduate School of Science, Gakushuin University, Japan

Presented by: Uyeda, Ei-ichi

Animal cells and fungal cells form the contractile ring at a division site during cytokinesis. The ring contracts to divide the cell into two by virtue of interaction between myosin and actin filaments. It has not well been known how the ring is formed. It has been known from analyses of cytokinesis mutant cells of fission yeast that a number of proteins in the ring in fission yeast cells and animal cells are common. Therefore, fission yeast cells have been used as good models to study mechanisms of cytokinesis. In this study we aimed to elucidate roles of the ADF (actin-depolymerizing factor)/cofilin Adf1 in formation and contraction of the ring. ADF is involved in severing actin filaments and also in depolymerisation of actin from the minus end of the filament in vitro. A temperature-sensitive mutant strain adf1-1 expresses mutated Adf1 and has been known to be unable to form the ring at a restrictive temperature 36˚C (Nakano and Mabuchi, MBoC 2006). We analysed behaviur of actin in live adf1-1 cells by expressing mRuby-tagged Lifeact (Riedl et al., Nat. Methods 2008). In mitotic adf1-1 cells actin patches and cables were clustered and the clusters moved from the ends to the mid region of the cell at 36˚C. However, the clusters were never reorganised into a ring structure at the mid region. When Adf1 was inactivated during formation of the ring, the ring disintegrated and the cell did not undergo cytokinesis. On the other hand, once the ring formation was completed, it contracted and cytokinesis was completed. The contraction speed of the ring in adf1-1 cells was significantly slower than that of wild-type cells. In summary, Adf1 is required in formation and maturation of the contractile ring, and also in its effective contraction.

Activation of a G1/S-checkpoint in fission yeast

Cathrine Arnason Bøe, Erik Boye, Beáta Grallert

Department of Cell Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, 0310 Oslo, Norway

Presented by: Bøe, Cathrine Arnason

Checkpoints are mechanisms that regulate progression of the cell cycle. The checkpoints can be activated by DNA damage or unfinished cell-cycle processes. Our group has previously identified a checkpoint activated after irradiation with ultraviolet light (UVC) in G1 cells. This G1/S checkpoint delays entry into S phase by inhibiting the formation of pre-replicative complexes (preRCs) on origins (Nilssen et al., PNAS 2003). The checkpoint is dependent on the stress-response kinase Gcn2 (Tvegård et al., Genes Dev 2007). However, both the upstream events leading to activation of Gcn2 and most of the downstream pathway leading to inhibition of preRC formation are unknown.

We have recently investigated what form of damage that leads to induction of the G1/S checkpoint (Bøe et al., PNAS 2012). UVC irradiation induces DNA damage, but can also damage other macromolecules. We hypothesized that some form of DNA damage is the most likely candidate to initiate the checkpoint response. Interfering with the repair of UVC-induced DNA damage should then affect the length of the cell-cycle delay. We therefore analysed cell-cycle progression after UVC irradiation of cells with different defects in the nucleotide excision repair (NER) and UV-damage endonuclease dependent excision repair (UVER), the two pathways responsible for repairing most of UVC-induced DNA damage. We find that the initial DNA damage does not elicit the checkpoint response, but certain repair intermediates do. The results and their implications will be discussed, with emphasis on what the checkpoint inducer might be and how Gcn2 is activated. A preliminary model for the G1/S checkpoint pathway will be presented.

Genetic analysis of mutants that are defective in hyphal development of *Schizosaccharomyces japonicus*

Nozaki Shingo[a], Furuya Kanji[b], Niki Hironori[a]

[a] *Microbial Genetics Laboratory, Genetic Strains Research Centre, National Institute of Genetics, Japan*
[b] *Division of Mutagenesis, Radiation Biology Centre, Kyoto University, Japan*

Presented by: Shingo, Nozaki

Many species of yeast change their growth mode from uni-cellular yeast to long filamentous hyphae, in response to environmental stress. Hyphal cells show invasive growth, which is closely related to pathogenicity for human and plants. In fact, infection of pathogenic dimorphic yeast, *Candida albicans* causes pathogenesis only when *C. albicans* grows as hyphal cells. Thus, understanding the mechanism of transition from yeast to hyphae is important for development of medicine. However, the detail of the molecular mechanism of it is still unclear.

Schizosaccharomyces japonicus is non-pathogenic dimorphic yeast, and conventional methods of yeast genetics and molecular biology can be applied for analysis of this yeast. We have investigated hyphal development of *Sz. japonicus*, and we revealed that DNA damage stress induces transition from yeast to true hyphae (Furuya and Niki, 2010). Transition to hyphal growth by DNA damage stress requires activation of Chk1. Because deletion mutant of*chk1* does not affect the hyphal transition upon nutrient stress, it seems that there are two independent pathways to induce hyphal development. In this study, we tried to isolate and characterize mutants that were defective in both nutrient- and DNA damage-dependent pathways. First, we screened 372 mutant strains that cannot transit to hyphal growth by DNA damage stress. Among them, 176 mutants were not able to transit to hyphal growth also by nutrient stress. Most of these mutants were sterile so that genetic analysis by mating was impossible. Other 28 mutants were able to mate and further investigated. Phenotypes of these mutants on the hyphal transition were divided into 2 categories: one group of mutants completely lost ability of the hyphal transition, the other group of mutants that showed delayed hyphal growth so that hyphal cells emerged after long incubation. By analyzing these mutants, mechanism of transition from yeast to hyphae will be discussed.

Mzt1/Tam4, a fission yeast MOZART1 homologue, is an essential component of the microtubule organizing centre

Deepsharan Kaur Dhani[a], Ben Goult[a], Daniel Rogerson[a], Danny A. Bitton[b], Crispin J. Miller[b], John Schwabe[a], Kayoko Tanaka[a]

[a] *University of Leicester, UK*
[b] *Paterson Institute of Cancer, UK*

Presented by: Dhani, Deepsharan Kaur

The recent systematic augmented annotation of the fission yeast genome (Bitton et al., Genetics, 187, 1207-1217 (2011)) has identified Tam4/Mzt1, the human homologue of which is referred to as MOZART1 that plays an essential role in spindle formation as a component of γ-Tubulin Ring Complex (γ-TuRC) (Hutchins et al., Science 328, 593-599, (2010)). Intriguingly, although Tam4/Mzt1 is highly conserved, its counterpart is apparently missing in the budding yeast. To gain more insights into Mzt1/Tam4 function, we generated a tam4/mzt1 shut-off strain harbouring GFP tagged α-tubulin. We found that the mzt1/tam4 is an essential gene and its depletion caused aberrant microtubule structure including malformed spindles and impaired post anaphase array. Localisation of the GFP-tagged Mzt1/Tam4 was found to be at microtubule organising centres (MTOCs) such as the spindle pole body, interphase MTOCs (iMTOCs) and equatorial MTOCs (eMTOCs). Mzt1/Tam4 interacts with Alp6, the GCP3 homologue, in the yeast two hybrid system and NMR analysis. It also co-immunoprecipitates with the γ-tubulin Gtb1 from the fission yeast cell extract. Our data support the idea that Mzt1/Tam4 contributes to the γ -Tubulin Complex function to regulate microtubule arrangement and dynamics.

Regulation of Srk1 nuclear import through the a-importin Imp1 in response to stress

David Giménez-Zaragoza, Rosa Aligué

University of Barcelona, IDIBAPS, Spain

Presented by: Giménez-Zaragoza, David

Previous works from our group have shown that the Srk1 kinase associates with Sty1/Spc1 MAP kinase and regulates the onset of mitosis by phosphorylating and inhibiting the Cdc25 phosphatase (Lopez-Aviles et al. 2005, Lopez-Aviles, Lambea et al. 2008). However, Cdc25 wouldn't be the only substrate of Srk1 in stress response because cells carrying a cdc25 gene with the majority of Srk1 phosphorylated sites mutated to alanine (cdc25-9A) displays less sensitivity to osmotic stress than Δsrk1 cells. Therefore, we were focused in uncovering additional substrates of Skr1 in stress response.

Identification of new substrates of Srk1 by TAP-analysis have revealed proteins such, Imp1 involved in nuclear protein import. Nuclear export components of the MAPK pathway have studied and associated to the nuclear export factor Crm1 (Gaits and Russell. 1999). However, less is known about the components involved in MAPK pathway nuclear import. Therefore we are pursuing this line of research. Imp1 protein belong to the importin alpha family, members of the importin-alpha (karyopherin-alpha) family can form heterodimers with importin-beta (Chen et al. 2004, Umeda et al. 2005). As part of a heterodimer, importin-beta mediates interactions with the pore complex. Imp1, contains 8 armadillo (ARM) repeats, which produce a curving structure with two NLS-binding sites, a major one close to the N terminus and a minor one close to the C terminus, and it also contains 1 importin beta-binding (IBB) domain (PomBase, Sistla et al. 2007). Imp1 is required for efficient nuclear import of Pap1 component of the stress response MAPK pathway (Umeda et al. 2005) and for the proper mitotic progression (Tallada et al. 2002).

Interaction of the fission yeast Mid1 Pleckstrin Homology domain with the major contractile ring element, F-actin

Marian J. Testori[a], Jennifer L. Jakubowski[a], David R. Kovar[b], Dawn M. Clifford Hart[a]

[a] *Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI 49401, USA*
[b] *University of Chicago, Department of Molecular Genetics and Cell Biology, Chicago, IL 60637, USA*

Presented by: Testori, Marian

The contractile ring in eukaryotic organisms is predominantly composed of filamentous actin (F-actin) and constricts to divide cells during mitosis. Proper formation of contractile ring is essential for the fate of the cell since aberrant cytokinesis can lead to uninhibited cell growth and division. In the fission yeast, Schizosaccharomyces pombe, actin polymerizes to form anti-parallel filaments in rings at the medial plane of the cell. During anaphase, the filaments constrict through the kinetic activity of the type 2 myosin, Myo2. The protein Mid1 is necessary for recruiting the proteins that bundle and nucleate actin filaments to the medial plane during the formation of the contractile ring. The Mid1 Pleckstrin Homology (PH) domain has been reported to stabilize Mid1 at the cell cortex by binding lipids in the membrane but the direct binding of the PH domain to contractile ring proteins has not been described. We now have evidence that Mid1 PH domain directly binds F-actin in vitro. To understand the significance of this association, the interaction of F-actin and the Mid1 PH domain was analyzed by spontaneous actin polymerization assays. The PH domain inhibited F-actin polymers from forming in a dose-dependent manner. This phenomenon was confirmed by rhodamine-phalloidin staining of spontaneously assembled actin filaments in the presence of the PH domain. The ability of the PH domain to regulate the stability of Mid1 in interphase nodes may not only be due to binding to lipids in the membrane, but also binding to F-actin. In addition, the effect of the Mid1 PH domain on F-actin may reveal important regulatory events for actin polymerization during the formation of the contractile ring.

Global cellular growth control

Louise Weston, Paul Nurse

Cell Cycle Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3LY, UK

Presented by: Weston, Louise

Cellular growth control is fundamental to the development of all living organisms yet surprisingly little is understood about the global regulation of growth. One challenge in growth control studies is the ability to eliminate the effect of nutrients on growth. In this study we have used a novel experimental system to overcome this challenge and employed it to systematically screen for cell growth regulators. This system utilises the pat1tsmei4Δ strain. At the restrictive temperature this strain enters the developmental program arresting the cell cycle in the G2 phase, switching off growth even when the cells are bathed in nutrients. This nutrient rich growth-arrest provides an opportunity to screen for genetic alterations that can promote growth. The Riken ORFeome is a plasmid library, which covers 99.2% of the fission yeast protein-coding genes and pseudogenes and enables the controlled expression of each fission yeast gene from the thiamine-regulatable nmt1 promoter. The pat1tsmei4Δ strain was transformed with this plasmid library and examined following the over-expression of each open reading frame at both the restrictive and non-restrictive temperatures, to identify cells that are able to continue growth at the developmental arrest only. This approach has identified 25 genes that act positively to promote cell growth. The candidate genes are involved in a range of functions and notably include a number of RNA polymerase subunits. The mechanisms involved in growth control are currently being explored and are discussed here.

Biochemical analysis of the Ndc80 kinetochore complex in *S. pombe*

Yuzy Matsuo[a], Hirofumi Takada[a], Sebastian Maurer[b], Thomas Surrey[b], Takashi Toda[a]

[a] *Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, 44 Lincoln`s Inn Fields, London WC2A 3LY, UK*
[b] *Laboratory of Microtubule Cytoskeleton, Cancer Research UK, London Research Institute, 44 Lincoln`s Inn Fields, London WC2A 3LY, UK*

Presented by: Matsuo, Yuzy

Accurate segregation of replicated chromosomes is critical for cell proliferation and development of organisms. During mitosis, the kinetochore, a large proteinaceous structure built on the centromeric DNA, must be properly captured by spindle microtubules. However, it remains poorly understood how the kinetochores interact with the plus end of spindle microtubules, thereby establishing stable bipolar attachment.
The KMN (stands for the Knl1 complex, the Mis12 complex and the Ndc80 complex) network locates within the outer kinetochore, by which it links the centromeric DNA to spindle microtubules. In particular, the Ndc80 complex is a direct point of contact. This complex is a long heterotetramer (~57 nm) consisting of two dimers, each comprising Ndc80/Nuf2 and Spc24/Spc25. Several studies suggest that both the Calponin Homology domain and N-terminal tail found in the Ndc80 protein directly bind the microtubule lattice. However, how stable interaction between the plus end of microtubules and the Ndc80 complex is established has remained enigmatic.
Our laboratory recently demonstrated that a conserved internal loop within Ndc80, interrupting the middle of the C-terminal coiled-coil domain, plays critical roles in proper kinetochore-microtubule attachment. This is achieved via loop-dependent recruitment of the TACC-TOG MAPs (Dis1/TOG and the Alp7/TACC-Alp14/TOG complex) to the outer kinetochore. However, it still remains unsolved as to whether the internal loop directly interacts with these MAPs and further how this loop helps to regulate the microtubule dynamics at the kinetochore-microtubule interface. To address these issues, it is necessary to explore the interplay between microtubules, the Ndc80 complex and TACC-TOGs in vitro. In this meeting, we will present our recent results of an in vitro reconstituted system using purified components.

The ERM-related protein Tea3 inhibits polarized growth and is phosphorylated by the PAK-like kinases Nak1 and Shk1

Marco Geymonat[a], Anatole Chessel[a], James Dodgson[a], Tara Finegan[a], Rafael Carazo-Salas[a]

Gurdon Institute and Genetics Department, University of Cambridge, UK

Presented by: Geymonat, Marco

In Schizosaccharomyces pombe polarized growth is tightly coordinated with cell cycle progression. After division, pombe cells grow monopolarly from their old end. During S/G2 a switch to bipolar growth occurs after which cells continue to grow until their size reaches ~14 μm. Then cells stop growing and re-localise their growth machinery to the cell centre where the septum is formed and cytokinesis occurs. Polarity factors are proteins that localise at the tips of the cells, which are important for polarity establishment and maintenance. Tea3 is a polarity factor that contains a kelch-repeat domain at its N-terminus and an ERM homologous domain at its C-terminus. It binds to Tea1 and Mod5 but its molecular role in polarization control is poorly understood. Here we show that the localization of Tea3 is cell cycle regulated. It is enriched at the non-growing end during monopolar growth, it is depleted from both tips during bipolar growth and repopulates the tips in mitosis. It also localises at the septum in late mitosis, in a Mod5 dependent manner. We demonstrate that Tea3 is a phosphoprotein in vivo and we find that phosphorylation is important for the tip-to-septum localisation at the end of mitosis. Phosphorylation is Mod5-dependent and is greatly reduced in strains where the PAK kinases Nak1 and Shk1 are inhibited. To study the role of Tea3 in regulating polarized growth, we over-express Tea3 full length and C-terminal truncations from an nmt1 promoter. When Tea3 is highly expressed polarized growth is inhibited, cells become round/lemon shaped and actin patches delocalized. Time-lapse movies show that those cells do not grow isotropically, as is the case in cells overproducing Gef1, but rather become round because of pressure build up within the cell unaccompanied by cell growth, which ultimately leads cells to burst. Interestingly, we find that Tea3 overexpression is lethal also in S. cerevisiae, suggesting a conserved role in proliferation control.

Meiotic nuclear movement in fission yeast is prolonged by stalled DNA replication through a Cds1-dependent checkpoint pathway

Kun Ruan[a], Takaharu G Yamamoto[b], Haruhiko Asakawa[a], Yuji Chikashige[b], Tokuko Haraguchi[b], Hisao Masukata[c], Yasushi Hiraoka[a]

[a] *Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita 565-0871, Japan*
[b] *Advanced ICT Research Institute Kobe, National Institute of Information and Communications Technology, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 651-2492, Japan*
[c] *Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka 560-0043, Japan*

Presented by: Ruan, Kun

In meiotic prophase, the fission yeast nucleus shows an elongated morphology, called a horsetail nucleus, and the horsetail nucleus moves back and forth within the cell; the horsetail nuclear movements continue about 140 min prior to the meiotic divisions. During the horsetail nuclear movements, telomeres remain clustered at the leading edge of the movements, providing a unique opportunity to examine chromatin structures within a defined orientation of the chromosome. Here we describe that mutants deficient in meiotic S phase showed abnormal morphology of the horsetail nucleus, indicating the longitudinal decompaction of chromosomes. This change of nuclear morphology occurred after the onset of DNA replication. Furthermore the duration of horsetail nuclear movements was strikingly prolonged to about 300 min in those mutants. We found that this prolonged duration of nuclear movements was caused by activation of replication checkpoint, revealing regulatory pathways linking horsetail nuclear movements to DNA replication in meiosis.

Role of PP2A in G1 establishment and mating response

Ruth Martin[a], Mari Nyquist-Andersen[a], Isabelle Jourdain[a], Sandra Lopez-Aviles[a]

The Biotechnology Centre of Oslo / University of Oslo, Norway

Presented by: Martin, Ruth

Under optimal growth conditions, Schizosaccharomyces pombe cell cycle is characterized by short G1 and long G2 phases. However, when deprived of a source of nitrogen, S. pombe cells arrest their cell cycle in G1. In this situation, given the presence of a mating partner, they differentiate in order to conjugate and undergo meiosis. During the cell cycle arrest in G1, Cdc2 activity needs to be kept low, and this is achieved through the stabilization of Rum1 and activation of the APC regulator Ste9. Both proteins are themselves inhibited by Cdc2 activity and, therefore, a phosphatase activity could be contributing to their timely activation. Moreover, Cdc2 also prevents the activation of the transcription factor Ste11 in order to inhibit sexual differentiation.

In Xenopus laevis and mammalian cells PP2A has been suggested as the main phosphatase counteracting CDK phosphorylation events. Hence, we decided to investigate the implication of fission yeast PP2A on the cell cycle arrest imposed upon N starvation and on the differentiation process. For this aim we generated temperature sensitive alleles of the essential scaffolding subunit paa1. The most severe allele, paa1-3, exhibited a marked mating defect at the permissive temperature of 25°C, and this phenotype could be partially rescued by further lowering the temperature to 20°C. In addition, these cells failed to arrest their cell cycle when starved for N, and neither did they show a decrease in the amount of the B type cyclin Cdc13. Importantly, the deprivation of the N source did not produce a substantial decrease in the average cell size.

All in all, our preliminary results suggest that PP2A plays an important role in the process of mating and the cell cycle response to nutritional depletion. We are currently exploring its implications in the sensing and transduction of the environmental cues that lead to sexual differentiation, as well as its role in counteracting Cdc2 activity during this process.

Linking pheromone signalling with cell polarity during mating: the role of Ras1 in fission yeast

Laura Merlini, Sophie Martin

University of Lausanne, Switzerland

Presented by: Merlini, Laura

Signal-induced polarized growth is a fundamental mechanism of cellular differentiation and environmental response. In conditions of nitrogen starvation fission yeast cells arrest in G1 phase of the cell cycle, express pheromone receptors on their surface and produce pheromones that are recognized by partner cells of opposite mating type. Cells can now polarize their growth in the direction of mating partners, but how this polarization occurs is still unclear. Mutations impairing the function of the conserved cell polarity factor Cdc42, or the lack of its regulators Scd1 and Scd2, cause sterility. Moreover, Cdc42 and its regulators undergo dynamic localization around the cell cortex during early stages of mating. This exploration is important for orientation of the mating projection, since mutants that constitutively activate pheromone signalling, fail to explore and choose by default a cell pole for growth.

Another regulator of mating in S. pombe is the human homolog of the Ras oncogene, Ras1, which plays roles in both MAPK pheromone signalling and in morphogenetic response of vegetative growing cells. Cells lacking Ras1 or its activator Ste6 are sterile and prevent Scd2 exploration in wildtype partner cells, that, in this condition, shmoo preferentially from one cell pole, suggesting ras1 mutants can produce pheromones. Since cells lacking components of the MAPK cascade do not induce responses in wt partner cells, the sterility of ras1 mutants could be due to defects in cell polarity rather than to lack of pheromone signalling. Interestingly, localization analyses show that Ras1, its putative GAP Gap1 and Ste6 dynamically scan the cell periphery in early stages of mating, and co-localize with Scd2 during this exploratory phase. Our data suggest that Ras1, beyond its known role in signalling regulation, could also promote polarized growth, most probably through Cdc42, during mating, thus linking MAPK pheromone signalling with cell polarity towards a partner cell.

Control of cell division in the fission yeast

Francisco J. Navarro[a], Paul Nurse[a,b]

[a] *Cell Cycle Control Lab. Cancer Research UK, London Research Institute, 44, Lincoln's Inn Fields, London WC2A 3LY, UK*
[b] *Laboratory of Yeast Genetics and Cell Biology, The Rockefeller University, New York, NY, 10065, USA*

Presented by: Navarro, Francisco

The process by which cells are able to coordinate cell growth and division is poorly understood. One approach to this problem is to identify rate-limiting steps for cell division which in turn may be modulated by cell growth. In the fission yeast Schizosaccharomyces pombe, the dephosphorylation of a conserved Tyr residue on the CDK Cdc2 is rate-limiting for mitotic entry. Alteration of the phosphorylation state of this residue, which depends on the kinases Wee1 and Mik1, and the phosphatase Cdc25, results in the uncoupling of cell growth and cell division, altering the size/mass at which the cell divides. In a screen of approximately 3000 viable gene deletion mutants for genes required for timely cell division, we found 18 mutants which divide with an abnormal small cell size. Genetic interaction analysis revealed that most of the identified genes act on pathways that ultimately regulate Tyr phosphorylation. Unexpectedly, we also identified several genes that have a gene-dosage effect on cell division without altering the levels of CDK Tyr phosphorylation. This finding suggests the existence of other mechanisms, in addition to CDK Tyr phosphorylation, that are required for the coordination between cell growth and cell division. Here we show a series of experiments aimed to help understand the CDK Tyr phosphorylation-independent regulation of cell cycle progression.

Identifying S-phase checkpoint targets responsible for damage-induced DNA replication slowing

Victor Liu, Nick Willis, Nick Rhind

University of Massachusetts Medical School, USA

Presented by: Liu, Victor

Eukaryotic cells have checkpoint mechanisms that detect and respond to DNA damage. The S-phase checkpoint responds to DNA damage by reducing the rate of DNA replication. However, the molecular mechanisms by which the S-phase checkpoint slows DNA replication are not fully understood.

In Schizosaccharomyces pombe, Cds1 is the S-phase checkpoint effector kinase. Cds1 plays an important role in damage-induced DNA replication slowing. cds1Δ cells are checkpoint deficient and—when treated with the alkylating agent MMS—are unable to slow DNA replication.

To identify the Cds1 targets responsible for checkpoint-dependent slowing of replication, we took a proteomic approach. First, we used mass spectrometry to obtain a list of peptides phosphorylated in S-phase of MMS-treated and untreated wildtype cells. We repeated this analysis in cds1Δ cells to derive a list of peptides phosphorylated in a checkpoint-dependent manner upon MMS treatment. We are currently validating this list of putative Cds1 targets by assaying for phosphorylation-mediated gel mobility shifts. Additionally, we are conducting bulk replication slowing assays in mutant strains of putative Cds1 targets. These investigations will advance our understanding of the mechanisms underlying the S-phase checkpoint response to DNA damage.

The assembly of CTP synthase into the cytoophidia - a newly discovered novel filamentous structure

Jing Zhang

Department of Physiology, Anatomy & Genetics, Oxford University, UK

Presented by: Zhang, Jing

Cytidine-5'triphosohate synthase (CTPsyn) is a critical metabolic enzyme involved in de novo synthesis of CTP. Although the catalytic function of CTPsyn has been well studied over decades, it is only recently that the cytoophidia, the filamentous form of CTPsyn, have been observed.

Cytoophidia is evolutionarily conserved from bacterial, yeast, fruit flies to mouse and human cells. There are several questions are waiting to be answered. First, is there any amino acid within the CTPsyn protein that is critical for cytoophidia assembly? Second, whether the filament forming property of CTPsyn can be separated from its enzyme activity? Third, is there any other component in cytoophidia other than CTPsyn? Last, and the most important, what are the functions of cytoophidia and why cells spend precious energy to produce this structure? Several different deletion strains and point mutation strains have been constructed and we have found critical regions in CTPsyn protein that are important of cytoophidia formation. We also find several amino acids that can affect cytoophidia formation. In addition, yeast two-hybrid and immuno-co-staining results give us indication of several proteins that has high possibility to be potential candidate of cytoophidia component. Several screening is now under taking and we are trying to establish a hyposis to answer the questions mentioned above. We are also trying to draw a whole picture of cytoophidia metabolic pathway map.

In addition of these core questions, we also find interesting phenomenon in our experiments, including that cytoophidia may related to cell cycle and cell life span, and that CTPsyn has uneven distribution after mitosis in S.pombe. The live imagine also shows interesting and novel move pattern of cytoophidia.

Although we have found some pieces of the cytoophidia puzzle, there is still a long way to go if we want to understand the whole story of this novel filamentous organelle.

Model of yeast actin cable distribution and dynamics

Haosu Tang, Dimitrios Vavylonis

Department of Physics, Lehigh University, Bethlehem PA, USA

Presented by: Vavylonis, Dimitrios

The growth of fission yeast relies on the polymerization of actin filaments at cell tips nucleated by formin For3p that localizes at tip cortical sites. These actin filaments bundle to form actin cables that span the cell and guide the movement of vesicles toward the cell tips. Since actin cables are structures whose dynamics can be monitored by fluorescence microscopy, and since yeast is a tractable genetic system, comparison of the results of theoretical models of actin cables to experiment could enable quantitative tests of the mechanisms of actin polymerization in cells. We used computer simulations to study the spatial and dynamical properties of actin cables. We simulated individual actin filaments as semiflexible polymers in 3D, composed of beads connected with springs. Formin polymerization was simulated as filament growth out of cortical sites located at cell tips. Actin filament severing by cofilin was simulated as filament turnover. We added attractive interactions between beads to simulate filament bundling by actin cross-linkers such as fimbrin. Comparison of the results of the model to prior experiments suggests that filament severing, nucleation and crosslinking are sufficient to describe the many features of actin cables. We found bundled and unbundled phases as cross-linking strength was varied and propose experiments to test the model predictions.

Co-operation of small molecules upon thermal stress management in *S. pombe*

Attila Glatz[a], Mária Péter[a], Gábor Balogh[a], Katalin Kontár[a], Imre Gombos[a], Ibolya Horváth[a], Annamária Pilbat[a], Zsolt Török[a], László Vígh[a]

Laboratory of Molecular Stress Biology, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, H-6723 Szeged, Temesvári krt. 62, Hungary

Presented by: Glatz, Attila

Using different prokaryotic models, we have earlier demonstrated that cellular membranes are not only the passive endurers of the heat shock, but might function as thermosensors, as well. Our "membrane as thermosensor" theory relies mainly on results showing that the expression of molecular chaperones can be induced under isothermal conditions by disturbing the physical state of the membranes. Furthermore, the chaperones (especially the small heat shock proteins, sHSPs) are able to bind and stabilize membranes both in vitro and in vivo. To extend our hypothesis, we have initiated similar research using Schizosaccharomyces pombe as a model.

S. pombe contains two sHSPs, both are iduced upon heat shock. The purified proteins are able to associate to yeast lipids with different affinity. Both sHSP null mutant cells show thermosensitive phenotype similar to the trehalose-deficient strain. The double sHSP mutant strain shows similar heat sensitivity when compared to the single mutants indicating that the two α-crystallin type HSPs might have different cellular functions. The temperature threshold of heat induction for both sHSPs is influenced by cellular trehalose level.The three mutant strains exhibit altered lipid composition under all temperatures tested, indicating the tight co-operation of the lipids, trehalose and sHSPs in this organism. Surprisingly, the level of fatty acid desaturation of triacylglycerol (TG) displayed opposite tendency compared to that of the membrane-forming lipids. TG is the main component of the lipid droplets, which where previously regarded as simple fat storage. Emerging number of experiments indicate that this should not be the case, and therefore cellular functions of lipid droplets need a revision. Our data strongly support this notion, suggesting that besides the known thermoprotectants, lipid droplets might also play a crucial role in thermal stress management.

Impaired phosphatidylcholine synthesis and aberrant nuclear division in mutants of SAM and SAH cycle enzymes

Takeshi Hayashi, Tomáš Pluskal, Mitsuhiro Yanagida

G0 Cell Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Presented by: Hayashi, Takeshi

S-adenosylmethionine (SAM or AdoMet) is an important compound related to many cellular functions because SAM serves as the methyl donor in most methyl transfer reactions, including methylation of proteins, nucleic acids and lipids. SAM is converted to S-adenosylhomocysteine (SAH) during methyl transfer reactions and then, SAH is hydrolyzed into homocysteine and adenosine. Because SAH is a potent product inhibitor of methyltransferase reactions, the ratio SAM/SAH is regarded as an indicator of cellular methylation capacity. Therefore, regulation of synthesis of SAM and hydrolysis of SAH could affect cellular methyl transfer reactions. Since cellular defects in genetic disruption of SAM synthesis or SAH hydrolysis are not well understood, we isolated temperature sensitive mutants of SAM synthetase (Sam1) and SAH hydrolase (Sah1) in S. pombe by a PCR-based random mutagenesis screen. sam1 and sah1 mutants showed the lsd (large and small daughter nuclei) phenotype in mitosis at restrictive temperature: DAPI-stained chromatin regions in the daughter nuclei differed in their size. However, sister chromatids appeared to be separated in cells showing the lsd phenotype. This phenotype resembles that of the mutant of fatty acid synthase Fas2. Metabolomic analysis revealed that the amount of some metabolites related to phospholipid metabolism dramatically decreased or increased in sam1-1 mutant cells. De novo phosphatidylcholine (PC) synthesis is reported to require three SAM-dependent methylation steps. Taken together, PC synthesis seems to be impaired in sam1 and sah1 mutants. Furthermore, we found that the lsd phonotype of sam1 and sah1 mutants was suppressed by choline, a precursor of PC in the methylation-independent pathway, suggesting that the lsd phenotype is caused by the defect in PC synthesis.

Elucidating the mechanisms of telomere bouquet dispersal

Hanna Amelina, Kazunori Tomita

UCL Cancer Institute, London, UK

Presented by: Amelina, Hanna

In the early stage of meiotic prophase, chromosome ends, telomeres, gather near the microtubule organizing center (MTOC) to form the so called 'bouquet' structure. As telomeres are clustered on the restricted region of the nuclear membrane, chromosomes are aligned. This widely conserved chromosomal rearrangement promotes pairing of homologous chromosomes. Recently it has been shown that the chromosomal bouquet also plays crucial role in regulating the meiotic MTOC and spindle (Tomita K & Cooper JP, Cell, 2007). Additionally, the timing of the end of the bouquet stage, namely 'telomere fireworks', appears to be connected to the completion of meiotic recombination. Therefore, telomere fireworks may couple completion of such a chromosomal event with meiotic spindle formation.

In this project we are using fission yeast as a model organism to elucidate molecular mechanisms of telomere bouquet dissociation, and to understand how telomere fireworks are regulated during meiotic prophase. We hypothesise that dissociation of telomeres is caused by phospho-modification, active-degradation or truncation of the binding domain(s) of the proteins composing bouquet. Therefore we focus on determining the states of telomere and bouquet proteins at the end of meiotic prophase. As a next step, we are going to search for factors involved in telomere fireworks and/or meiotic spindle regulation, and investigate the relationship between the chromosomal bouquet/telomere fireworks and the regulation of the meiotic spindle.

Revealing the mechanism underlying the end of the bouquet stage will provide a high-resolution picture of chromosome dynamics during meiotic prophase, and will provide significant foothold to investigate how telomeres control the meiotic spindle.

Studying the roles of the kinetochore component Ndc80 in the spindle assembly checkpoint

Aldona Chmielewska, Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, 44 Lincoln`s Inn Fields, London WC2A 3LY, UK

Presented by: Chmielewska, Aldona

During cell division, chromosome segregation is facilitated by the kinetochore, a multiprotein structure, which assembles around the centromeric region of the chromosome. Kinetochore-microtubule attachment is critical for the segregation of sister chromatids to the opposite poles during mitosis. The outer kinetochore, which contains the KMN network (KNL-1/Mis12 complex/Ndc80 complex) of which the Ndc80 protein is a primary microtubule-binding site, links chromosomes to the mitotic spindle. Any improper attachments are recognized by the spindle assembly checkpoint (SAC), a surveillance pathway, which blocks premature segregation of chromosomes by preventing anaphase onset. Incorrect kinetochore-microtubule attachments lead to SAC activation, which is coupled with localization of its components (Mad1, Mad2, Mad3/BubR1, Bub1 and Bub3) to the kinetochores. Recent studies have shown binding of Bub1 and Bub3 to KNL-1 (fission yeast Spc7), however the interacting partners of other SAC components at the kinetochore still remain largely elusive.

In this current study, we have identified ndc80 mutants (ndc80-AK01 and ndc80-AK05) that were hypersensitive to microtubule poisons with no apparent growth defects in the absence of drugs. Preliminary analysis supports the notion that ndc80-AK01 and ndc80-AK05 are defective in SAC signaling, as these mutants do not arrest at mitosis and show high septation indexes in absence of microtubules. Cytological and genetic analysis of these new mutants will be presented.

Cell size, cell cycle, and the cytoskeleton

Stephen Huisman, Damian Brunner

Institute of Molecular Life Sciences, University of Zürich, Switzerland

Presented by: Huisman, Stephen

Cells double their size during each cell cycle but maintain a constant size between cycles. This process of cellular size homeostasis is directed by interactions between the cell cycle machinery and a size measuring system. While the cell cycle is now fairly well understood, not a lot is know about how cells measure their size. We are using the S. pombe system to investigate pathways determining cell size homeostasis. We found a surprising link between cell size and the cytoskeleton. We discovered a strong synthetic interaction between a cell cycle regulator (cdc25) and a microtubule plus end binding protein (tip1). Phenotypic suppressors were identified in a genetic screen and a via a candidate based approach. Suppressors include genes in known cell size determining pathways like Pom1 and the Cdc25. Interestingly we also identified proteins involved in the microtubule and actin cytoskeleton, RNA-binding, proteolysis, and cell polarity. We are currently investigating the mechanisms behind the suppression to describe novel pathways and or players involved in cell size measurement.

Ubr11 ubiquitin ligase regulates modulation of cell growth by extracellular oligopeptides and amino acids

Kenji Kitamura

Center for Gene Science, Hiroshima University, Japan

Presented by: Kitamura, Kenji

The evolutionarily conserved Ubr11 ubiquitin ligase is essential for utilization of extracellular oligopeptides because it is required for expression of major peptide transporters Ptr2 and Isp4. An extragenic suppressor mutation, referred to as upa1, was isolated which completely recovered the peptide uptake defect in a ubr11Δ strain. Interestingly, ubr11 and upa1 mutations also affected the utilization of monomeric amino acids. With these and wild-type strains, we found several dipeptides which positively or negatively modulated the growth. Remarkably, some dipeptides had an effect only in the upa1 mutant. Representatives are artificial sweetener aspartame, and carnosine, the naturally occurring imidazole dipeptide in animals, which exerts various anti-aging effects in normal mammalian cells but is toxic for some cancer cells. Analysis of molecular mechanisms underlying the upa1 mutant-specific action of these dipeptides, and also the cloning of the upa1 gene are currently in progress. Ubr11 is responsible for the N-end rule pathway (NeRP)-mediated proteolysis, although physiologically important substrates for peptide uptake have not been identified yet in S. pombe. To explore the relationship between NeRP and peptide uptake, roles of highly conserved domains in Ubr11 were examined. Activity of Ubr11 is influenced by its binding to oligopeptides harboring N-terminal basic (type-1: R, K, H) or bulky hydrophobic (type-2; Y, W, F, L, I) amino acid. Mutations in UBR box prevented uptake of oligopeptides and NeRP-dependent degradation of both type-1 and type-2 model substrates, as in ubr11Δ cells. In contrast, an N-domain mutant was completely normal for the type-1 peptide-related function and specifically failed to recognize type-2 peptide, but nevertheless failed to express peptide transporters. These data suggest the importance of recognition of the type-2 oligopeptides and/or a putative type-2 NeRP substrate by Ubr11 for its in vivo function.

Regulatory mechanism to activate the M-phase promoting factor during meiosis by an RNA-binding protein Spo5 in fission yeast

Mayumi Arata, Masamitsu Sato, Akira Yamashita, Masayuki Yamamoto

Kazusa DNA Research Institute, Japan

Presented by: Arata, Mayumi

Meiosis consists of two consecutive nuclear divisions with no DNA replication between them. Cells reduce the M-phase promoting factor (MPF) activity at the MI/MII transition but they have to prevent complete loss of it to ensure MII entry. In fission yeast, Mes1 is known to facilitate the maintenance of the MPF activity at this transition by blocking degradation of cyclin. However, much remains unclear about the regulation of MII progression. To obtain further insight into the regulatory mechanisms of MII progression, we analyzed a meiotic RNA-binding protein Spo5. spo5-deleted cells (spo5Δ) do not form spores and more than half of them arrest at the binucleate stage in meiosis. Thus, Spo5 is supposed to be involved in the regulation of MII, but its molecular function in this regard remains elusive. We found that the B-type cyclin Cdc13 was degraded at an earlier stage of meiosis in spo5Δ than in wild-type cells. Quantitative real-time PCR assay showed that the level of cdc13 mRNA expression was lower in spo5Δ during MI/MII transition than in wild-type cells. Some other mRNAs also showed altered expression patterns in the spo5Δ strain. In addition, the MII defect of spo5Δ was partially suppressed by the introduction of active CDK. These results suggest that the MII defect of spo5Δ is caused by a decrease in the MPF activity. Taken together, it is presumable that Spo5 is involved in the maintenance of the MPF activity during meiosis through controlling the expression of several mRNAs to ensure the progression of consecutive nuclear divisions.

Importance of posttranscriptional and transcriptional regulations by Spo5 and Pcr1 during fission yeast meiosis

Naoyuki Togashi, Akira Yamashita, Masamitsu Sato, Masayuki Yamamoto

Kazusa DNA Research Institute, Japan

Presented by: Togashi, Naoyuki

Meiotic cells undergo two rounds of nuclear division and generate eggs, sperms, or spores in eukaryotes. Previous studies showed that a number of transcription factors dramatically change the transcriptome and thereby coordinate progression of meiosis and sporulation in fission yeast. In contrast, mechanisms for post-transcriptional regulations during meiosis are poorly understood. The spo5+ gene encodes a meiosis-specific RNA-binding protein, which is essential for the progression of meiosis II and sporulation. However, target RNA molecules of Spo5 have not been identified so far.

Here we show that Spo5 is a nucleocytoplasmic shuttling protein that mainly localizes to the cytoplasm in a manner dependent on mRNA binding. We found that localization to the cytoplasm is essential for the function of Spo5. To further elucidate the cytoplasmic function of Spo5, we searched for downstream factors of Spo5. We identified mRNAs of some transcription factors and cell cycle regulators as targets of Spo5. These include cdc13+ encoding cyclin and pcr1+ encoding an ATF/CREB family transcription factor. Spo5 binds to these mRNAs through their 3'-UTR and boosts their expression level, indicating that Spo5 may stabilize target mRNAs. In addition to receiving posttranscriptional regulation by Spo5, Pcr1 directly binds upstream of the cdc13+ gene and promotes its transcription, suggesting that the function of Pcr1 may be partly mediated by Cdc13. We also found that the Cdc13 expression level must be precisely controlled for the execution of meiotic cell division and sporulation. These results demonstrate that the coordination of posttranscriptional and transcriptional regulations is important for gametogenesis.

Regulation of the G2/M transition in response to UVC-irradiation

Christiane Rothe, Erik Boye, Beáta Grallert

Institute for Cancer Research, Oslo University Hospital, Oslo, Norway

Presented by: Rothe, Christiane

The G2/M checkpoint is an active mechanism to ensure that cells with damaged DNA arrest before entry into mitosis, at least partly to allow time for DNA repair. The consensus in the field is that the protein kinases Rad3 and Chk1 are absolutely required for the G2/M checkpoint response following exposure to ultraviolet light (UVC) (AlKhodairy et al, EMBO J, 1992).

We have investigated the cell-cycle progression of checkpoint mutants and observed that a rad3 deletion mutant delays entry into mitosis after UVC-irradiation, although the delay is much shorter than in wild-type cells. Like rad3 cells, a mutant lacking the checkpoint effector kinase Chk1 also displays a short delay. These results suggest that even in the absence of Rad3/Chk1 there is a UVC-induced delay in G2 phase.

We have investigated where in the cell cycle rad3 deletion cells delay, in particular whether they stand in G2 or in early mitosis (metaphase). We have also considered a number of possible mechanisms as reasons for the delay. Since UVC-irradiation is known to reduce general translation, we explored whether a size-control mechanisms might bring about the delay. Furthermore, we investigated a possible involvement of the mitotic kinases Fin1 (NIMA related kinase) and Ark1 (Aurora kinase), a possible contribution by the spindle assembly checkpoint, as well as the possibility that Tel1 (ATM checkpoint kinase) might be able to delay entry into mitosis in the absence of Rad3.
The results of these investigations will be presented.

Mitochondrial stress response extends chronological lifespan via autophagy activation in fission yeast

Masayuki Harada, Fuyuki Ishikawa

Graduate School of Biostudies, Kyoto University, Japan

Presented by: Harada, Masayuki

Mitochondria are the major source of reactive oxygen species (ROS) in cells, and are continuously damaged by the self-producing ROS. Recent studies have revealed that upon mitochondrial insult, cells elicit adaptive stress responses leading to restructuring of cellular metabolisms, activation of cellular defense mechanisms and in some cases lifespan extension. However, it is largely unknown how such responses to mitochondrial damages operate in S. pombe. We established fission yeast cells that inducibly produce endonuclease EcoRI fused to mitochondria-targeting sequence (MTS). MTS-EcoRI specifically cleaved mitochondrial DNA, but not genomic DNA, resulting in inhibition of respiration. We found that MTS-EcoRI expression extends lifespan of fission yeast in the stationary phase. We hypothesize that the extended longevity may represent adaptive responses to mitochondrial dysfunction.

We found that autophagy-related genes atg1, atg2 and atg8 are required for the lifespan extension. The expression level of Atg8 and the autophagic activity were increased in MTS-EcoRI-expressing cells. Furthermore, atg8 overexpression alone was sufficient to extend the lifespan of wild type cells. These results suggest that induction of autophagy mediates the lifespan extension in MTS-EcoRI-expressing cells. We also found that antioxidant treatment abolished the autophagy activation and the lifespan extension, suggesting that ROS plays a role in inducing the responses involving autophagy activation. We propose that ROS-mediated mitochondrial stress signaling promotes cell survival through autophagy activation.

Spatial control of the microtubule-associated protein complex Alp7/TACC-Alp14/TOG

Naoyuki Okada[a], Yasutaka Kakui[a], Masayuki Yamamoto[a], Masamitsu Sato[b]

[a] *Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan*
[b] *1.Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo 2 Center for Advanced Biomedical Sciences/TWIns, Waseda University, Japan*

Presented by: Okada, Naoyuki

Microtubules change their formation and location drastically during the cell cycle. In interphase, the cytoplasmic array of microtubules is formed, and then reorganized into spindle microtubules within the nucleus at mitotic onset. Alp7/TACC (transforming acidic coiled-coil protein)-Alp14/TOG (tumor overexpressed gene) is a conserved MAP (microtubule-associated protein) complex, which undergoes nucleocytoplasmic shuttling throughout the cell cycle. The nuclear transport of the Alp7-Alp14 complex is crucial for the spindle integrity. However, it remains largely unknown how this complex achieves nuclear accumulation upon mitotic entry.

At mitotic entry, the Alp7-Alp14 complex accumulates in the nucleus using the nuclear localization signal (NLS) of Alp7. To further investigate how the localization of Alp7-Alp14 is determined, we analyzed a series of truncation and point mutants of Alp7 and Alp14. One of these mutants, Alp14-L615A, was found to accumulate in the nucleus together with Alp7 throughout the cell cycle. Thus, the nuclear import of the Alp7-Alp14 complex is dependent on Alp7, and the export is dependent on Alp14. We also found that Alp7 is phosphorylated by the cyclin-dependent kinase (CDK) in vitro, and phosphorylation sites were biochemically identified. The phospho-resistant Alp7 mutant protein failed to accumulate in the nucleus during mitosis, whereas the phospho-mimetic version accumulated in the nucleus even in interphase. These results suggest that the CDK-phosphorylation of the Alp7 play a key role for its nuclear accumulation during mitosis.

Based on these results, we would like to discuss transport mechanisms of the Alp7-Alp14 complex and its contribution to the spatiotemporal regulation of the microtubule organization during the cell cycle.

Removal of centrosomal PP1 by NIMA kinase unlocks the MPF feedback loop to promote mitotic commitment in *S. pombe*

Kuan Yoow Chan[a], Agnes Grallert[a], Marisa Alonso-Nuñez[a], Marisa Madrid[a], Ashapurno Biswas[a], Isabel Alvarez-Tabarés[a], Yvonne Connolly[b], Kayoko Tanaka[a], Alasdair M. Robertson[a], Iain M Hagan[a]

[a] *Cell Division Group, Paterson Institute for Cancer Research, Manchester, UK*
[b] *Biological Mass Spectrometry, Paterson Institute for Cancer Research, Manchester, UK*

Presented by: Chan, Kuan Yoow

The Mitotic Promoting Factor (MPF) complex Cdc2/CyclinB is regulated by the coordinated action of inhibitory phosphorylation by Wee1 kinase and Cdc25 phosphatase which removes this inhibitory phosphorylation. A number of mitotic kinases (e.g. Polo, Aurora and NIMA related kinases) influence MPF activity through feedback amplification loops. Mutations in either the wee1+ or the spindle body component Cut12 can suppress cdc25.22. The cut12.s11 allele is a gain of function mutant, which elevates Plo1 activity and allows for mitotic entry in the absence of Cdc25 activity.

The cut12.s11 mutation resides in a highly conserved PP1 bi-partite binding motif (GILKtpgtlqikKtVnF). Cut12 binds to Dis2 (one of the two PP1 isoforms) in immunoprecipitation, in vivo BiFc and yeast two hybrid assays. Mutations that remove the PP1 binding motif block PP1(Dis2) association allowing mitotic entry in the absence of Cdc25 activity. Phosphomapping and phosphospecific antibodies show two conserved tyrosine T75 and T78 within the PP1 binding motif are phosphorylated. Mutation of these sites to acidic residues to mimic phosphorylation, blocks PP1(Dis2) association to Cut12 and suppress cdc25.22. Western blot and immunoprecipitation assays of synchronous cells show the NIMA kinase Fin1 phosphorylates T78 in late G2 phase, at the point at which PP1(Dis2) disassociates from Cut12. Cdc2/CyclinB phosphorylates T75.
In summary, 30 minutes before mitosis, Cut12 is phosphorylated by MPF and Fin1 to block PP1(Dis2) recruitment to Cut12. Departure of PP1(Dis2) promotes the association of Plo1 with the SPB and the subsequent activation of a positive feedback-loop that will ultimately promote mitotic commitment.

Analyses of Vps901 and Vps902, VPS9 domain-containing proteins in fission yeast

Yuta Tsukamoto[a], Tomo Matsuda[a], Hiromi Tsuji[a], Takuya Goto[a], Masaaki Miyamoto[b]

[a] *Division of Biology, Graduate School of Science, Kobe University, Japan*
[b] *Division of Biology, Graduate School of Science and CSREA, Kobe University, Japan*

Presented by: Tsukamoto, Yuta

Vesicular trafficking is necessary for the proper transport of various molecules to the target membrane, and the Rab family of small GTPases plays important roles in the selective transport in eukaryotic cells. Rab proteins act as molecular switches by cycling between inactive form (GDP-bound form) and active form (GTP-bound form). Rab5, one of the over 60 Rab proteins, is implicated in the regulation of early endosomal transport in mammalian cells. Recent studies suggest that Rab5 is implicated with various cellular events such as phagosome maturation, chromosome alignment and actin remodeling. Rab GTPases are activated by guanine nucleotide exchange factors (GEFs), and VPS9 domain-containing proteins are considered to be Rab5 GEFs. To gain a better understanding of Rab5 signaling system, it is important to analyze Rab5 GEFs. In mammalian cells, at least nine VPS9 proteins are known, however the relationship between VPS9 proteins remains unknown. In fission yeast *Schizosaccharomyces pombe*, only two VPS9 proteins, Vps901 and Vps902, are predicted from genome analysis. We think *S. pombe* is a simple model organism for analyzing the relationship of VPS9 proteins.
We tried to examine the function and the localization of Vps901 and Vps902. In order to explore the function of Vps901 and Vps902, we constructed *vps901* deletion mutant, *vps902* deletion mutant and *vps901 vps902* double deletion mutant cells. We found that *vps901* mutant cells had defects in mating, vacuole fusion and response to ion stresses. Our results using genetic analyses suggest that two VPS9 proteins work in cooperation in cellular events such as cell growth, sporulation and vacuolar morphogenesis.

Investigating the quantitative model for cell cycle progression in fission yeast

Matthew Swaffer, Paul Nurse

Cell Cycle Lab, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3LY, UK

Presented by: Swaffer, Matthew

The quantitative model for cell cycle progression proposes that oscillations in a single CDK activity are sufficient to order cell cycle events. As CDK activity rises across the cycle, low CDK activity initiate S-phase and higher levels are required to drive mitotic entry. We have adopted a phosphoproteomics approach, as well as focusing on candidate CDK substrates, to describe the behavior of CDK substrate phosphorylation at these two activity thresholds.

Preliminary findings indicate that the phosphorylation of different classes of substrates is differentially sensitivity to these two activity thresholds. To study the potential role of CDK counteracting phosphatase(s) in establishing this difference we have evaluated the kinetics of CDK substrate dephosphorylation upon CDK inactivation. This suggests CDK substrates are dephosphorylated with very similar kinetics, independently of the dynamics of their initial phosphorylation.

Identification of Rgf1p and Rgf3p domains involved in function and subcellular targeting

Elvira Manjón, Sofía Muñoz, Virginia Tajadura, Patricia García, Sandra Cruz, Yolanda Sánchez

Instituto de Biología Funcional y Genómica, CSIC/Universidad de Salamanca and Departamento de Microbiología y Genética, Universidad de Salamanca. C/ Zacarías González, s/n. Salamanca, Spain

Presented by: Manjón, Elvira

In fission yeast, three guanine nucleotide-exchange factors (GEFs) activate Rho1p: Rgf1p, Rgf2p and Rgf3p. Rgf1p localizes to the cell tips in interphase cells and to the division septum in mitotic cells. Rgf1p is not essential for viability, but it does play a role in cell integrity and is also necessary for the establishment of bipolar growth. Rgf3p localizes exclusively to the septum and is essential for maintaining cell integrity during cell separation. The three GEFs contain a RhoGEF domain, which contacts the Rho GTPase to catalyze nucleotide exchange, and an associated Pleckstrin homology (PH) domain, which fine-tunes the exchange process. Rgf1p and Rgf2p also contain a putative Dishevelled, Egl-10 y Pleckstrin (DEP) domain N-terminal to the DH domain and a Citron Homology (CNH) domain at the C-terminus. Rgf3p also contains a CNH domain but lacks a DEP domain.The function of the DEP and the CNH domains is still unknown.

We tested the functional relevance of the different domains in Rgf1p and Rgf3p. As expected, the RhoGEF and the PH domains are both essential for function. However, the N-terminus and the DEP domain are dispensable for full function.

From these studies we have learnt that the CNH domain is essential for function in both GEFs. Rgf1pΔCNH cells, carrying a deletion in the CNH domain and Rgf1p ΔC45 cells lacking the last 45 aa of the Rgf1p, showed similar phenotypes than the null mutant regarding polarity and cell integrity. In addition, for the Rgf1p ΔC45 construct, the normal localization of Rgf1p at the two tips was disrupted and the signal was mainly monopolar. Our results demonstrate that the CNH domain of the Rgf1p and particularly the C-end (last 45 aa), is required for Rgf1p anchorage to the new growing tip allowing the establishment of bipolar growth. A similar situation was shown for Rgf3p; deletion of the CNH domain in Rgf3p renders the cells unviable and this domain is also required for localization of the protein to the septum.

MAPK-dependent Rnc1 localization to stress granules

Yuna Ito[a], Ryosuke Satoh[a,b], Mari Higa[a], Hajime Naruse[a], Makoto Takada[a], Reiko Sugiura[a]

[a] *School of Pharmaceutical Sciences, Kinki University, Japan*
[b] *Microbial Chemistry Research Foundation, Japan*

Presented by: Ito, Yuna

MAPK signaling pathways regulate various physiological processes, such as cell growth, differentiation and apoptosis. Our laboratory has been studying the MAPK signaling pathway and its cross-talk mechanisms with RNA-binding proteins using the fission yeast S. pombe, as an excellent model organism to study the regulatory mechanisms of the signaling pathway common to higher eukaryotes. One of the signaling pathways we are focusing is Pmk1 MAPK, a homologue of ERK MAPK in mammals and identified Rnc1 as a regulator of the MAPK signaling. Rnc1 encodes a K-homology (KH)-type RNA-binding protein. Previously, we reported that Rnc1acts as a negative regulator of the Pmk1 MAPK signaling through the mRNA stabilization of Pmp1, the MAPK phosphatase for Pmk1. Interestingly, Pmk1 phosphorylates Rnc1 and thereby promotes the RNA-binding activity of Rnc1 for Pmp1 mRNA (Nature, 2003).

Here, we show that Rnc1 localizes to stress granules in response to various stimuli, including heat shock and oxidative stress. Notably, in Rnc1 deletion cells, the formation of the Pab1-positive RNA granules were markedly delayed, and Rnc1 deletion cells exhibited a significant growth delay as compared with the wild type cells under these stresses. These results suggest that Rnc1 localization to the stress granules reflects its role in the stress responses in fission yeast.

Hpz1 and Rad3 modulate the G1-S transition

Jon Halvor J Knutsen, Cathrine A Bøe, Erik Boye, Beáta Grallert

Department of Cell Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway

Presented by: Knutsen, Jon Halvor

The hpz1 gene is expressed in the G1 phase and the corresponding protein localizes to the nucleus. Hpz1 is important for resistance to ultraviolet light in G1 phase and to treatment with hydroxyurea. Furthermore, the absence of Hpz1 results in an advancement of S-phase entry after a G1 arrest as well as earlier recovery from a hydroxyurea block, which suggests that the firing of replication origins is facilitated. The gene encoding Hpz1 is, in many fungi, fused with and in the same reading frame as that encoding Rad3, the homologue of the human ATR checkpoint protein. This suggests that the two proteins share function or participate in the same biological pathways (Bøe et al., 2012, PLoS ONE).

We have shown that also absence of Rad3 results in an advancement of S-phase entry after G1 arrest. Our data suggest that Rad3 and Hpz1 cooperate to regulate the initiation of DNA replication and that they both have a negative effect on initiation.

Phosphorylation-dependent assembly and coordination of the DNA damage checkpoint complex by Rad4 (TopBP1)

Meng Qu[a], Mathieu Rappas[b], Christopher Wardlaw[b], Antony Carr[b], Antony Oliver[b], Li-Lin Du[a], Laurence Pearl[b]

[a] *National Institute of Biological Sciences, Beijing, China*
[b] *Genome Damage and Stability Centre, University of Sussex, Falmer, UK*

Presented by: Wardlaw, Christopher

The S.pombe BRCT-domain protein Rad4 (TopBP1) is known to facilitate activation of the DNA damage checkpoint by coupling the Rad9-Rad1-Hus1 (9-1-1) complex and the mediator protein Crb2 (53BP1) resulting in Chk1 activation. We have identified a cluster of phosphorylation sites in the N-terminal region of Crb2 that mediate the binding between Crb2 and the Rad4 BRCT domains and determined the crystal structure of these phospho-dependent interactions. The phosphorylations of these Crb2 sites are required for the activation of the DNA damage checkpoint and are phosphorylated by Cdc2 (CDK) in a hierarchical manner. Phosphorylation of two canonical Cdc2 sites promotes phosphorylation of a non-canonical Cdc2 site by forming an initial weak interaction between Crb2 and Rad4 which recruits Cdc2. Finally we show that interaction of a single Rad4 molecule with a dimer of phosphorylated Crb2 molecules is essential for DNA damage checkpoint activation.

Nonmedially assembled F-actin cables incorporate into the actomyosin ring in fission yeast

Junqi Huang[a], Yinyi Huang[b], Haochen Yu Yu[c], Dhivya Subramanian[d], Anup Padmanabhan[d], Rahul Thadani[d], Yaqiong Tao[a], Xie Tang[d], Roland Wedlich-Soldner[c], Mohan Balasubramanian[a,d]

[a] *Department of Biological Sciences, National University of Singapore*
[b] *Mechanobiology Institute, Singapore*
[c] *Cellular Dynamics and Cell patterning Research Group, Max Planck Institute of Biochemistry, Martinsried 82152, Germany*
[d] *Temasek Life Sciences Laboratory, Singapore*

Presented by: Huang, Yinyi

In many eukaryotes, cytokinesis requires the assembly and constriction of an actomyosin-based contractile ring. Despite the central role of this ring in cytokinesis, the mechanism of F-actin assembly and accumulation in the ring is not fully understood. In this paper, we investigate the mechanism of F-actin assembly during cytokinesis in Schizosaccharomyces pombe using lifeact as a probe to monitor actin dynamics. Previous work has shown that F-actin in the actomyosin ring is assembled de novo at the division site. Surprisingly, we find that a significant fraction of F-actin in the ring was recruited from formin-Cdc12p nucleated long actin cables that were generated at multiple nonmedial locations and incorporated into the ring by a combination of myosin II and myosin V activities. Our results, together with findings in animal cells, suggest that de novo F-actin assembly at the division site and directed transport of F-actin cables assembled elsewhere can contribute to ring assembly.

Asymmetric DNA segregation and diffusion barrier in fission yeast

Haochen Yu, Yves Barral

Institute of Biochemistry, ETH Zürich, Switzerland

Presented by: Yu, Haochen

Extrachromosomal circular DNA (ecDNA) have been shown to contribute to replicative aging in budding yeast. During asymmetric division of budding yeast cells, non-centromeric plasmids and rDNA circles are retained with high frequency in the mother cells via at least two distinct mechanisms. Nothing is known about the fate of ecDNA in symmetric cell division. Here, we studied the segregation and dynamics of ecDNA in S. pombe by constructing a tetO-tetR based plasmid visualization system. We discovered that like in budding yeast, plasmids predominantly localized to nuclear periphery. Such localization was disrupted when LEM domain proteins Lem2 and Man1 were deleted. By following the tetR-GFP labelled plasmids through mitosis, we demonstrated that plasmids segregated in a non-random asymmetric manner. The non-random asymmetry was abolished in lem2Δ man1Δ, and was reduced in condensin mutant cut3-477. Our data lead to the hypothesis that diffusional confinement on NE could contribute to plasmid asymmetry. We therefore probed the presence of diffusion barrier on S. pombe NE by performing FLIP experiments using Cut11-GFP as a marker. In anaphase cells, the exchange of Cut11-GFP across the two imminent daughter nuclei was significantly slower than the exchange within the same hemisphere. Such difference was not observed in interphase and early mitotic cells. These data strongly suggests that a diffusion barrier was established between the two hemispheres at early anaphase. Interestingly, the timing of the barrier establishment coincides with a major membrane-remodelling event, whereby NE comes into extensive contact with the cortical ER at the equatorial region, forming a ring-like structure. As the nucleus elongates, this ring-like structure seems to constrict and form a thin band between NE and ER. We are currently investigating the possible role of such event on the formation of diffusion barrier, as well as the link between barrier formation and ecDNA asymmetry.

Cytoplasmic freezing in fission yeast

Maria Heimlicher[a], Chieze Ibeneche[b], EL Florin[b], Damian Brunner[a]

[a] *IMLS, University of Zürich, Switzerland*
[b] *University of Texas at Austin, USA*

Presented by: Heimlicher, Maria

The maintenance of spatial organization in eukaryotic cells is a highly dynamic process. This ensures flexibility in response to changing needs as imposed for example by changes in the local environment. However, the constant remodeling and maintenance of such anisotropic organization requires energy. In nutrient limited conditions this raises the questions whether, and if so how, cytoplasmic organization is maintained.

When fission yeast cells run out of glucose they enter a quiescent state until new nutrients become available. In optical tweezer experiments we found that this quiescent state is accompanied by a drastic increase in the viscosity of the cytoplasm. In the end, the diffusive motion of lipid granules and other observable structures is fully restricted. It seems as if the cytoplasm reorganizes from a fluid-like state into a solid-like state. We term this novel phenomenon "cytoplasmic freezing". As soon as glucose becomes available again, the cytoplasm reverts to the fluid like state.

We are investigating the frozen state of the cytoplasm in a collaborative, interdisciplinary approach. Biophysics tools such as single particle tracking and optical tweezers are used to quantify the magnitude and time course of cytoplasmic freezing (Florin group, University of Texas, Austin). With 3-D cryo-electron tomography the structural changes taking place when cells undergo cytoplasmic freezing are investigated, in particular to visualize the predicted filamentous network (Hoenger group, University of Colorado, Boulder). In Zurich we are identifying and characterizing the molecular machinery underlying the cytoplasmic state transitions. Therefore we combine genetics and live bright field and fluorescence microscopy techniques to describe cellular architecture and dynamics and to acquire quantitative data.

Regulation of DNA replication in fission yeast by Hsk1 kinase through physical and functional interactions with Mrc1

Seiji Matsumoto, Kyosuke Ueda, Motoshi Hayano, Yutaka Kanoh, Michie Shimmoto, Hisao Masai

Genome Dynamics Project, Department of Genome Medicine, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8613, Japan

Presented by: Matsumoto, Seiji

Phosphorylation of Mcm by Cdc7 kinase is crucial for initiation of DNA replication and thus for selection of active origins. Critical roles for Cdc7 kinase have also been shown in various chromosome transactions other than DNA replication. We have shown that deletion of mrc1+ or rif1+ can bypass the requirement of Hsk1 kinase, the fission yeast homolog of Cdc7, for DNA replication and growth. We previously reported that both checkpoint-dependent and -independent functions of Mrc1 are involved in the bypass of the Hsk1 function. In this study, we analyzed checkpoint-independent functions of Mrc1 that are involved in regulation by Hsk1 kinase.

Through comprehensive deletion analyses of Mrc1 (total 1019 aa), we found that 98-amino acid sequence (residues 782-880) is responsible for the growth inhibition of an hsk1 mutant but not essential for checkpoint function of Mrc1. This segment was found to be required for Mrc1 binding to Hsk1-Dfp1/Him1 and thus named HBS (Hsk1-binding-sequence). Mrc1 undergoes phosphorylation not only upon HU-treatment, but also during normal progression of S phase, and this phosphorylation depends on Hsk1 and HBS. A mutant Mrc1 with all the potential phosphorylation sites within HBS replaced with Alanine still underwent phosphorylation, suggesting that Hsk1 binds Mrc1 through HBS and phosphorylates Mrc1 at unidentified sites outside the HBS. We are also examining the effects of phosphorylation of Dfp1/Him1 on the binding of Hsk1 to Dfp1/Him1 or Mrc1, which is reduced in Cds1 overproducer but increased in cds1Δ. We will discuss potential regulatory mechanisms of initiation of DNA replication by Hsk1 through its physical and functional interactions with Mrc1.

Role of the conserved NDR kinase Orb6 in control of polarized cell growth

Illyce Suarez, David Wiley, Maitreyi Das, Fulvia Verde

University of Miami Miller School of Medicine, Department of Molecular and Cellular Pharmacology, USA

Presented by: Suarez, Illyce

The conserved NDR kinase Orb6 controls cell morphogenesis in different organisms, ranging from yeast to neuronal cells. We previously found that Orb6 kinase regulates cell polarity by spatial control of Cdc42 GTPase. (Das et al, Curr Biol, 2009) Here we report that Orb6 kinase has a genetically separable function in the control of cell growth that is mediated by the RNA-binding protein Sts5.

We found that Sts5 is a cytoplasmic protein that rapidly localizes to the P-bodies, sites of RNA storage and degradation, following cell stress or starvation. Consistent with our finding that Sts5 is a substrate of Orb6 kinase, loss of Orb6 kinase activity leads to localization of Sts5 to the P-bodies.

We have identified several mRNAs that associate with Sts5 by microarray and q-PCR analysis. These mRNAs encode for conserved signaling proteins involved in morphology and bipolar growth activation, as well as cargos with a role in cell wall formation and nutrient transport. The cellular levels of Sts5-associated mRNAs increase in sts5Δ mutants and decrease upon Orb6-kinase inhibition, suggesting that Sts5 may promote mRNA degradation and that Orb6 kinase regulates this process.

In addition, a synthetic genetic array screen (SGA) revealed genetic interactions between Orb6 and several signaling pathways involved in nutritional responses, including the glucose-sensing PKA pathway. We found that the catalytic subunit Pka1 plays a role in limiting P-body formation and that Orb6 kinase and Pka1 synergize in the control of P-body formation.

Our findings suggest that Orb6 kinase promotes polarized cell growth by preventing Sts5 localization to the P-bodies and consequent mRNA degradation and indicate that Orb6 kinase may play a role in modulating cellular responses to stress in concert with nutritional response pathways.

Regulation of telomerase activity by the telomeric nucleoprotein complex in fission yeast

Christine Armstrong, Sian Pearson, Kazunori Tomita

Chromosome Maintenance Group, Cancer Institute, University College London, UK

Presented by: Armstrong, Christine

Telomerase is active in a wide range of cancers and essentially confers unlimited proliferative capacity upon the cells. As such, factors that regulate telomerase activity are potential therapeutic targets. However, very little is known about the regulation of telomerase activity in normal or cancer cells.

Telomeric nucleoprotein structure and function is well conserved between fission yeast and mammals. Recent studies in mammalian cells indicate that the telomeric protein TPP1 is directly involved in both telomerase binding and enhancement of processivity. In S. pombe, telomerase associates with Tpz1 (the TPP1 ortholog) in cell extracts. However, this association is dependent upon the telomerase accessory protein, Est1, binding to the Tpz1-binding protein, Ccq1. Moreover, the current model suggests that recruitment of telomerase leads to activation. However, our results indicate that there are additional factors regulating telomerase activity after recruitment in S. pombe.

Our results indicate that Ccq1 not only acts as a site for Est1 binding and, therefore, telomerase recruitment: it can also modulate telomerase activity and/or processivity. We have found that the N-terminal of Ccq1 is required for telomerase activation in addition to recruitment. Furthermore, the C-terminal region of Ccq1 has the ability to restrict telomere lengthening to WT levels.

Previous work suggests that Trt1 can retain an association at the telomere independently of Est1 (Tomita and Cooper, 2008). We have found that Trt1 may directly bind to Tpz1, and that mutation of the predicted Trt1 binding domain results in telomere shortening.

Since Est1 is also required for telomerase activity in humans, we suggest a potential model of telomerase regulation involving both Est1 and Tpz1. Although an ortholog of Ccq1 has not yet been found in mammals, our results indicate that telomerase activity in fission yeast may also require interaction with the TPP1 ortholog, Tpz1.

Role of the F-box protein Pof8 in telomere length homeostasis

Hanna Amelina, Christine Armstrong, Siân Pearson, Vera Moiseeva, Kazunori Tomita

[a] *University College London, UCL Cancer Institute, UK*

Presented by: Tomita, Kazunori

Chromosome maintenance is a major issue for all dividing cells. Telomeres are known to protect chromosome ends from degradation as well as chromosome end-to-end fusions. However, chromosomes lose telomeric DNA progressively with every round of replication. Shortened telomeres elicit checkpoints that arrest the cell cycle so as not to lose the protection ability. To extend cell proliferation, germ cells and about 90% of cancer cells activate telomerase to replenish telomeric repeats. However, the basic mechanism of telomerase regulation largely remains a mystery.

In fission yeast, pof8 mutant cells maintain critically short telomeres, but which mechanism/pathway for telomere length homeostasis is compromised is not known. Pof8 is first reported as an F-box protein that binds to Skp1 and Cullin to form so-called the SCF complex. F-box protein family binds to the substrate, yet none of Pof8 substrate has been reported yet. Our genetics experiments suggested that Pof8 is involved in telomerase dependent telomere length homeostasis. We will discuss how Pof8 is involved in regulation of telomerase activity.

Fission yeast as a test tube for complex structures assembly

Paulo Duarte[a], Zita Carvalho-Santos[b], Claudia C. Bicho[c], Mónica Bettencourt-Dias[a]

[a] *Instituto Gulbenkian de Ciência, Oeiras, Portugal*
[b] *Champalimaud Foundation for the Unknown, Lisboa, Portugal*
[c] *Department of Cell & Tissue Biology, UCSF School of Dentistry, San Francisco, USA*

Presented by: Duarte, Paulo

The Centriole Basal Body (CBB) is a microtubule-based structure whose ancestral function is seeding the formation of cilia and flagella. CBBs are also found at the core of the animal centrosome where they work as the principal Microtubule Organizing Center (MTOC). CBBs usually assemble in close proximity to pre-existing centrioles. However, they can assemble in a CBB-free environment in what is called the de novo pathway. This self-assembly property suggests that CBBs could potentially be formed in a simple system just by adding the key components.

What is the minimal set of proteins required to form the CBB? Heterologous expression of different components can be used as a valuable tool to study the assembly dynamics of particular cellular structures. Therefore, we decided to use a simple biological model system where "core" mechanisms can be dissected. Schizosaccharomyces pombe not only has a simple MT cytoskeleton but also many experimental tools have already been developed. Additionally, no flagella, and consequently, no CBB structure is formed in the fission yeast. Interestingly, the genes encoding most of the already identified CBB components are also absent in the fission yeast.

In order to address the minimal set of proteins required for CBB biogenesis, we turned the fission yeast our test tube: we expressed four Drosophila Centriole components-coding genes in the fission yeast and found that these four proteins not only localize to the Spindle Pole Body (SPB), but also regulate the stability of MTs.

Despite their difference in structure, similar rules seem to govern animal and yeast MTOC assembly and separation, suggesting the existence of a conserved "core" program that ensures bipolar spindle formation.

We want to explore these findings and reveal core mechanisms regulating the localization and assembly of MTOC components. We also aim to uncover centriole unique properties, including MT organization and stability.

Nuclear microtubules and TOGs ensure accurate meiotic segregation through chromosomal repositioning

Yasutaka Kakui[a], Masamitsu Sato[b], Naoyuki Okada[c], Takashi Toda[d], Masayuki Yamamoto[e]

[a] *Chromosome Segregation Laboratory, Cancer Research UK, London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London, WC2A 3LY, UK*
[b] *Laboratory of Cytoskeletal Logistics, Department of Life Science and Medical Bioscience, Graduate School of Advanced Science and Engineering, Waseda University, 2-2 Wakamatsucho, Sinjuku, Tokyo, 162-8480, Japan*
[c] *Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo. 7-3-1 Hongo, Tokyo 113-0032, Japan*
[d] *Cell Regulation Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London, WC2A 3LY, UK*
[e] *Kazusa DNA Research Institute, 2-6-7 Kazusa-kamatari, Kisarazu, Chiba 292-0818, Japan*

Presented by: Kakui, Yasutaka

Position of chromosomes in the nucleus is differentiated to accomplish various cellular processes. During interphase of mitotic cell cycle, kinetochores, the platform for microtubule binding upon chromosome segregation, are attached to the Spindle Pole Body (SPB; a centrosome equivalent in fungi). This spatial proximity between the SPB and kinetochores is an elegant preparation for accurate segregation in mitosis, because microtubules emanated from the SPB easily capture adjacent kinetochores. In contrast to that, kinetochores are apart from the SPB for promoting pairing and recombination of homologous chromosomes, which facilitate generation of diversity among progenies during meiotic prophase. As cells no longer gain the spatial benefit of the proximity, this kinetochore positioning could be disadvantageous for subsequent meiotic segregation,. It remains largely unknown how cells overwhelm the potential risk of chromosome missegregaion after completion of recombination.

Here, our multicolour live-cell imaging reveals dramatic repositioning of kinetochores right before the onset of meiotic segregation. We discovered that the radial array of microtubules is assembled in the nucleus especially in this period and collect all the kinetochores for accurate meiotic segregation. Furthermore, TOG (Tumour Overexpressed Gene) family proteins, Dis1 and Alp14, play crucial roles for the chromosomal repositioning. We like to discuss the molecular mechanism to conquer the spatial disadvantage of kinetochores.

Mechanistic control of the Microtubule Organizing Center γ-tubulin ring complex: a fission yeast to human perspective

Zachary Olmsted, Timothy Riehlman, Andrew Colliver, Adam Winnie, Janet L Paluh

College of Nanoscale Science & Engineering, University at Albany - State University of New York, USA

Presented by: Olmsted, Zach

In eukaryotes the assembly of the mitotic spindle into a bipolar structure is a critical control point in chromosome segregation. The multi-protein γ-tubulin ring complex (γ-TuRC) acts as a microtubule organizing center (MTOC) assisting transformation of the microtubule cytoskeleton into a functional spindle as well participating in other key eukaryotic roles in immune cell targeting, neurogenesis and development. We identified the first mechanism of γ-TuRC MTOC regulation by a Kinesin-like protein (Klp). S. pombe Kinesin-14 Pkl1 binds the complex and removes γ-tubulin subunits to alter γ-TuRC function (Olmsted et al. 2013). We also analyzed human γ-tubulin small complex (γ-TuSC) proteins in fission yeast, expanding the repertoire of cross-species functional information for the MTOC (Riehlman et al. in revision). The γ-TuSC subunit of γ-TuRC provides the template for growth of uniform 13 protofilament microtubules. Our analysis supports the Kollman-Agard structure as a general eukaryotic model, while revealing species-specific protein constraints in the γ-TuSC between yeasts, or yeasts and man. The ability of mitotic Klps to coordinate MTOC function with microtubule-based events is not well understood. We determined that the primary role of Kinesin-5 Cut7 is to regulate Kinesin-14 Pkl1 function and that a double knockout of genes encoding these Klps is viable. Kinesin-6 Klp9 and Pkl1 functions are also balanced, regulating prometaphase spindle length. Our goal is to utilize MTOC and motor studies to master mitotic control and to apply our findings to cancer therapies. In addition we are using microtubule cytoskeleton proteins as a classic model for self-assembling nano to micro-scale networks towards development of new in vitro-based responsive biomaterials. Our research combines genetic analysis, site-directed mutagenesis, timelapse microscopy, cross-species studies, biochemical purification and nucleation assays, atomic force microscopy and nanotechnology.

A quantitative study on fission yeast growth and division behavior in response to nitrogen deprivation

Qi Liu, Chao Tang

Center for Quantitative Biology and School of Life Science, Peking University, 100871, Beijing, China

Presented by: Liu, Qi

Growth and division are two basic biological events in cells. Cells can adapt to and survive in various stressful environments by adjusting their growth and division behavior. It has been reported that after nitrogen deprivation, most of the proliferating fission yeast cells exit vegetative cell cycle through two rounds of cell division without growth. Given the individual diversity on cell size and in cell cycle progression, how different cells exit proliferation remains to be understood. Using time-lapse microscopy and quantitative measurements we find that there exists a correlation between cell length and division number after EMM-N shift. Long cells divide twice while short cells exhibit only one round division, and interestingly cells of mediate length divide “one and a half” times -- only one of the two daughters divide again after mother cell division. Furthermore, a few cells with greatly extended length but otherwise looked normal in morphology display a third division in granddaughter cells. This correlation is preserved in the population with mother cell length distribution greatly shifted by growth switch from YE5S to EMM medium. Upon the medium shift, the division numbers do not increase accordingly with the cell length, no more than three divisions were observed. We further investigated this correlation in cell size mutants. All of the five deletion mutants (rum1, wee1, cig1, cig2, and puc1) show the same growth and division phenotype as the wild type strain. We proposed two hypotheses for its mechanism based on our observation and the examination to these hypotheses is ongoing.

An auxiliary, RNAi-independent chromosome stability maintenance mechanism regulated by Translin (Tsn1) and TraX

Nasser Almobadel, Zafer Al-shehri, Natalia Gomez-Escobar, Alessa Jaendling, Ramsay McFarlane

North West Cancer Research Institute, UK

Presented by: McFarlane, Ramsay

Translin is a highly conserved protein which has been implicated in a numerous biological functions ranging from cancer-associated chromosomal rearrangements through to control of mRNA transport in the mammalian brain. It has a binding partner protein, Translin-associate factor X (TRAX), the stability of which is dependent upon Translin. Two recent finding have started to give a more detailed insight into the function of Translin and TRAX. Firstly, in Drosophila these two proteins were reported to make up the C3PO complex, which functions to facilitate the removal of the passenger strand in RNAi mediated gene silencing. Secondly, studies in Neurospora crasssa and mice have demonstrated that Translin and TRAX serve to endonucleolytically process immature RNAs, predominantly processing pre tRNAs to mature tRNA molecules; this finding has resulted in the suggestion that these conserved proteins are implicated in a range of biological function due to their tRNA processing activity. However, deletion of the genes encoding these two proteins in Schizosaccharomyces pombe does not result in any measurable phenotype in mitotically proliferating cells.

He we address the question of whether Translin and TRAX play a redundant role with RNAi regulators. S. pombe uses the RNAi machinery to maintain the heterochromatic state of key functional genomic regions, such as the centromeres. Using standard genetics we make some observations which indicate the existence of a novel auxiliary pathway for maintaining chromosomal stability in the absence of RNAi regulation. Interestingly, whilst TRAX does not seem to be essential for this novel pathway, loss of TRAX results in a suppression of the genome instability phenotype of an Argonaute-deficient mutant, a suppression which is Translin-dependent. This indicates that TRAX functions to repress a pathway that can partially substitute for Ago1 to maintain chromosome stability.

MAPK signalling regulates stress-dependent formation of RNA-granules in fission yeast

Mari Higa[a], Hajime Naruse[a], Yuna Ito[a], Akira Doi[a], Ryosuke Satoh[a,b], Ayako Kita[a], Reiko Sugiura[a]

[a] *Laboratory of Molecular Pharmacogenomics, School of Pharmaceutical Sciences, Kinki University, Japan*
[b] *Laboratory of Basic Biology, Institute of Microbial Chemistry, Japan*

Presented by: Higa, Mari

Mitogen-activated protein kinases (MAPKs), found in all eukaryotes, are signal-transducing enzymes playing a central role in a variety of biological processes. The Pmk1 MAPK is a homologue of ERK/MAPK in mammals and regulates cell integrity in fission yeast. We have been studying the Pmk1 MAPK signaling and identified several RNA-binding proteins as direct targets of the Pmk1 MAPK signaling pathway. These include rnc1+ encoding a KH-type RNA-binding protein and nrd1+ encoding an RRM-type RNA-binding protein (Nature 2003, Mol. Biol. Cell 2009). Nrd1 acts as an important regulator of the posttranscriptional expression of Cdc4 myosin in fission yeast. Notably, Pmk1 MAPK-dependent phosphorylation negatively regulates the RNA-binding activity of Nrd1, and Nrd1 can localize to poly(A)-binding protein (Pabp)-positive RNA granules in response to various stress stimuli in a Pmk1 MAPK mediated phosphorylation-dependent manner, thus suggesting that the phosphorylation of Nrd1 by MAPK enhances its localization to stress-induced cytoplasmic granules (Mol. Biol. Cell 2009; PLOS ONE 2012). In addition, Nrd1 deletion leads to defects in RNA granule formation, and overexpression of Nrd1 results in increased size and number of granules, indicating that Nrd1 regulates stress-induced RNA granule formation. Notably, Nrd1 binds to Cpc2 (fission yeast RACK) in a MAPK phosphorylation-dependent manner and Cpc2 regulates the formation of Nrd1-mediated stress granule formation. Thus, environmental stress promotes sequestration of the Nrd1/Cpc2 complex into stress granules, which may serve as a platform for the nucleation of Pab1-positive RNA granules. Thus, the phosphorylation-dependent sequestration of Nrd1 to stress granules might represent a mechanism to suppress the ability of Nrd1 to bind and stabilize target mRNAs such as Cdc4. In conclusion, our results indicate that Nrd1 plays a role in stress-induced granule formation, which affects stress resistance in fission yeast.

Inhibitory effect of Scoposide C, a triterpene glycoside on IMPDH activity in *Schizosaccharomyces pombe*

Nazli Arda[a], Semian Karaer[a], Aysegul Topal Sarikaya[b], Merve Yilmazer[a], Yagmur Kiraz[a], Nazli Boke Sarikahya[c], Suheyla Kirmizigul[c]

[a] *Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, 34134 Vezneciler, Istanbul, Turkey*
[b] *Yeni Yuzyil University, School of Medicine, Department of Molecular Biology and Genetics, 34010, Zeytinburnu, Istanbul, Turkey*
[c] *Ege University, Faculty of Science, Department of Chemistry, 35100, Bornova, İzmir, Turkey*

Presented by: Arda, Nazli

Scoposide C is an oleanane type triterpene glycoside, isolated from a n-BuOH fraction of the aerial part of *Cephalaria scoparia*, an endemic plant for Turkey. This compound was found to possess moderate antimicrobial activity. Here we aimed to investigate its inhibitory effect on inosine monophosphate dehydrogenase (IMPDH), which is regarded as an important target in antiviral, antibacterial, antiparasitic, immunosuppressive and anticancer drug discovery. A transformant, which overexpress gua1 gene that encodes excess IMPDH alike many tumor cells, was obtained by transformation of gua1 gene to host strain sp292h-, using pDUAL vector. Yeast cells (10^6 cells/mL) were treated with 200 µg/mL Scoposide C and 20 µg/mL mycophenolic acid (MPA), as a specific inhibitor, at the midlog phase for 24 h. Scoposide C inhibited cell viability by 25.1 % in host and by 48.3 % in transformant. It was more effective in reducing cell viability on both cell types than MPA, at least for the dose used in this study. IMPDH activity was also diminished by Scoposide C in both strains. It was reduced by 41.7 % in host and 36.2 % in transformant. The IMPDH inhibitory activity of Scoposide C in transformant cells was similar to that of MPA whereas it was grater than MPA in host cells. The results of this study provide the first evidence for the inhibitory effect of Scoposide C on IMPDH activity. As inhibitors of IMPDH are sought as potential pharmacological agents, Scoposide C seems to be a potential natural drug, which can be used in cancer as well as immunosuppressive chemotherapy.

Condensin *de novo* accumulation at the transcriptional active genes from metaphase to anaphase

Norihiko Nakazawa, Alejandro Villar-Briones, Kenichi Sajiki, Orie Arakawa, Xingya Xu, Mitsuhiro Yanagida

The G0 Cell Unit, Okinawa Institute of Science and Technology Graduate School, Japan

Presented by: Nakazawa, Norihiko

Condensin plays essential roles for chromosome dynamics and diverse DNA metabolism. Previously, we showed that condensin removes the replication protein A (RPA) and RNA molecules from single-stranded DNA through its reannealing activity *in vitro*. However, how the molecular activities of condensin contribute to chromosome segregation is largely unknown. Here, we report that *S. pombe* condensin accumulates at highly transcriptional active sites in both interphase and mitosis. This accumulation is found more prominent in chromatin immunoprecipitation-sequencing (ChIP-seq) of condensin (Cut14/SMC2), than in that of cohesin (Rad21) and topoisomerase II (Top2) proteins. Consistent with continuous requirement of condensin throughout mitosis, condensin *de novo* associates with the heat shock responsible genes from metaphase to anaphase and gradually accumulates around 3'-UTR region. Significantly, this accumulation is diminished in condensin temperature sensitive mutant *cut14-208*. This mutant neither affects the transcriptional activation nor the termination of heat shock responsible genes. These and other results suggest that condensin in mitosis associates with the highly transcribed regions and may resolve the RNA- and/or protein-bound obstructive sister chromatid DNAs into the smoothly-separable chromatid DNAs for faithful chromosome segregation during mitosis.

The Cdk8 module of the Mediator and its function during mitotic gene transcription

Gabor Banyai, Marcela Dávila López, Zsolt Szilagyi, Claes M Gustafsson

Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Gothenburg, Sweden

Presented by: Szilagyi, Zsolt

The Mediator complex is required for control of gene transcription in many eukaryotes. Its Cdk8 module contains four proteins, Med13, Med12, Cdk8 and CycC. This module and its association with the Mediator complex is highly conserved from yeast to man, and its components are found associated with a growing number of human diseases, including cancer. Our recent work has uncovered that the Mediator co-activator complex plays a direct role in the control of gene transcription at mitosis. Cdk8-dependent phosphorylation of the forkhead 2 (Fkh2) protein controls mitotic entry and mutations in Fkh2, which mimic protein phosphorylation, lead to premature mitotic entry.

We have now extended these previous studies and found that mutations affecting other components of the Cdk8 module have distinct effects on mitotic commitment and the timing of gene activation at mitosis. Whereas Cdk8 kinase inactivation delays mitotic entry, deletion of the Med12 and Med13 subunits have an opposite effect, i.e. advancing mitotic commitment through modulating the Y15 phosphorylation status of Cdc2. In addition, the periodic association of Cdk8 to mitotic promoters is lost in Med12 Med13 mutants. Our findings thus suggest that Med12 and Med13 regulate Cdk8 activity, which in turn influence mitotic commitment.

Based on these and other findings, we suggest that the Cdk8 module directly regulates Cdc2 activity in fission yeast. Our data also suggest that Mediator and the Cdk8 module provide a link that coordinates mitotic signaling required for a faithful mitotic progression with control of mitotic gene transcription.

Cbf11 is required for rDNA array stability

Martin Převorovský[a], María Rodríguez-López[b], Martina Oravcová[a], Pablo Hernández[b], Jürg Bähler[c], František Půta[a], Petr Folk[a]

[a] *Department of Cell Biology, Faculty of Science, Charles University in Prague, Viničná 5, 128 43 Prague 2, Czech Republic*
[b] *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Ramiro de Maeztu 9, 28040 Madrid, Spain*
[c] *Department of Genetics, Evolution & Environment and UCL Cancer Institute, University College London, Gower Street, London WC1E 6BT, UK*

Presented by: Převorovský, Martin

CSL (CBF1/RBP-Jκ, Suppressor of Hairless, LAG-1) protein family comprises well-established transcription factors that regulate metazoan development via the Notch signalling pathway. Cbf11 and Cbf12, the two S. pombe CSL paralogs, were previously shown to play antagonistic roles in the regulation of cell cycle progression and cell adhesion. Here we describe an unexpected role of these proteins in the maintenance of genome integrity.

Both Δcbf11 and Δcbf12 cells are sensitive to exogenous genotoxic stress, and Δcbf11 cells show pronounced nuclear defects, fragmented DNA and increased chromosome loss and Rad52 foci formation. Genome-wide ChIP-seq analyses demonstrate overlapping binding patterns of Cbf11 and Cbf12 in the vicinity of replication origins, including the rDNA ars3001, and at repetitive elements. Curiously, the rDNA array size is smaller in Δcbf11 cells compared to wild-type and shows high clonal variability. Notably, Cbf11 and Cbf12 interact physically and genetically with factors involved in rDNA biology.

Our data indicate a novel role for CSL transcription factors in the chromosome biology of fission yeast.

Towards a global perspective of cellular ageing

Charalampos Rallis, Luis López-Maury, Sandra Codlin, Danny Asher Bitton, Sophie Atkinson, Jürg Bähler

University College London, Department of Genetics, Evolution & Environment and Institute of Healthy Ageing, Gower Street, Darwin Building, London WC1E 6BT, UK

Presented by: Rallis, Charalampos

Aging is a multi-factorial process depending on both genetic and environmental factors that are remarkably conserved, and it is the biggest risk factor for a number of complex pathologies such as cancer and neurodegeneration. Research using unicellular organisms is a driving force in the still young research field. Fission yeast is increasingly used in lifespan studies and is bound to make unique contributions to defining both universal and specialized processes relevant to cellular aging. We have previously analysed the effects of rapamycin and caffeine, singly and combined, on fission yeast cells. The drugs led to several specific phenotypes caused by TORC1 signaling inhibition, including prolonged chronological lifespan, inhibition of global translation, cell growth and division, and reprogramming of genome expression mimicking nitrogen starvation. We screened the deletion library comprising ~84% of all non-essential genes for drug-resistant mutants identifying 33 genes. Among the corresponding mutants, 5 showed shortened and 21 showed increased lifespans; 14 of the latter mutants showed no further lifespan increase with rapamycin. Intriguingly, amid the other mutant phenotypes, slow growth did correlate with longevity but oxidative stress resistance did not. We analyzed cellular, translational, and transcriptome aspects of chronological lifespan, revealing the changes occurring at different layers of gene regulation. We also performed unbiased screens to systematically reveal the non-essential, pro-aging and anti-aging fission yeast genes. These studies provide a rich resource and the basis to guide aging research in fission yeast and beyond.

The role of MRN and other DNA repair pathways in removal of the replication-blocking nucleoside analogue Gemcitabine from DNA

Andrea Keszthelyi, Marie Gasasira Uwamahoro, Ellen Vernon, Rick Beardmore, Edgar Hartsuiker

NWCR Institute, Bangor University, UK

Presented by: Hartsuiker, Edgar

Replication fork blockage interferes with cell division and contributes to genome instability. While DNA damage response mechanisms that deal with blockages caused by obstacles on the template (e.g. DNA damage or tightly bound proteins) or nucleotide pool depletion (e.g. after hydroxyurea treatment) have been more widely studied not much is known about replication blockage caused by integration of modified nucleotides into the nascent strand during DNA replication. The Mre11/Rad50/Nbs1 (MRN) protein complex is highly conserved among eukaryotes and together with CtIP is involved in a wide range of early responses to DNA damage, often mediated by its role in DNA end processing. Central to DNA-end processing are the Mre11 single strand endonuclease and 3'-5' exonuclease activities. While these activities have been implicated in the repair of DNA double strand breaks (DSBs), their contribution to other DNA damage responses remains largely unknown. We present data suggesting that MRN of Schizosaccharomyces pombe is able to remove the replication-blocking nucleoside analogue 2'-2' difluorodeoxycytidine (Gemcitabine, dFdC) from the nascent strand during DNA replication. An mre11 nuclease dead mutant (mre11-D65N) is also defective in dFdC removal, suggesting that the nuclease activity is important for dFdC removal. We have also extended our studies to MRN-independent repair pathways, and found that proteins involved in Nucleotide Excision Repair (NER) and Base Excision Repair (BER) contribute to dFdC removal. Finally, we obtained data suggesting that dFdC treatment is mutagenic and that dFdC-induced mutation rates are further increased in rad32mre11-D65N. Mutation rates are strongly reduced in a rev3Δ mutant.

Meiotic recombination is regulated by the posttranslational modification SUMO

Lærke Holm[a], Gerry Smith[b], Genevieve Thon[a]

[a] *Copenhagen University, Denmark*
[b] *Fred Hutchinson Cancer Research Center, USA*

Presented by: Holm, Lærke

Post-translational modifications of proteins can lead to degradation, subcellular translocation, or altered interaction with partner proteins. The small ubiquitin-related modifier SUMO (Pmt3 in S. pombe) is such a modification involved in numerous cellular processes. Pmt3 attachment to target proteins can be catalyzed by the SUMO E3 ligase Pli1. Target proteins for Pmt3 are many; however, Pmt3 and Pli1 have a meiosis-specific function regulating meiotic recombination. We found that in meiosis the effect of Pli1 in the rDNA repeats differs from its effect in the bulk of the genome. We have found that meiotic recombination in euchromatic regions is stimulated by Pli1, as reported previously [a,b,c] and by Pmt3, but meiotic recombination is inhibited by Pli1 in the rDNA arrays. The inhibition in the rDNA appears to occur through Pli1 preventing double-strand break (DSB) formation by Rec12. In euchromatic regions DSBs are formed and repaired with the same efficiency in pli1Δ and wild-type cells. DSBs can be repaired using either the sister chromatid or the homolog as template [d,e], but only repair with the homolog produces an observable recombinant. We speculate that Pli1 is involved in determining whether a DSB in euchromatic regions is repaired using the sister chromatid or the homolog as template. A direct repeat ade6 assay showed that during mitotic growth Pli1 inhibits intrasister chromatid recombination and unequal sister chromatid exchange. However, the accuracy of this assay was not sufficient to determine whether Pli1 has the same role in meiosis. We are now investigating whether the pairing of homologs is affected in pli1Δ mutant cells.

a Watts et al., BST, 2007
b Spirek et al., Chromosoma 2010
c Ellermeier et al., PNAS, 2010
d Cromie et al., Cell, 2006
e Hyppa and Smith, Cell, 2010

Proteasome-mediated degradation of replisome components facilitates faithful replication of the genome

Laura Roseaulin[a], Chiaki Noguchi[b], Takashi Toda[c], Eishi Noguchi[b]

[a] *Drexel University College of Medicine, USA (present address: Curie Institute, France)*
[b] *Drexel University College of Medicine, USA*
[c] *Cancer Research UK, London Research Institute, UK*

Presented by: Noguchi, Eishi

Replication stress interferes with the normal progression of the replication fork. Under these conditions, cells activate the replication checkpoint to coordinate DNA repair with cell cycle arrest. The current understanding is that, in response to replication block, this checkpoint stabilizes replication forks and replisome structures to achieve accurate DNA replication. However, it would also be advantageous for the cell to stop DNA replication and reorganize the replisome structures when conditions are not ideal, but such mechanisms have not been explored. In our present study, we describe mechanisms that regulate stability of replisome components in response to replication stress. We found that Pol2 (a major DNA polymerase) and Mrc1 (a mediator of the replication checkpoint) undergo proteasome-dependent degradation during DNA replication. Our investigation has revealed the involvement of the SCF-Pof3 ubiquitin ligase in Pol2 and Mrc1 degradation, which occurs in a chromatin-enriched fraction. Interestingly, degradation of Pol2 and Mrc1 are also dependent on a Polo-like kinase (Plo1) and Cdc7-related kinase (Hsk1), respectively. Considering that these kinases regulate cell cycle progression, coordinated regulation of Pol2 and Mrc1 levels during the cell cycle plays an important role in preservation of genomic integrity. Indeed, when we forced cells to stabilize replisome components, cells underwent abnormal DNA replication, leading to mitotic catastrophes. Our study provides novel mechanistic insights into understanding how the replication machinery is regulated to achieve faithful duplication of the genome upon replication stress.

Arsenic genotoxicity in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

Ireneusz Litwin[a], Dorota Dziadkowiec[b], Robert Wysocki[a]

[a] *Institute of Experimental Biology, University of Wroclaw, Poland*
[b] *Faculty of Biotechnology, University of Wroclaw, Poland*

Presented by: Wysocki, Robert

Arsenic is a well-established human carcinogen of poorly understood mechanism of genotoxicity. It is generally accepted that arsenic acts indirectly by generating oxidative DNA damage that can be converted to replication-dependent DNA double-strand breaks (DSBs) as well as by interfering with DNA repair pathways and DNA methylation. Here we show that in yeast species arsenic causes DSBs in all phases of the cell cycle. This is accompanied by DNA damage checkpoint activation resulting in Mec1 and Tel1-dependent phosphorylation of histone H2A (yeast H2AX) and Rad53 kinase that leads to cell cycle delays in S and G2/M phases. Arsenic-induced DNA breaks are processed by homologous recombination (HR) as shown by Rfa1 and Rad52 nuclear foci formation and requirement of HR proteins for survival of S. cerevisiae and S. pombe cells during arsenic exposure. Interestingly, in G1 phase arsenic induces phosphorylation of H2A and Rad53 as well as formation of Rfa1 foci only in the absence of the Yku70-Yku80 complex which normally binds to DNA ends and inhibits resection of DSBs. This strongly indicates that DSBs are produced by the action of arsenic in this phase independently of replication but DNA ends are protected by Yku70-Yku80 and thus invisible for the checkpoint response. Importantly, arsenic treatment does not lead to enhanced production of reactive oxygen species or accumulation of oxidative DNA lesions in yeast. We show further that arsenic greatly sensitizes both budding and fission yeast to phleomycin as simultaneous treatment results in profound accumulation of DSBs. In sum, we propose that arsenic is a direct DSB inducer and suggest that in addition to its usage in the therapy of acute promyelocytic leukemia, arsenic could be combined with phleomycin-like antitumor drugs as a sensitizer to treat other types of cancer.

Short telomeres lower the viability of *Schizosaccharomyces pombe* quiescent cells

Stephane Coulon[a], Julien Audry[a], Benoit Arcangioli[b], Vincent Geli[a]

[a] *Cancer Research Center of Marseille, INSERM UMR1068, France*
[b] *Genome Dynamic, Pasteur Institute Paris, France*

Presented by: Coulon, Stephane

Schizosaccharomyces pombe can be experimentally maintain for weeks in quiescence in the absence of nitrogen and therefore appears as a great model to study DNA repair during quiescence. Published and preliminary results indicate that the mechanisms required for genetic stability and cell viability in the absence of cell division differs from those of proliferating cells (Ben Hassine and Arcangioli, EMBOj 2009). Telomeres contain repeated sequences rich in guanines. These telomere G-rich sequences are highly sensitive to damage by oxidative stress or ultraviolet-irradiation. This raises the question of the stability of telomeres in quiescence.

We have investigated how eroded telomeres are processed during quiescence and the fate of quiescent cells harbouring these short telomeres. By deleting the RNA component of the telomerase, we have monitored the progressive telomere shortening after successive dilution of cells growing in minimum medium. Thanks to this appraoch we have been able to program the entry in G0 of presenescent cells that have moderately short or short telomeres. We observed that telomeres were rearranged with high efficiency independently of genome replication. This rearrangement of telomeres increased proportionally with the shortening of telomeres and the incubation time in quiescence. Remarkably, the viability of cells in G0 decreases when telomeres shorten indicating that unstable telomeres might trigger a programmed cell death. Our results indicate that addition of caffeine lowers the telomeric rearrangements and restores cell viability to some extent, suggesting that checkpoint pathways may control these mechanisms.

A novel role for the microtubule cytoskeleton in fission yeast DNA repair

Jacob Zhurinsky[a], Antonio Torres-Mendez[a], Fred Chang[b], Rafael Daga[a]

[a] *Centro Andaluz de Biologia del Desarrollo, Univ. Pablo de Olavide, Seville, Spain*
[b] *Columbia University, NY, USA*

Presented by: Zhurinsky, Jacob

DNA damage repair is key to maintaining genomic integrity and preventing cancer. While biochemical mechanisms involved in DNA damage signaling and repair have been well characterized, the role of nuclear architecture and in vivo DNA dynamics in damage response is poorly understood. In the fission yeast Schizosaccharomyces pombe, cytoplasmic microtubule bundles that are attached to the nuclear envelope and spindle pole body move the nucleus in an oscillatory manner during interphase. Here, we show that these cytoplasmic microtubules are required for efficient DNA repair. Time lapse imaging show that chromosomal movement and pairing and the dynamics of DNA repair centers during interphase are microtubule-dependent. We propose that cytoplasmic microtubules exert forces on nuclear envelope proteins that move the chromosomes, and that these movements affects steps in DNA repair such as homology search during recombination. Our results reveal an unexpected role of cytoplasmic microtubules in genomic integrity.

Functional characterization of Upf1 targets in *Schizosaccharomyces pombe*

Ana M. Matia-González[a], Ayesha Hasan[b], Gøril H. Moe[a], Juan Mata[b], Miguel A. Rodriguez-Gabriel[a]

[a] *Centro de Biología Molecular Severo Ochoa. UAM- CSIC. 28049, Madrid, Spain*
[b] *Department of Biochemistry. Hopkins Building. University of Cambridge, CB2 1QW, UK*

Presented by: Rodriguez-Gabriel, Miguel

Nonsense-mediated mRNA decay (NMD) is a highly conserved mechanism of mRNA degradation. NMD eliminates mRNAs containing premature termination codons (PTCs), preventing the production of truncated proteins with possible deleterious effects. However, there is mounting evidence that NMD factors, like Upf1, Upf2 and Upf3, participate in general regulation of gene expression, affecting the expression of genes lacking PTCs. We have used the fission yeast Schizosaccharomyces pombe to identify mRNAs directly regulated by NMD. Using a combination of genetic and biochemical approaches, we have defined a population of fission yeast mRNAs specifically regulated by Upf1. We show that other components of the Upf complex, Upf2 and Upf3, are required for binding of Upf1 to its RNA targets and for the proper response of fission yeast to oxidative stress. Finally, we investigated the physiological importance of this phenomenon, and demonstrate that the Upf1-dependent down-regulation of some of its direct targets is necessary for normal resistance to oxidative stress.

Fission yeast Reb1 more than a replication termination protein

Maria Rodriguez-López[a,b], Martin Převorovský[c], Jorge B Schvartzman[b], Dora B Krimer[b], Jürg Bähler[a], Pablo Hernández[b]

[a] *Research Department of Genetics, Evolution and Environment, University College London, UK*
[b] *Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28040 Madrid, Spain*

[c] *Department of Cell Biology, Faculty of Science, Charles University, 128 43 Prague 2, Czech Republic*

Presented by: Rodriguez-López, Maria

The fission yeast Reb1 protein was formerly known to bind to two specific sites near the 3´-end of the rRNA genes participating in the termination of rDNA transcription by RNA polymerase I, and in the blockage of replication forks approaching from the direction opposite to transcription.

This protein also binds in vivo and in vitro to a sequence upstream the promoter of *ste9*+ participating in the up-regulation of the expression of *ste9*+ under nitrogen starvation. In response to nutritional stresss Ste9 activates the anaphase-promoting complex/cyclosome targeting B-type cyclin for proteasome in G1. This enables cells to proceed through sexual differentiation, mating and spore formation to resist the harsh environment. Deleting *reb1*+ reduces the cell´s capacity to arrest in G1 leading to a diminish fertility.

We have also found that Reb1 becomes necessary to maintain cell viability when cells need to lengthen the G1 phase to reach the minimal size to begin replication, as in the termosensitive *wee1-50* mutant. The combined *reb1*Δ *wee1-50* mutants die of mitotic disaster after a couple of rounds of premature entrance into replication and mitosis. Our results demonstrate that Reb1 is also required for the viability of cells with defects on proteins required for the initiation of replication, as in *cdc10-129* mutants. These data indicates that Reb1 seems to be required to avoid a premature passage through start.

We are currently working on a high-throughput screening to look for other Reb1 binding sites on the fission yeast´s genome. Our preliminary study has revealed that Reb1 binds upstream many genes, preferentially at the vicinity of the transcription start sites. Indicating that Reb1 may be acting as transcriptional regulation protein for multiple genes.

Capturing spontaneous telomere fusions in fission yeast

Jose Escandell Planells, Hugo Almeida, Miguel Godinho Ferreira

Instituto Gulbenkian de Ciência, Oeiras, Portugal

Presented by: Escandell Planells, Jose

Telomeres are specialized structures at the end of the chromosomes composed by a repetitive DNA sequence and a specific protein complex, the shelterin. These structures are critical to protect the linear chromosomes from erosion and end-joining. Telomere dysfunction can occur as a consequence of telomere shortening or after the loss of telomeric components leading to telomere-to-telomere fusions, responsible for genomic instability. Thus, maintaining telomere integrity is fundamental in order to prevent cancer development.

We recently developed a novel assay to study, in a quantitative manner, telomere end-to-end fusions [1]. In normal unperturbed cells, this is rare and understudied event that can only be detected due to the assay's positive selection nature. Using this system, we now show that wt cells undergo replication stress at telomeres and how this process induces genome instability trough telomere nonhomologous end joining (NHEJ).

Levels of expression of the telomere protein Taz1 modulate chromosome-end fusions and genome stability. Increasing the number of telomeres in normal cells results in limiting levels of Taz1 that, in turn, provokes telomere-to-telomere fusions. In addition to extra telomeres, we address the frequency of telomere end-joining in replication defective rqh1 cells and rap1Δ mutants.

[1] H. Almeida and M. Godinho Ferreira, "Spontaneous telomere to telomere fusions occur in unperturbed fission yeast cells.," Nucleic Acids Res, 41(5): 3056-67, Jan. 2013.

Pdc1 functions in the assembly of P-bodies in *Schizosaccharomyces pombe*

Chun-Yu Wang[a], Wen-Ling Chen[a], Shao-Win Wang[a]

Institue of Molecular and Genomic Medicine, National Health Research Institutes, 35 Keyan Road, Zhunan Town, Miaoli County, 350, Taiwan

Presented by: Wang, Shao-Win

P-bodies are cytoplasmic RNA granules containing the Dcp1-Dcp2 decapping-enzymes where mRNA decay occurs. Here we described the characterization of P-bodies in the fission yeast Schizosaccharomyces pombe. Most information on the property and function of P-bodies stems from studies in the distant related budding yeast Saccharomyces cerevisiae. In yeast, Edc3 was identified as an essential component in P-body assembly. However, we found that, unlike in S. cerevisiae, fission yeast Edc3 is dispensable for P-body formation. Pdc1, a novel partner of the decapping-enzyme, required for the assembly of fission yeast P-body was identified. Pdc1 interacts with Dcp2 through its C-terminal and contains a colied-coli region for self-interaction and P-body formation. In line with the model that, Pdc1 acts as a scaffold protein cross-bridging different components to stimulate P-body assembly, additional protein-protein interactions can be demonstrated between Pdc1 and components such as Edc3 and Ste13. Although not required for the interaction between Dcp1 and Dcp2, our data suggests that Pdc1 acts as a functional homologue of Edc4, a third component of the high eukaryote decapping-enzymes that is thought to be absent from fungi. Similar results were found in human HeLa cells, depletion of Edc4, but not Edc3, leads to decrease of P-bodies indicating that, in fission yeast through mammals, the essential function of Edc3 in P-body assembly was substituted with the development of Edc4-like proteins. Our results highlight the diverse P-body protein composition between different species and might help to provide insight into their evolutional path.

P-TEFb/Cdk9 promotes opposing functions of Rtf1 and the core PAF complex in transcription elongation

Jean Mbogning, Stephen Nagy, Viviane Page, Jillian Burston, Beate Schwer, Stuart Shuman, Robert Fisher, Pierre-Etienne Jacques, Jason Tanny

McGill University, Canada

Presented by: Tanny, Jason

Positive transcription elongation factor b (P-TEFb) is a highly conserved cyclin-CDK complex that promotes elongation by RNA polymerase II, mRNA processing, and chromatin modification. P-TEFb-dependent chromatin modifications, including mono-ubiquitylation of histone H2B (H2Bub1), can in turn oppose P-TEFb activity, suggesting that they comprise an auto-regulatory mechanism that limits P-TEFb function during RNAPII elongation. The molecular details of the regulatory interplay between P-TEFb and H2Bub1 are unknown. Here we show that the opposing functions of P-TEFb and H2Bub1 in the model eukaryote Schizosaccharomyces pombe are mediated by distinct subunits of the Polymerase Associated Factor (PAF) complex. We find that the "core" S. pombe PAF complex is composed of Paf1, Leo1, Cdc73, and Tpr1/Ctr9; Rtf1, which co-purifies with PAF in budding yeast, is biochemically distinct. Furthermore, whereas Rtf1 recruitment to transcribed genes requires phosphorylation of Spt5, core PAF recruitment instead depends upon phsophorylation of the Rpb1 C-terminal domain repeat at serine 5, suggesting that core PAF and Rtf1 engage distinct P-TEFb targets on chromatin. Although core PAF and Rtf1 are both necessary for H2Bub1 formation, they have different functions: core PAF mutants (particularly cdc73Δ and tpr1Δ) phenocopy reduction of P-TEFb activity, whereas rtf1Δ phenocopies the loss of H2Bub1 or its cognate enzymatic machinery. Finally, we find opposing genetic interactions between core PAF and either Rtf1 or the H2Bub1 machinery, suggesting that the opposing functions of P-TEFb and H2Bub1 are mediated by core PAF and Rtf1. Our data provide mechanistic insight into how P-TEFb activity can direct distinct and opposing functions to ensure optimal RNAPII elongation.

The telomere bouquet regulates meiotic kinetochore assembly

Michael Klutstein, Alex Fennell, Julia Promisel Cooper

Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3LY, UK

Presented by: Klutstein, Michael

The role of the conserved meiotic telomere bouquet has been enigmatic for over a century. We showed previously that disruption of the fission yeast bouquet impairs spindle formation in about half of meiotic cells. Surprisingly, we find that bouquet deficient cells with a functional meiotic spindle have further meiotic defects including chromosomes that delay or fail to achieve spindle attachment, suggesting an impairment of kinetochore function. The kinetochore proteins Mis6 and Dad1, as well as the centromeric histone H3 variant Cnp1, fail to co-localize with those centromeres that display spindle attachment defects in the absence of the bouquet. The HP1 ortholog Swi6 also fails to localize to these centromeres, suggesting that a defect in peri-centromeric heterochromatin formation underlies the kinetochore defects. Our data suggest a model in which the transient co-localization of telomeres and centromeres during initial stages of bouquet formation creates an environment conducive to robust heterochromatin formation and centromere assembly. We further show that meiotic centromeres are more sensitive to heterochromatin impairment than mitotic centromeres, explaining why the telomere bouquet may be critical in the establishment of this conductive microenvironment specifically in meiotic cells. To further explore the function of this microenvironment, we investigated the phenotype of cells harboring circular (telomere-less) chromosomes lacking the bouquet. Insertion of an internal telomere repeat stretch on one of the circular chromosomes rescues centromere function specifically on the chromosome carrying the telomere stretch, verifying that proximity of a meiotic centromere to a functional telomere and to the bouquet promotes its assembly.

Analysis of DNA re-replication in single cells

Manuel Ramírez, Marianna Rapsomaniki, Nickolaos Nikiforos Giakoumakis, Stavros Taraviras, Zoi Lygerou

Laboratory of Biology, School of Medicine, University of Patras, Rio, Patras, Greece

Presented by: Ramírez, Manuel

In every cell cycle, DNA has to be copied once and only once before cell division. To prevent any region of the genome from being replicated a second time, cells have developed multiple overlapping control mechanisms which ensure that origins of replication can only fire once per S phase and that passively replicated origins cannot get activated. These controls can be overriden by certain mutations affecting proteins involved in the initiation of DNA Replication. For example, the over-expression of the pre-replication complex component Cdc18 leads to DNA re-replication as origins fire repeatedly. These cells show a typical elongated cdc phenotype and their DNA content is several folds higher than a wild type strain. Previous works with these mutants, based on microarray analysis of populations of yeast cells, have shown that increase in DNA content is not homogeneous throughout the genome, but some regions show a higher number of copies than others.

Our research analyzes DNA replication and the effects of DNA re-replication at the single-cell level using imaging techniques. The strategy for this analysis involves tagging specific regions of the genome with the lac operator (lacO) sequence in mutants that express Cdc18 under the control of the nmt1 promoter. These cells also express the lac inhibitor protein (lacI) fused to GFP, which binds to the tagged region. With this system, DNA replication and re-replication events can be followed at the single cell level with high spatio-temporal resolution. The analysis of how uncontrolled replication affects different regions of the genome will provide useful information about the mechanism of re-replication in fission yeast.

Centromere integrity is preserved by Rad51-dependent recombination in fission yeast

Faria Zafar, Atsushi Onaka, Akiko Okita, Rei Asai, Takeshi Shitanda, Tatsuro Takahashi, Hisao Masukata, Takuro Nakagawa

Department of Biological Science, Graduate School of Science, Osaka University, Japan

Presented by: Nakagawa, Takuro

An intriguing feature of centromere observed in many eukaryotes is the presence of DNA repeats that is a potential threat to the genome integrity as it causes gross chromosomal rearrangements (GCRs) such as deletion and translocation. In fission yeast, spontaneous GCR between the inverted repeats in centromere produces the isochromosome in which one of its arms is replaced by a copy of the other. Paradoxically, a deletion of Rad51 increases the formation of isochromosomes, indicating that Rad51 is important to maintain the integrity of centromere. However, the molecular mechanism behind that remains unclear. To assess recombination between the centromere repeats, we introduced a pair of ade6 heteroalleles into centromere 1 and detected spontaneous formation of Ade prototrophs. We found that Rad51 is essential for the recombination in centromere, and that the recombination is rarely associated with crossover as compared to the arm region. A deletion of Rad51 increased the fraction of crossovers among the recombinants, suggesting that crossovers and isochromosomes are different outcomes of the same Rad51-independent reaction. Interestingly, a mutation of the H3K9 methyltransferase Clr4 or the centromere-specific H3 variant CENP-A did not affect the crossover suppression. However, a mutation of the centromere-specific histone-fold protein CENP-S increased crossovers although the requirement of Rad51 was not relieved. These data suggest that Rad51 with the aid of CENP-S promotes noncrossover-type of recombination, thereby preventing the isochromosome formation.

Role of the Ccr4-Not complex in the Spc1-dependent stress response

Marina Portantier[a], Elena Hidalgo[b], Miguel Ángel Rodríguez-Gabriel[a]

[a] *Centro de Biología Molecular Severo Ochoa, UAM-CSIC. Madrid, Spain*
[b] *Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, Barcelona, Spain*

Presented by: Portantier, Marina

MAPK pathways are the principal connection between external stimuli and internal responses in all eukaryotic cells. The MAPK pathway of Spc1, p38 ortholog in mammals, plays an important role in the response to stress in S. pombe. This pathway has been well studied in fission yeast but there are still open question about its regulation and especially how the pathway achieves a specific response to the variety of stimuli. Upon oxidative stress, the MAPK Spc1 and the RNA binding protein Csx1 present a coordinated response. Looking for candidates that interact with Spc1 and Csx1, we observe that they interact with components of the Ccr4-Not complex in TAP purification assays. This complex has gained importance in the last years because it is thought to be a global regulator of gene expression and it is conserved along all eukaryotes. Ccr4-Not complex regulates processes like mRNA synthesis, mRNA degradation and protein degradation and interacts with other important cell complexes like SAGA, PolII, exosome and the proteasome. It is formed by nine core subunits, a Ccr4 group (Ccr4, Caf1) involved in deadenylation and a Not group (Not1 (scaffold), Not2, Not3, Not4) involved in transcripcional regulation and protein modification, a RNA binding protein Caf40, and Caf130 only present in S. cerevisiae. Other proteins seem to be part of the complex like Caf4, Caf16, Btf3 and Dhh1.

We show the results concerning Ccr4-Not complex regulation of the Spc1-dependent stress response. Several components of the complex present stress sensitivity and regulate the expression of Spc1- dependent stress genes. The mechanism seems to be at the level of transcription elongation since some of the components regulate the binding of PolII to stress genes and are sensitive to elongation inhibition drugs. At the moment we are looking for the functional link between Ccr4-Not complex and Spc1 MAPK to elucidate how is the regulation of the complex in response to stress.

Fission yeast Mhf1 (CENP-S) and Mhf2 (CENP-X) play a role in genome stability

Marta Tormos-Pérez, Nathalia Chica, Livia Pérez-Hidalgo, Javier Botet, Sergio Moreno

Instituto de Biología Funcional y Genómica (IBFG), CSIC/Universidad de Salamanca, 37007 Salamanca, Spain

Presented by: Tormos-Pérez, Marta

Our laboratory is interested in understanding the complex molecular mechanisms that regulate cell division and its coordination with cell growth and DNA replication in eukaryotes. We have undertaken a functional genomic approach to identify novel cell cycle regulators and gain greater insight into the mechanisms that control cell cycle progression in fission yeast. For this purpose we have used a synthetic lethal approach (SpSGA) to investigate the genetic interactions between the collection of non-essential fission yeast mutants (Bioneer version 3.0) and the query mutant strains wee1-50 and cdc2-3w that have wee phenotypes characterized by a short G2 phase and small cell size.

We identified mhf1Δ and mhf2Δ among the 70 mutants that displayed negative genetic interactions with wee1-50 and cdc2-3w. Mhf1 (CENP-S) and Mhf2 (CENP-X) are two histone-fold proteins, highly conserved from yeast to mammalian cells, that play an only partially understood role in the kinetochore architecture and kinetochore-microtubule dynamics. In mammalian cells MHF1 and MHF2 are also involved in DNA repair, specifically in the Fanconi anaemia pathway. For these reasons, we are functionally characterising these proteins in fission yeast. Our preliminary results show that both Mhf1 and Mhf2 are required for proper chromosome segregation during mitosis and meiosis. We have observed that the mutants lacking mhf1+ or mhf2+ show genomic instability and a significant increase in the rate of chromosome loss. We are now beginning to understand the role of these proteins in the maintenance of genome stability during chromosome segregation at cell division and during DNA repair/remodelling processes upon replicative stress.

Characterization of the exoribonuclease Dis3L2 discloses a novel degradation pathway in eukaryotes

Michal Malecki[a], Sandra Viegas[b], Tiago Carneiro[c], Pawel Golik[d], Clementine Dressaire[b], Miguel G. Ferreira[c], Cecilia M. Arraiano[b]

[a] *1. Instituto de Tecnologia Quimica e Biologica (ITQB) Oeiras, Portugal*
2. Institute of Genetics and Biotechnology, University of Warsaw, Poland
3. Current address: University College London, Department of Genetics, Evolution & Environment, London, UK
[b] *Instituto de Tecnologia Quimica e Biologica (ITQB) Oeiras, Portugal*
[c] *Instituto Gulbenkian de Ciencia (IGC) Oeiras, Portugal*
[d] *Institute of Genetics and Biotechnology, University of Warsaw, Poland*

Presented by: Malecki, Michal

Two general pathways of mRNA degradation were identified using S. cerevisiae model. First step in both is transcript deadenylation, after which mRNA can be degraded either in 5'-3' direction by Xrn1 exonuclease or in 3'-5' direction by the exosome complex. Exosome complex active subunit Dis3/Rrp44 – is the 3'-5' exonuclease which contains also endonucleolytic activity. Recently it was shown that in higher eukaryotes, in contrast to budding yeast, there is more than one copy of DIS3 encoded in the genome. In humans three different DIS3 isoforms share different localization, biochemical properties and functions.

In this work we identified a novel S. pombe RNA exonuclease. Our extended phylogenetic analyses prove that identified nuclease belongs to the same protein family as human Dis3L2, and that members of this family are conserved throughout eukaryotes with the exception of some fungi species including S. cerevisiae. Fission yeast Dis3L2 localizes in the cytoplasm where it coexists with the cytoplasmic exosome but do not co-localize or co-purify with the exosome complex. Our in vitro and in vivo data suggest that in S. pombe Dis3L2 is involved in the cytoplasmic mRNA degradation, it works independently from the exosome complex and degrades specifically uridylated RNAs. This indicates that in S. pombe and possibly in other eukaryotes, in contrast to the situation in S. cerevisiae, mRNAs can be degraded either in 5-3' direction by Xrn1 exonuclease, or by 3-5' pathway by either exosome complex or Dis3L2 exonuclease.

Involvement of *rrp1+* and *rrp2+* in the Swi5/Sfr1- and Srs2 dependent pathway in response to DNA damage and replication inhibition in *Schizosaccharomyces pombe*

Karol Kramarz[a], Karolina Kanik[a], Piotr Wiśniewski[b], Antony M. Carr[c], Dorota Dziadkowiec[b]

[a] *Faculty of Biotechnology, Faculty of Biological Sciences, University of Wrocław, Poland*
[b] *Institute of Low Temperature and Structure Research, Polish Academy of Sciences, Wrocław, Poland*
[c] *Genome Damage and Stability Centre, University of Sussex, United Kingdom*

Presented by: Dziadkowiec, Dorota

Previously we have shown that Rrp1 and Rrp2 proteins are required for the Sfr1/Swi5-dependent branch of Homologous Recombination (HR) in S. pombe. Here we demonstrate using a yeast 2-hybrid approach that Rrp1 and Rrp2 can interact with each other and with Swi5, an HR mediator protein. Rrp1 and Rrp2 form co-localizing MMS-induced foci in nuclei, further suggesting they function as a complex. To place the Rrp1/2 proteins within one of HR sub-pathways, we carried out epistasis analysis between mutants devoid of Rrp1/2, Rad51 (recombinase), Swi5 and Rad57 (HR-mediators) and the anti-recombinogenic helicases Srs2 and Rqh1. We confirm that Rrp1 and Rrp2 act together with Srs2 and Swi5 and independently of Rad57 and also show that Rqh1 acts independently of Rrp1/2. Mutants devoid of Srs2 are characterized by elevated recombination frequency with a concomitant increase in the percentage of conversion-type recombinants. Strains lacking Rrp1 or Rrp2 did not show a change in HR frequency, but the number of conversion-type recombinants was increased, suggesting a possible function for Rrp1/2 with Srs2 in counteracting Rad51 activity. We propose a model based on our data placing Rrp1 and Rrp2 together with Swi5 and Srs2 in a synthesis-dependent strand annealing (SDSA) HR repair pathway.

Red5: an essential factor for selective elimination of meiotic mRNAs in vegetative fission yeast

Tomo Sugiyama[a], Nobuyoshi Watanabe[b], Eri Kitahata[b], Tokio Tani[b], Rie Sugioka-Sugiyama[c]

[a] *Life Science Center of Tsukuba Advanced Research Alliance, Univ. of Tsukuba, Japan*
[b] *Department of Biological Sciences, Faculty of Science, Kumamoto University, Japan*
[c] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan*

Presented by: Sugiyama, Tomo

Meiosis is the process of cell division that produces gametes from germ cells. Meiosis involves (1) the reduction of chromosome number by half and (2) the diversification of genetic information by meiotic recombination. The fission yeast Schizosaccharomyces pombe is an excellent model organism with which to study sexual differentiation, that is, meiosis. Although meiosis in S. pombe was long believed to be regulated only at the transcriptional and post-translational levels, at least some of numerous meiosis-upregulated genes are transcribed even in vegetative cells, and these transcripts are selectively degraded to prevent the ectopic expression of meiotic mRNAs. Mmi1, an RNA-binding protein with a YTH domain, forms nuclear dots and promotes the selective removal of meiotic mRNAs containing determinant of selective removal (DSR) sequences (UUAAAC/UCAAAC) by the nuclear exosome in mitotic cells (Chen et al., 2011; Harigaya et al., 2006; McPheeters et al., 2009; Yamashita et al., 2012). However, the detailed mechanism of DSR-directed mRNA decay is not yet understood fully.

We previously reported Red1, which forms nuclear foci, as an essential factor for the selective elimination of meiotic mRNAs in mitotically dividing cells (Sugiyama and Sugioka-Sugiyama, 2011). In addition, we have identified Red5 as another nuclear protein that is required for suppressing meiotic mRNAs in vegetative S. pombe. We will show that Red5 is involved in both meiotic mRNA decay and mRNA export, and would like to discuss a possible link among mRNA decay, mRNA export, and the nuclear pore complex.

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Topoisomerase II-interacting gene network prevents mitotic catastrophe and cell death induced by the anti-cancer drug doxorubicin in fission yeast

Zoey Tay, Thi Thuy Trang Nguyen, Ee Sin Chen

Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore

Presented by: Chen, Ee Sin

Doxorubicin is an anthracycline antibiotic that is employed in the treatment of a wide range of cancers. Doxorubicin acts by intercalating into DNA helices and inhibiting the religation of cleaved DNA ends during the decatanation process of Topoisomerase II (Top2), thus resulting in the formation of profuse double stranded DNA breaks and induction of cell death. The chemotherapeutic efficacy of doxorubicin is often undermined by the development of resistance, however the underlying mechanisms are not fully understood. To address this issue, we embarked on a screen to identify genes and universal molecular mechanisms that are required for conferring doxorubicin resistance (DXR) using the S. pombe single gene deletion collection. From the screen we have identified 91 genes that are interconnected to form a "safety net" that span multiple cellular compartments to protect the cells against this cytotoxic agent. Because of the nature of the initial screen, genes that are essential for growth were excluded and among these is the major target of doxorubicin, Top2. We therefore interrogated the role of Top2 with respect to the characterized DXR network. Here we present evidence to show that Top2 formed a cluster of interactions that synergizes in parallel with the majority of the components of the DXR network. Partially downregulating the activity of Top2 using a point mutation in several DXR mutants resulted in cumulative death of cells that was accompanied by upregulation of chromosome missegregation. This observation shows that Top2 cooperates with the DXR factors, many of which regulate chromatin architectural organization, to prevent cell death arising from mitotic catastrophe. We will discuss a model in which Top2 may control the structural integrity of the centromeric chromatin, and in so safeguard the genomic stability of cells exposed to doxorubicin.

RNA processing machinery targets facultative heterochromatin to safeguard silencing of meiotic genes

Martin Zofall[a], Soichiro Yamanaka[b], Francisca Reyes-Turcu[a], Ke Zhang[c], Chanan Rubin[d], Shiv I.S. Grewal[a]

[a] *Laboratory of Biochemistry and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*
[b] *Institute of Integrated Medical Research, Department of Molecular Biology, Keio University, School of Medicine, Tokyo, Japan*
[c] *Department of Biology, Wake Forest University, Winston Salem, NC, USA*
[d] *Evogene*

Presented by: Zofall, Martin

Facultative heterochromatin is important for regulating gene silencing in response to environmental cues and to coordinate gene expression patterns during cellular differentiation and development. Despite the important role of facultative heterochromatin in regulating adaptive and developmental responses, the processes required for assembly and maintenance of facultative heterochromatin are poorly understood. We examined genome-wide heterochromatin distribution in Schizosaccharomyces pombe and discovered that in addition to blocks of constitutive heterochromatin at centromeres, telomeres and mating type locus, fission yeast chromosomes contain isolated heterochromatin assemblies, which we have termed heterochromatin islands that coat various transcription units, including several meiotic genes. Heterochromatin assembly at meiotic genes requires RNA processing factors that eliminate meiotic mRNAs and contribute to meiotic gene silencing during mitotic growth. We found that the loss of RNA elimination factors, including the sequence specific RNA binding protein Mmi1 or the Red1 RNA processing factor, or blocking transcription of meiotic mRNA abolishes heterochromatin at meiotic genes. RNA processing factors associate with heterochromatin coated meiotic loci and interact with the histone methyltransferase Clr4/SUV39h, a critical component of the heterochromatin assembly process. Heterochromatin islands augment silencing of meiotic genes during vegetative growth, and disassemble at the onset of meiosis in response to nutritional signals that induce sexual differentiation. We have found that fission yeast contains dynamic heterochromatic assemblies that respond to developmental signals. Importantly, these findings establish fission yeast as a useful model organism for investigating the mechanisms that regulate facultative heterochromatin.

Roles of Rqh1 and Exo1 at uncapped telomere and circular chromosomes

Noaya Hirata, Shinobu Ukimori, Tomoko Nanbu, Masaru Ueno

Department of Molecular Biotechnology, Hiroshima University, Higashi-Hiroshima Japan

Presented by: Ueno, Masaru

Pot1 is a telomere binding protein. Deletion of pot1 causes telomere uncapping and rapid telomere degradation, resulting chromosome circularization mediated by single-strand annealing (SSA) (1). Rqh1 helicase is involved in the several steps in homologous recombination (HR), including processing of DNA double-strand break (DSB) ends, suppression of inappropriate recombination, and resolution of recombination intermediate. The pot1Δ rqh1Δ double mutant is lethal, but the reason remains unclear. As Rqh1 is suggested to be involved in SSA, one explanation for the lethality might be its inefficient SSA. To test this possibility, we asked whether Rqh1 is required for SSA when Pot1 is shut off. To do this, we combined nmt81 promoter and auxin-inducible protein depletion system (We call it nmt81-pot1-aid strain) (2). We found that both nmt81-pot1-aid and nmt81-pot1-aid rqh1Δ cells lost their telomeric DNA and viability after Pot1 shut off. Moreover, chromosome end fusion was detected in both cells after Pot1 shut off. These results suggest that the function of Pot1 can be efficiently knocked down and that Rqh1 is not required for SSA. Both nmt81-pot1-aid rqh1Δ and nmt81-pot1-aid exo1Δ cells lost their telomeric DNA and viability after Pot1 shut off. In contrast, nmt81-pot1-aid rqh1Δ exo1Δ cells did not lose the telomeric DNA and viability, suggesting that Exo1 and Rqh1 process uncapped telomere independently.

We also found that the synthetic lethality of the pot1Δ rqh1Δ double mutant is suppressed by deletion of either rad51 or exo1 (2). Rqh1 shut off in a pot1 disruptant with circular chromosomes caused chromosome missegregation. Crossovers between circular chromosomes generate dimers that cannot segregate properly in E. coli. Taken together, we propose that Rqh1 inhibits crossovers between circular chromosomes to suppress the generation of circular dimers.

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Set1 cooperates with CENP-B in genome organization and transcriptome regulation

David R. Lorenz[a], Irina V. Mikheyeva[a], Peter Johansen[a], Lauren Meyer[a],Anastasia Berg[a], Shiv I. S. Grewal[b], Hugh P. Cam[a]

[a] *Boston College, Biology Department Chestnut Hill, MA 02467, USA*
[b] *Laboratory of Biochemistry and Molecular Biology, National Cancer Institute National Institutes of Health, Bethesda, MD 20892, USA*

Presented by: Cam, Hugh

Chromatin modifiers impose regulatory controls over diverse chromosomal processes including transcription, nuclear organization, and genome stability. Here we reveal an unexpected role for the histone methyltransferase Set1 (KMT2) as a general transcriptional repressor of the fission yeast Schizosaccharomyces pombe genome. Set1 localizes to repetitive elements and represses both forward and reverse transcripts associated with centromeric heterochromatin and Tf2 long terminal repeat (LTR) retrotransposons distinct from its H3K4 methylation (H3K4me) activity. Set1 cooperates with Abp1, the S. pombe homolog of the mammalian centromere binding protein B (CENP-B), to mediate repression of heterochromatin and Tf2. Intriguingly, Set1 helps organize dispersed Tf2 into distinct nuclear foci termed Tf bodies, the integrity of which requires class I/II histone deacetylases (HDACs) and Sirtuins. Our study uncovers dual roles for Set1 in the maintenance of euchromatin and heterochromatin, and its cooperation with a transposase-containing transcription factor to mediate repression and genome organization of repetitive elements associated with heterochromatin and interspersed retrotransposons.

Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription

Bianca P. Hennig[a], Katja Bendrin[a], Yang Zhou[a], Tamas Fischer[a]

Biochemistry Center (BZH), Heidelberg University, Heidelberg, Germany

Presented by: Fischer, Tamas

Proper chromatin organization is essential for defining transcription units and maintaining genomic integrity in eukaryotes. Mutations affecting the chromatin structure can lead to increased cryptic transcription and genomic instability. In an effort to understand how transcription units are correctly defined, we screened a selection of deletion mutants in Schizosaccharomyces pombe, by monitoring cryptic transcript levels at selected loci. We found that deletion of hrp1 and hrp3, the S.pombe paralogs of the Chd1 subfamily of ATP-dependent chromatin remodelers, causes strong accumulation of antisense transcripts at the tested loci. High resolution genome-wide expression profiles revealed that these mutants show significant accumulation of antisense transcripts at about 40% of the genome, but remarkably, the level of coding mRNAs is mostly unaffected. Nucleosome mapping experiments uncovered a specific role for Chd1 remodelers in the positioning of nucleosomes in gene coding regions. While the arrangement of nucleosomes in promoter regions was similar to WT, nucleosome organization within coding regions was remarkably irregular in hrp1Δhrp3Δ strain. Such irregular nucleosome structure can lead to enhanced cryptic promoter activity within transcription units. We extended our analysis to other mutations associated with enhanced cryptic transcription activity, such as set2Δ, alp13Δ, and FACT complex subunit pob3Δ. While nucleosomes were severely depleted in the pob3Δ strain, nucleosome positioning was less affected. In sharp contrast, nucleosome organization in the alp13Δ and set2Δ strains was indistinguishable from WT. These data indicate multiple mechanisms in the repression of cryptic promoter activity in eukaryotic cells.

Error-prone progression of homologous recombination-restarted replication forks

Ken'Ichi Mizuno[a], Johanne Murray[a], Antony Carr[a]

Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, BN1 9RQ, UK

Presented by: Mizuno, Ken'Ichi

Impediments to DNA replication are associated with gross chromosomal rearrangements which are linked to cancer development or genomic disorders. To investigate the mechanisms that lead to genome instability we have developed systems to arrest a replication fork at a specific site using the DNA replication fork terminator sequence RTS1. Homologous recombination proteins are necessary for restarting DNA replication at RTS1 (Lambert et al., 2010), but in systems where replication was stalled in both directions using inverted RTS1 sequences, faulty template choice led to non-allelic recombination generating acentric and dicentric chromosomes. We set up a single RTS1 system to test the effect on chromosomal rearrangement of inverted repeats in close proximity to the restart site. We predicted that chromosomal rearrangement such as inverted fusion would be abolished. However, inverted fusion took place frequently in a manner dependent on replication fork arrest showing that progression of a replication fork restarted through homologous recombination was error-prone. The frequency of inverted fusion depended on repeat size and potential to form structured DNA. Furthermore, fidelity of the restarted fork progression improved gradually with distance from the restart suggesting that restarted forks mature over time. Our results show that resumption of DNA replication by homologous recombination leads to gross chromosomal rearrangements by two distinct mechanisms, firstly the restart event can result in ectopic recombination and secondly the restarted fork is error prone.

Identification of genetic factors affecting the dynamic transition from cellular quiescence to proliferation

Bruno Lages[a], Charalampos Rallis[a], Jürg Bähler[a]

University College London, Department of Genetics, Evolution & Environment, London WC1E 6BT, UK

Presented by: Lages, Bruno

Quiescence, the most common cellular state, can be regarded as a proliferation stand-by. When permitted, a rapid and determined transition from quiescence to proliferation confers a competitive advantage. Unicellular organisms in the wild thus exhibit an efficient regulation of this critical transition.

Upon nitrogen removal, fission yeast cells stop growth and arrest as small, quiescent cells that survive for months (Yanagida 2009, Trends Cell Biol 19:705). Upon re-feeding with nitrogen, quiescent cells start to grow and divide again after a certain delay (lag phase). Intriguingly, the lag phase increases as a function of the time the cells have spent in quiescence. The fascinating lag-phase phenomenon is appreciated anecdotally but not understood, but it raises the possibility of an epigenetic memory of the previous growth state.

We want to identify genetic factors, notably those involved in genome regulation, that determine the kinetic transition from cellular quiescence to growth. We are screening the genome-wide fission yeast deletion library for factors determining the lag-phase duration and/or variability. We pooled all mutants for massively parallel profiling of the quiescence-growth transition, and detect the relative abundance of all mutants by Bar-seq (Han et al. 2010, Genome Biol 11:R60). We sequenced aliquots of the pool at multiple timepoints after nitrogen re-feeding to uncover mutants that re-start growth earlier or later than wild-type cells. We identified initial candidate genes, includeing those encoding chromatin regulators, which may be involved in determining the lag phase. Initial data of this ongoing study will be presented.

Experimental Evolution of Fission Yeast Genomes to Repeated Environmental Changes

Laurent van Trigt[a], Ville Mustonen[b], Daniel Jeffares[a], Jürg Bähler[a]

[a] *University College London, Department of Genetics, Evolution & Environment, UK*

[b] *Wellcome Trust Sanger Institute, Hinxton, UK*

Presented by: van Trigt, Laurent

Evolutionary biology has mainly relied on comparative studies. Supported by advances in sequencing, several groups are now using microorganisms for experimental evolution to directly analyse the evolution process and its genetic basis. To understand how cells adapt to an environmental stress, we developed experimental evolution assays during which fission yeast populations were repeatedly exposed to short heat-shocks. Samples were frozen after each selection cycle for subsequent phenotypic and genotypic analyses. Initially, 10 such experiments were performed for up to 150 selection cycles. In all experiments, cells acquired a markedly increased survival to heat within only 10-20 selection cycles, while during later cycles there was a more subtle but continuous increase in stress resistance. Surprisingly, all populations, including a mock-treated control, also evolved a more rapid re-initiation of exponential growth following heat shock. We sequenced the genomes of 96 strains at selected time points over the course of the evolution experiments. The genetic changes occurring in regulatory and coding regions in the different independent experiments were then analyzed, with the ultimate goal of uncovering the specific changes that are causing the striking alteration in phenotype. We determined dynamic allele frequency trajectories that had swept through each population. This analysis led to the discovery of a common haplotype, consisting of 9 single-nucleotide polymorphisms (SNPs), which is likely associated with the more rapid recovery of growth after heat shock. Because some of these SNPs may be neutral and hitchhiking along with selected, causative alleles, large pools of segregants are being generated in which linkage is broken up. By repeating evolution assays on these populations, as well as determining allele frequency trajectories, we should be able to dissect which combinations of alleles from the haplotype are required for the adapted phenotype.

Prp1 and Prp4 kinase control splicing of essential cell cycle genes

Daniela Eckert[a], Angel Guerra-Moreno[b], Susanne Zock-Emmenthal[a], Franzisca Klinger[a], José Ayté[b], Norbert F. Käufer[a]

[a] *Institute of Genetics, Technische Universität Braunschweig, Spielmannstrasse 7, 38106 Braunschweig, Germany*
[b] *Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, C/Doctor Aiguader 88, Barcelona 08003, Spain*

Presented by: Eckert, Daniela

Prp1, which has been found exclusively associated with precatalytic spliceosomal particles, is a physiological substrate of Prp4 kinase. In contrast, the kinase is not stably attached to any of these spliceosomal complexes. We have identified and characterised two extragenic suppressors of Prp1, namely Lin1 and Spp42. In both suppressor strains, cells transiently arrested in the G1 and G2 phases of the cell cycle. Analyses of splicing profiles in these strains revealed that unspliced pre-mRNAs of the res1 gene, which encodes a cell cycle regulatory component that acts at the Start transition, and the tbp1 gene, which encodes the TATA-binding protein, were transiently accumulated. Investigation of pre-mRNA profiles using temperature-sensitive Prp1 strains and a strain expressing an analogue-sensitive Prp4 kinase demonstrated that splicing of res1 and tbp1 is regulated via phosphorylation of Prp1 by Prp4 kinase.

Global profiling of cells following inhibition of Prp4 kinase revealed that the res1 and tbp1 genes belong to a large group of intron-containing genes in which splicing of the pre-mRNAs is dependent on Prp4 kinase activity. Our results suggest that phosphorylation of Prp1 by Prp4 kinase improves intron recognition. We propose a model how Prp4 might be involved in the quality control of pre-mRNA splicing by acting at “checkpoints” during the recognition of introns and the activation of pre-catalytic spliceosomal complexes.

The investigation of glucose repression and stress response pathways in *Schizosaccharomyces pombe*

Bedia Palabiyik[a], Farinaz Jafari Ghods[a], Semian Karaer Uzuner[a]

Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, Turkey

Presented by: Jafari Ghods, Farinaz

Glucose is the preferred carbon and energy source in most organisms which playing critical roles in the regulation of many cellular and biological processes. However, excessive glucose consumption leads to diabetes and other age-related disease such as cancer, cardiovascular disease, glaucoma and Alzheimer. The idea of investigation about glucose repression and stress response relationship in Saccharomyces cerevisiae and Schizosaccharomysec pombe was motivated by the fact that calorie intake restriction extends the life span of a variety of model organisms from yeast to mammals. Most studies in parallel with our previous study confirmed the existence of relationship between glucose sensing/signaling and oxidative stress response pathways. In the light of our foregoing study indicated the evidences supporting presence of additional pathway different from general stress response pathways, it was suggested that in S. pombe ird mutants, which are resistant to glucose repression, resistance to oxidative stress is not dependent on Atf1 and Pap1 transcription factors.

The Aim of this study is to analyze the expression level of genes involved in glucose repression and oxidative stress response pathways in S. pombe ird mutants, in which the genes encoding Atf1 and Pap1 transcription factors and Sty1-MAPK are being disrupted via “knock out” technique, through real-time polymerase chain reaction. Following this, we are planning to determine levels of gene expression in double mutants to learn more about other possible regulatory machineries which make these mutants resistant to oxidative stress.

Chromatin architecture and chromatin remodelling at stress genes in fission yeast

Patricia García[a], Elena Hidalgo[a]

Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, Barcelona, Spain

Presented by: García, Patricia

Cells have the capacity to adapt to harsh external conditions and to induce massive changes on their gene expression patterns to allow survival. Schizosaccharomyces pombe displays a large transcriptional response common to several stress conditions, ruled by the MAPK Sty1 and the transcription factor Atf1 (Chen et al., 2003; Vivancos et al., 2006; Sanso et al., 2011a). Both proteins regulate up to 400 CESR (core environmental stress response) genes binding to their promoters in a stress-dependent manner. One key point in the regulation of gene expression involves post-transcriptional modifications of histones, especially the acetylation of histone H3. Regarding the chromatin architecture of CESR genes, we recently established that: (i) their promoters have a wide nucleosome depleted region (NDR) prior to stress imposition; (ii) the histone acetyltransferase Gcn5, a component of the SAGA complex, is recruited to stress genes in a Sty1 and Atf1-dependent manner to promote Pol II progression (Sanso et al., 2011b). We have further investigated both issues. On one hand, nothing is known about the establishment of NDRs at the promoters of these particular set of genes. Since the expression of CESR genes is Atf1-dependent, and it has been shown that Atf1 is bound to these promoters even prior to stress, we propose based on genome-wide ultrasequencing that this basal binding of Atf1 to promoters may be sufficient to keep chromatin open at these regions. On another hand, we have searched how other chromatin regulators affect Pol II transcription along the CESR genes. Chromatin configuration has to be tightly regulated to prevent cryptic transcription prior to stress, and to re-establish a closed configuration once Pol II has passed through the genes. We have found two histone deacetylases, Clr3 and Clr6, to close chromatin after Pol II passage and to avoid cryptic transcription under basal conditions.

Modulating the level of the seventh largest subunit of RNA Polymerase II affects multiple phenotypes in *Schizosaccharomyces pombe*

Deepak Kumar[a], Nimisha Sharma[b]

[a] *University School of Biotechnology, G.G.S.Indraprastha University, Sector 16C, Dwarka, New Delhi -110078, India*
[b] *University School of Biotechnology, G.G.S. Indraprastha University, Sector 16C, Dwarka, New Delhi -110078, India*

Presented by: Kumar, Deepak

Eukaryotic RNA polymerase II is a twelve-subunit enzyme that plays an important role in the control of gene expression in response to both external and internal stimuli. Its seventh largest subunit, Rpb7p, is highly conserved from yeast to humans and associates with the fourth largest subunit, Rpb4p, to form a heterodimer. Deletion of rpb7+ is lethal for the yeast cells. The function(s) of the Rpb7p subunit and the Rpb4p/Rpb7p complex are still poorly understood in S. pombe and other higher eukaryotic organisms. In this work, we have initiated experiments to elucidate the function(s) of the Rpb7p subunit in S. pombe. Towards this goal, we expressed rpb7+ under the control of thiamine-regulated nmt promoter of varying strengths. It was observed that under optimum conditions Rpb7p influenced the growth of cells in a dose-dependent manner when its expression reduced below a critical level in the presence of thiamine. We also show that low levels of Rpb7p affected the growth of cells under a variety of stress conditions, including heat stress, osmotic stress, nitrogen limiting conditions as well as survival during stationary phase. Our results further demonstrate that S. pombe cells expressing low levels of Rpb7p were elongated and showed cell separation defects. Yeast two hybrid analysis has also been carried out to determine if Rpb7p can interact with the mediator subunits that are also involved in regulating the process of cell separation. Taken together, our results suggest that the Rpb7 subunit of RNA polymerase II is important for survival under stress conditions and also, for proper separation of cells in S. pombe.

Klf1, a C2H2 zinc finger-transcription factor, is required for cell volume maintenance in the fission yeast G0 phase

Mizuki Shimanuki, Tomáš Pluskal, Mitsuhiro Yanagida

Okinawa Institute Science and Technology Graduate University, Japan

Presented by: Yanagida, Mitsuhiro

Fission yeast S. pombe is an excellent model for studying cellular quiescence induced by starvation. Shifting to medium without a nitrogen-source induces proliferative cells to enter long-term G0 quiescence. Klf1 is a Kruppel-like transcription factor with a 7-amino acid-spaced C2H2-type zinc finger motif. The deletion mutant Δklf1 normally divides in vegetative medium, but proliferation is not restored after long-term G0 quiescence. Cell biologic, transcriptomic, and metabolomic analyses revealed a unique phenotype of the Δklf1 mutant in quiescence. Mutant cells had diminished transcripts related to signaling molecules for switching to differentiation. In contrast, proliferative metabolites for cell-wall assembly and antioxidants significantly increased. Further, the size of the Δklf1 cells is dramatically increased during quiescence due to the aberrant accumulation of calcofluor-positive chitin-like materials beneath the cell wall. After 4 weeks quiescence, the ability for reversible proliferation is lost, but energy metabolism is maintained. Klf1 thus plays a role in G0 phase longevity through enhancing the differentiation signal and suppressing metabolism for growth. If Klf1 is lost, S. pombe fails to maintain a constant cell size during quiescence.

Newly identified components of the Mis16-Mis18 complex promote CENP-A assembly at *S. pombe* centromeres

Lakxmi Subramanian, Flavia de Lima Alves, A. Arockia Jeyaprakash, Juri Rappsilber, Robin Allshire

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, UK

Presented by: Subramanian, Lakxmi

The histone H3 variant CENP-A specifies eukaryotic centromeres, and provides a specialized chromatin environment that is essential to maintain centromere integrity. A number of centromeric proteins and protein complexes that are required to deposit and maintain CENP-A at centromeres have been identified in a wide variety of model systems. In the fission yeast Schizosaccharomyces pombe, these include Scm3 (HJURP), the Mis6 (CENP-I)-Sim4 (CENP-K) complex, and the Mis16 (RbAp46/48)-Mis18 complex. While Scm3 (HJURP) has been described as a CENP-A specific chaperone, no specific functions have been attributed thus far to the proteins within the Mis6 (CENP-I)-Sim4 (CENP-K) complex or to Mis18. In an attempt towards dissecting the exact molecular function(s) of these factors that lie upstream of CENP-A in maintaining centromeric chromatin integrity, we performed large-scale interaction screens and identified two novel Mis18-interacting proteins. These proteins bind centromeres and co-localise with Cnp1 (CENP-A). We will present our ongoing efforts towards further characterising these proteins and understanding their modes of action at fission yeast centromeres.

The effects of glucose sensing and glucose intake on the oxidative stress response in *Schizosaccharomyces pombe*

Bedia Palabiyik, Farinaz Jafari Ghods, Evren Önay Uçar[a]

Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, Istanbul, Turkey

Presented by: Önay Ucar, Evren

The resistant invertase mutants of Schizosaccharomyces pombe (ird5, ird13 and ird14) have high tolerance to oxidative stress induced by H2O2, when they were compared to wild type. The increased expression level of ght3 gene, which have highest affinity among hexose transporter genes and fbp1 gene in all ird mutants can be interpreted as lack of glucose repression in ird mutants. To investigate if an oxidative stress response comes from glucose depletion occurred in ird mutants, we analysed transcript of stress response related genes, such as sod1, ctt1, atf1, pap1 and sty1, under stressed and non-stressed conditions. Then, we studied the phosphorylated Sty1-MAP kinase in ird mutants comparing to wild type. These findings altogether support the concept that there was an adaptive response to oxidative stress in these mutants. In addition, it has been drawn attention to the links between glucose signalling, glucose intake and the oxidative stress response.

A possible mechanism for the antagonistic functioning of fission yeast CSL transcription factors

Jarmila Tvaruzkova, Martina Oravcova, Martin Převorovský

Department of Cell Biology, Faculty of Science, Charles University in Prague, Czech Republic

Presented by: Tvaruzkova, Jarmila

CSL (CBF1/Suppressor of Hairless, Lag-1) proteins are the major effectors of the Notch receptor signaling pathway with a crucial role in metazoan development. These transcription factors can act both as transcriptional activators and repressors. Although it lacks the Notch receptor pathway, fission yeast contains two CSL homologues, namely Cbf11 and Cbf12, with the ability to trigger transcription in vivo. Cbf proteins play antagonistic roles in the coordination of cell and nuclear division, cell adhesion, and maintenance of chromosome integrity. Up to date the molecular mechanism of the antagonism of CSL proteins in fission yeast is not understood.

We have studied DNA binding and protein level changes of Cbf11 and Cbf12 in response to the absence of the other paralogue using chromatin immunoprecipitation and western blot analysis, respectively. Our results suggest that the stability of Cbf proteins is affected by the presence of their paralogue. In conclusion, our data indicate a possible mechanistic explanation of the antagonism observed between the two fission yeast CSL transcription factors.

Identification of novel centromere proteins Mis19 and Mis20 as Mis18-interacting partners in *S. pombe*

Takeshi Hayashi, Masahiro Ebe, Koji Nagao, Aya Kokubu, Mitsuhiro Yanagida

G0 Cell Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Presented by: Hayashi, Takeshi

CENP-A (Cnp1/CenH3) is a centromere specific variant of histone H3, which is required for accurate chromosome segregation. Mis16 and Mis18 form a complex and are essential for CENP-A localization at centromeres in fission yeast and mammals. The histone acetylation-deacetylation process is crucial for the regulation of CENP-A deposition. The Mis16-Mis18 complex is accumulated at the centromeres in late anaphase. Priming of the telophase centromere chromatin by the Mis16-Mis18 complex is needed for recruiting CENP-A through regulating the acetylation status in the centromere. How the Mis16-Mis18 complex achieves this function remains obscure. Here we report identification of fission yeast Mis19 and Mis20 as Mis16 and Mis18-interacting proteins by mass spectrometry. Mis19 and Mis20 are localized at centromeres during interphase, but not in mitosis, just like Mis18. Inactivation of Mis19 leads to CENP-A delocalization and massive chromosome missegregation, whereas Mis20 is dispensable for proper chromosome segregation. Mis19 directly interacts with both Mis16 and Mis18, and enables the interaction between Mis16 and Mis18, suggesting that Mis19 acts as a bridge between Mis16 and Mis18 to recruit CENP-A to centromeres.

Two levels of telomeric chromatin regulation

Jessica Greenwood, Julia Promisel Cooper

London Research Institute, Cancer Research UK, Telomere Biology, 44 Lincolns Inn Fields, London WC2A 3LY, UK

Presented by: Greenwood, Jessica

Despite the prime importance of telomeres in chromosome stability, the chromatin structure of telomeres is poorly understood. The composition of the telomere provides a specific and regulated means to protect the chromosome end and to avert the end replication problem through telomerase recruitment. Epigenetic regulation of the telomeric region also influences telomere function. Telomeric regions have been shown to silence transcription of genes placed in the vicinity through telomere position effect (TPE) and marks of heterochromatin, such as Histone H3K9Me, have been localized to subtelomeric regions. Here we show that the chromosome end in fission yeast is assembled into a protected region encompassing both the telomere repeats themselves and over half a kilobase of subtelomeric DNA. This structure is dependent upon conserved telomeric proteins Taz1 and Rap1. Surprisingly, this structure is also regulated by RNA, as the telomeric chromatin footprint is altered by RNase treatment. Finally, we have investigated the role of heterochromatin and have found that while the telomere structure is not dependent on the histone methylation machinery, it does, in fact, contain histones that contain heterochromatic marks. Therefore, telomeric chromatin is organized at at least two different levels: 1) an architectural level reflected by protection from micrococcal digestion, and 2) a histone level, where histone marks are superimposed upon the higher order architecture.

Heterochromatin transcription and alternative chromosome end-protection mechanisms in the absence of canonical telomeres

Martina Begnis, Julie Cooper

CRUK, London Research Institute, UK

Presented by: Begnis, Martina

The discovery of HAATI chromosomes in fission yeast has recently shaken the dogma that canonical telomeres are essential to maintain chromosome linearity. HAATI cells survive telomerase loss by rearranging the genome such that blocks of generic heterochromatin jump to the ends of chromosomes and acquire the ability to recruit the canonical end-protection factor Pot1 to maintain chromosome linearity (Jain et al.; 2010). These survivors amplify either of two classes of repetitive sequences, the rDNA for rDNA-HAATI or the subtelomeric repeats for STE-HAATI. The HAATI survival mode is reminiscent of the chromosome end-protection system in flies and is likely to be widely conserved, for instance in human telomerase-minus cancer cells. RNAi is one of the pathways that contribute to the formation of heterochromatin in the genome, but it has been shown to be dispensable for the establishment of heterochromatin at canonical telomeres (Kanoh et al.; 2005). Here we demonstrate that small RNAs are required for the jumping of rDNA repeats that allows the formation of rDNA-HAATI chromosomes. On the contrary, STE-HAATI generation appears to be actively promoted by loss of Dicer (Dcr1). We propose that Dcr1 might play a role in repressing those mechanisms that affect STE mobility, possibly involving transposition-like events. Our results implicate heterochromatin transcription in the formation of specific types of alternative chromosome end-protection complexes and suggest avenues for establishing the mechanisms underlying HAATI survival.

Epigenetic inactivation of a centromere to stabilize dicentric chromosomes

Hiroshi Sato[a], Fumie Masuda[a], Yuko Takayama[b], Shigeaki Saitoh[a]

[a] *Division of Cell Biology, Institute of Life Science, Kurume University*
[b] *Department of Biosciences, Faculty of science and Engineering, Teikyo University*

Presented by: Sato, Hiroshi

The kinetochore is a multi-protein complex that forms on a chromosomal locus designated as the centromere and links the chromosome to the spindle during mitosis and meiosis. Most eukaryotes except holocentric species have a single centromere per chromosome, and the presence of multiple centromeres on a single chromosome causes aberrant spindle - chromosome attachment resulting in breakage and/or loss of the chromosome. Recent studies, however, implicate the presence of latent centromeres on a chromosome, which are normally repressed by a hitherto unknown mechanism.

To gain insight into the mechanism, we generate the artificial dicentric chromosome, which contains two centromeres, by fusing two chromosomes in fission yeast Schizosaccharomyces pombe. Although the majority of cells harboring the artificial dicentric chromosome are arrested with elongated cell morphology in a manner dependent on the DNA structure checkpoint genes, a portion of the cells survive by converting the dicentric chromosome into a stable functional monocentric chromosome; either centromere was inactivated epigenetically or by DNA rearrangement. Mutations compromising kinetochore formation increased the frequency of epigenetic centromere inactivation. The inactivated centromere is occupied by heterochromatin and frequently reactivated in heterochromatin- or histone deacetylase-deficient mutants. These results suggest that chromosomes with multiple centromeres are stabilized by epigenetic centromere inactivation, which consists of initiation step induced by disassembly of the kinetochore and subsequent heterochromatinization preventing reactivation of the inactivated centromere.

Screening for genes involved in nucleotide synthesis and homologous recombination repair

Elizabeth Blaikley, Timothy Humphrey

Gray Institute for Radiation Oncology & Biology, Department of Oncology, University of Oxford, UK

Presented by: Blaikley, Elizabeth

Repair of DNA double strand breaks (DSB) by homologous recombination (HR) is important for genome stability. It has previously been shown that nucleotide synthesis, a universal response to DNA damage, is required for efficient HR and to prevent loss of heterozygosity, a causal event in tumourigenesis. Nucleotide synthesis is highly regulated and in fission yeast is dependent on damage-induced degradation of the ribonucleotide reductase inhibitor, Spd1, by the Ddb1-Cul4Cdt2 ubiquitin ligase complex.

The aim of this project was to identify new genes involved in nucleotide synthesis and HR. 346 DNA damage sensitive deletion strains were screened for those exhibiting altered damage sensitivity when nucleotide synthesis was promoted by deleting spd1. This led to the identification of two classes of mutants, those exhibiting increased or reduced damage sensitivity. Further analysis of the suppressed mutants revealed a requirement for the DNA damage checkpoint pathway in promoting expression of Cdt2. This facilitates the Ddb1-Cul4Cdt2 dependent degradation of Spd1. However other mutants functioned independently of Cdt2. The role of these genes in nucleotide synthesis or DNA damage response processes that are more efficient in the presence of high nucleotide pools is currently under investigation. As perturbed nucleotide levels can affect mutation rates, these genes are predicted to play an important role in genome stability.

Insights into Stc1-interactions bridging RNAi and chromatin modification

Sreerekha S. Pillai[a], Francesca Taglini[a], Chao He[b], Yunyu Shi[b], Elizabeth H. Bayne[a]

[a] *Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, UK*
[b] *Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China*

Presented by: Pillai, Sreerekha

In fission yeast, as in higher eukaryotes, centromeres are flanked by heterochromatin that serves pivotal functions in genome integrity. Chromatin modification at fission yeast centromeres involves the RNAi pathway: siRNAs generated from centromeric non-coding RNAs target the Ago1-RITS complex to homologous nascent transcripts. This RNAi targeting is required for the recruitment of the Clr4-H3K9methyl-transferase complex (CLRC) to chromatin, promoting binding of chromo-domain proteins like Swi6 (HP1) and hence heterochromatin formation at the pericentromere.

Stc1 was recently identified as a key pathway component that recruits CLRC to chromatin in an RNAi-dependent manner. Understanding the interactions of Stc1 is important since it acts as the key factor which connects RNAi to chromatin modification. Here we present the solution structure of Stc1 determined by NMR, and provide data on how it mediates the interaction between RITS and CLRC complexes. Our study reveals that association with RITS is mediated by a novel type of tandem zinc-finger domain in the N-terminus of Stc1, while the structurally disordered C-terminus of Stc1 is involved in binding to CLRC.

Assembly of CENP-A at fission yeast centromeres

Alison Pidoux, Alexander Kagansky, Diego Folco, Lakxmi Subramanian, Nick Toda, Sandra Catania, Georgina Hamilton, Robin Allshire

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, UK

Presented by: Pidoux, Alison

Despite the conserved essential function of centromeres, centromeric DNA is not conserved between species. There is strong evidence indicating that centromeres are epigenetically regulated. Although centromeres normally assemble on preferred sequences, these sequences are neither necessary nor sufficient for centromere assembly. For instance, neocentromeres can form upon sequences that previously showed no centromere function. The prime candidate for the epigenetic mark that specifies centromere identity is the histone H3 variant, CENP-A, which is present at all centromeres, including neocentromeres.
We aim to understand how CENP-A assembly is influenced by sequence and by chromatin context. Schizosaccharommyces pombe centromeres are composed of a central domain which is assembled in CENP-A chromatin and forms the kinetochore, flanked by the heterochromatic outer repeat regions. We have previously shown that heterochromatin is required for establishment of CENP-A chromatin, but not for its maintenance. We are dissecting the process of CENP-A chromatin establishment to understand what features are required to promote assembly on central domain sequences.

The genomes of three additional Schizosaccharomyces species have been sequenced and contain no sequences homologous to S. pombe centromeres. However, chromatin immunoprecipitation indicates that like S. pombe centromere regions, S. octosporus and S. cryophilus contain heterochromatic histone modification (H3K9me2) and CENP-A. Similarities and differences in centromere organization and function in Schizosaccharomyces will be discussed.

Characterization of telomere protein Tpz1 phosphorylation in fission yeast

Yating Chang[a], Aaron Aslanian[b], John Yates[b], Toru Nakamura[a]

[a] *University of Illionis at Chicago, Department of Biochemistry and Molecular Genetics, USA*
[b] *The Scripps Research Institute, Department of Chemical Physiology, USA*

Presented by: Chang, Yating

Telomeres, specialized protein-DNA complexes at the ends of eukaryotic chromosomes, are essential for preventing uncontrolled DNA damage responses and loss of telomeric DNA during DNA replication. Previous studies have identified a six-protein complex (Pot1, Ccq1, Tpz1, Poz1, Rap1 and Taz1) called shelterin as playing key roles in both telomere protection and recruitment of the telomerase complex (Trt1 catalytic subunit, Est1 regulatory subunit and TER1 telomerase RNA). We have recently shown that deletion of Shelterin subunits Taz1, Rap1 and Poz1 (involved in negative regulation of telomere extension by telomerase) causes hyper-phosphorylation of Ccq1 by the DNA damage checkpoint kinases Rad3 and Tel1, and that Rad3/Tel1-dependent phosphorylation at Thr93 of Ccq1 is critical for Est1-Ccq1 interaction and telomerase recruitment.

Here, we report that Tpz1 also undergoes Rad3/Tel1-dependent hyper-phosphorylation upon loss of Taz1, Rap1 or Poz1. Furthermore, we found that trt1 deletion or ccq1-T93A mutation, which cause telomere shortening and therefore loss of Taz1 binding sites, also lead to Tpz1 hyper-phosphorylation. Mutation of all six putative Rad3/Tel1 phosphorylation sites (SQ or TQ sites) caused substantial reduction in Tpz1 hyper-phosphorylation induced by rap1 or taz1 deletion. However, a tpz1-6AQ strain retained normal telomere length and residual phosphorylation of Tpz1 was still observed, suggesting that other sites besides SQ/TQ are subjected to phosphorylation. Moreover, our analysis indicated that kinase(s) other than Rad3 and Tel1 are involved in Tpz1 phosphorylation. To clarify roles of Tpz1 phosphorylation in fission yeast telomere function, we thus performed mass spectrometry analysis of Tpz1. Our analysis so far identified 20 Rad3/Tel1-dependent and 10 Rad3/Tel1-independent putative phosphorylation sites on Tpz1. We will provide our analysis of fission yeast cells carrying mutations at those newly identified Tpz1 phosphorylation sites.

Analysis of the RNA targets for the exosome co-factors Mmi1 and Red1

Cornelia Kilchert[a], Sander Granneman[b], Lidia Vasiljeva[a]

[a] *Department of Biochemistry, University of Oxford, UK*
[b] *Institute for Structural and Molecular Biology, University of Edinburgh, UK*

Presented by: Kilchert, Cornelia

Fission yeast has developed an elaborate system to prevent activation of the meiotic programme during mitotic growth. During mitosis, meiotic mRNAs are transcribed but selectively and rapidly removed from the cell in an exosome-dependent manner. Removal can be triggered by the presence of so-called determinants of selective removal (DSRs), specific sequence elements that recruit Mmi1, which in turn recruits the exosome. Not all Mmi1-dependent RNAs contain a large number of DSRs and it is not clear how Mmi1 is recruited in the absence of the DSR. Some, but not all of the meiotic transcripts also recruit Red1, which induces formation of heterochromatin at the gene locus to silence transcription. How Red1 is recruited to target RNAs remains elusive. Using UV cross-linking and analysis of cDNA (CRAC), we want to map RNA targets of Mmi1 and Red1 genome-wide, and identify the relevant binding motifs. In addition to the wild-type, we will use cells deleted for the exosome component Rrp6 to enrich mRNAs that are targeted for degradation. Comparison to a dataset of exosome-associated RNAs will also allow us to identify possible targets of an exosome-independent pathway mediated by Mmi1/Red1.

Feedback regulation of TORC1 signaling by Npr2, but not by Tsc2

Yan Ma[a], Ning Ma[a], Qingbin Liu[a], Lili Zhang[a], Elizabeth Henske[b], Takayoshi Kuno[a]

[a] *Division of Molecular Pharmacology and Pharmacogenomics, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Japan*
[b] *Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, USA*

Presented by: Ma, Yan

The target of rapamycin (TOR) is a highly conserved protein kinase that regulates cell growth and metabolism. Both of TSC2 and Npr2 are identified as negative regulators of TORC1 signaling, however the distinct roles of the two regulators are not well understood. We used a genome-wide screen to identify negative regulators of TORC1. We isolated mutants that phenocopy Δtsc2, in which TORC1 signaling is known to be up-regulated. We discovered that Δnpr2 displayed similar phenotypes to Δtsc2 in terms of amino acid uptake defects and increased intracellular localization of the Cat1 permease. However, Δnpr2 and Δtsc2 clearly showed different phenotypes in terms of Isp5 transcription and Rps6 dephosphorylation upon nitrogen depletion. Furthermore, we showed that Tor2 controls amino acid homeostasis at the transcriptional and posttranscriptional levels. Genetic epistasis analyses suggest that Npr2 functions downstream of Tsc2 and Rag GTPases. Our data reveal that both Npr2 and Tsc2 negatively regulate TORC1 signaling, and that Npr2, but not Tsc2, may be involved in the feedback loop of a nutrient-sensing pathway.

Subnuclear localisation during nitrogen starvation

Alejandro Rodriguez, Dennis Larsson, Manou Engels, Pernilla Bjerling

[a] *Dept. of Medical Biochemistry and Microbiology (IMBIM), University of Uppsala, Sweden*

Presented by: Rodriguez, Alejandro

Transcriptional activation of genes is often correlated to chromatin reorganisation within the cell nucleus. During nitrogen depletion in the fission yeast, Schizosaccharomyces pombe, gene clusters that reside close to the nuclear membrane (NM) tend to move to the nuclear interior. These gene clusters contain genes that are upregulated during nitrogen depletion (Alfredsson-Timmins Chromosoma 2009). Furthermore, this is followed by a concomitant nucleosome loss in promoter and coding regions of genes that are activated by nitrogen depletion. Two gene clusters whose subnuclear localisation is affected in this way are named Chr1 and Tel1.

In order to study how subnuclear localisation affects gene activation the Chr1 and Tel1 gene clusters were tethered to the nuclear membrane. This was done by first introducing binding sites for Gal4 DNA binding domain (Gal4-DBD) next to the gene clusters. Gal4-DBD, under the control of the nmt promoter, fused to a CVCC peptide that serves as a farnesylation signal, allows for the fusion to the NM, and thus preventing the clusters to move away from the nuclear periphery. To be able to follow the changes in subnuclear localisation, lacO arrays were also integrated near the gene clusters. When the LacI-GFP fusion protein then binds to the lacO arrays these can be monitored by using live cell microscopy. In addition, the nuclear membrane protein Pom152 and the spindle pole body component Cut12 were also labelled.

By using live cell microscopy the Chr1 cluster was observed to remain at the nuclear periphery when anchored by Gal4-CVCC under nitrogen depletion. In addition, two genes that are normally induced by nitrogen depletion within the Chr1 cluster namely, urg1+ and urg3+, had a reduced expression compared to a wild type strain lacking Gal4-CVCC.

Transcriptional regulation by glucose starvation-induced lncRNAs

Arisa Oda[a], Naomichi Takemata[b], Tomoichiro Miyoshi[b], Yutaka Suzuki[c], Sumio Sugano[c], Kunihiro Ohta[a]

[a] *Graduate School of Science, The University of Tokyo, Japan*
[b] *Graduate School of Arts and Sciences, The University of Tokyo, Japan*
[c] *Graduate School of Frontier Sciences, The University of Tokyo, Japan*

Presented by: Oda, Arisa

Eukaryotic cells transcribe a myriad of non-coding RNAs. Some of them are RNA polymerase II-dependent long transcripts that are very similar to mRNA: 5' capped and polyadenylated. Such long non-coding RNAs (lncRNAs) have been suggested to play a critical role in gene regulation possibly by chromatin and epigenetic modulation. However, their physiological functions are not fully uncovered.

We have been investigating Schizosaccharomyces pombe lncRNAs that couple with gene activation and chromatin remodeling during glucose starvation. When cells face with glucose starvation, a cascade of lncRNAs transcription starts from the 1.3-0.7 kb upstream noncoding segment of fbp1+ (gene encoding fructose-1,6-bisphophatase, a key regulatory enzyme in gluconeogenesis), followed by stepwise chromatin remodeling (Hirota et al., Nature, 2008). We refer to such lncRNAs as mlonRNAs (metabolic stress-induced long non-coding RNA). Using directional RNA-seq analysis with next generation sequencers, we found that similar lncRNAs are transcribing from a number of other S. pombe loci in response to glucose starvation. Surprisingly, directional RNA-seq also revealed the existence of antisense RNA at fbp1+ under glucose rich condition. This indicates that not only mlonRNAs but antisense RNAs play a role in the fbp1+ transcriptional control. Moreover, ChIP-seq analysis revealed that such lncRNAs are accompanied with changes in histone H3 density. Hence, we propose that mlonRNAs have a function in chromatin alterations in response to metabolic stress signals induced by glucose starvation.

Centromere-specific regulation of recombination in fission yeast

Okita Akiko, Onaka Atsushi, Katahira Yasuhiro, Takahashi Tatsuro, Masukata Hisao, Nakagawa Takuro

Dept. of Biol. Sci., Grad. Sch. of Sci., Osaka Univ., Japan

Presented by: Akiko, Okita

Centromere is essential for proper segregation of chromosomes. Many eukaryotic centromeres are consisted of DNA repeats, and kinetochore chromatin and heterochromatin form in the central and surrounding regions of centromere, respectively. In fission yeast, a rad51 deletion increases gross chromosome rearrangement (GCR) in centromere, suggesting that maintaining the integrity of centromere requires homologous recombination. Analysis of spontaneous recombination between ade6 heteroalleles that are inserted into the central region of centromere, revealed that Rad51-dependent recombination exclusively occur in centromere. Analysis of recombinant DNA showed that crossovers of the intervening sequence were suppressed in centromere as compared to the arm region. However, what regulates recombination in centromere is still unclear.

The surrounding repetitive sequences and heterochromatin formed on the outer repeats might affect recombination in the central region of centromere. To test this possibility, we introduced the entire region of centromere 1 (cen1) of 40-kb into the arm region. Ectopic formation of heterochromatin was confirmed by ChIP experiments, although the kinetochore-specific H3 variant CENP-A was missing. In this cen1(arm) construct, Rad51 was not essential for the recombination and crossovers were increased as compared to the original centromere, suggesting that kinetochore chromatin is crucial for centromere-specific regulation of recombination. Recently, FANCM helicase has been shown to localize to centromere in human cells. Interestingly, a deletion of fml1, FANCM homolog in fission yeast, was found to increase crossovers in centromere. These results suggest that Fml1 might be recruited to centromere and promotes noncrossovers, thereby suppressing crossovers and GCRs in centromere.

An experimental evolution approach to gene regulation

Andrew Higgins, Christos Andreadis, Jürg Bähler, Max Reuter

University College London, Department of Genetics, Evolution & Environment, London WC1E 6BT, UK

Presented by: Higgins, Andrew & Andreadis, Christos

How genes are regulated is subject to a huge amount of study and has relevance to all aspects of biology. It seems logical to conclude that all regulated genes evolved from unregulated ones, whether in recent evolutionary time or further back when life was very simple. If we could view this process of regulatory evolution in the same way that palaeontologists can view the evolution of a fish fin into a mammalian limb then we would stand to learn many interesting details of how regulatory mechanisms came to be as they are. This may lead to further discoveries about the function of these diverse mechanisms, since only by understanding the functions which the mechanisms were selected to serve can we understand what these mechanisms are actually doing from a phenotypic perspective.

Using experimental evolution in S. pombe we are attempting to artificially select for a gene to evolve from being unregulated to regulated. We have found that expression of the ura4 gene can have strong negative and positive correlations with fitness in two different environments. We have placed the ura4 gene under the control of a novel promoter so that it is constitutively expressed across these environments. By selecting S. pombe alternately in these two environments we hope to evolve de novo regulation of the ura4 gene.

Identification of the Ubiquitin-fusion degradation protein 1 (Ufd1) as a player in the STUbL pathway and DNA damage response

Julie Bonne Køhler, Maria Mønster Jørgensen, Gabriele Beinoraite, Michael Thorsen, Geneviève Thon

University of Copenhagen, Denmark

Presented by: Køhler, Julie Bonne

We examine how sumoylation and the ubiquitin/proteasome degradation system cooperate to mediate the cellular response to DNA damage. Many factors participating in the DNA-damage response catalyze protein sumoylation or ubiquitylation. These modifications drive the sequential recruitment of proteins to sites of DNA damage and influence the choice of repair pathway. However, the timely and selective removal of modified factors is likely to be equally crucial for successful repair. A pathway through which sumoylated proteins are turned over involves their modification by a class of SUMO-targeted ubiquitin ligases (STUbLs), represented by the Slx8/Rfp1 and Slx8/Rfp2 dimers in fission yeast. Our results shed light on this pathway by identifying the ubiquitin-fusion degradation protein (Ufd1), a conserved eukaryotic protein capable of binding mono-ubiquitin and K48-linked ubiquitin chains, as an interactor of both the SUMO E3 ligase Pli1 and STUbL protein Rfp1. Ufd1 forms together with the Npl4 protein a major co-factor to the Cdc48 ATPase, involved in various ubiquitin-dependent processes. We found that deleting a part of Ufd1 responsible for its interactions with Pli1 and Rfp1 lead to an accumulation of sumoylated species and genomic instability, phenotypes shared by STUbL mutants. Based on presented data we discuss a role for Ufd1 (and by extension of the Cdc48/Ufd1/Npl4 complex) in the STUbL pathway and the DNA damage response.

Functional analysis of meiRNA, a non-coding RNA species essential for meiosis in fission yeast

Yuichi Shichino[a,b], Akira Yamashita[b], Masayuki Yamamoto[a,b]

[a] *Kazusa DNA Res. Inst., Japan*
[b] *Dept. Biophys. Biochem., Schl. of Sci., Univ. Tokyo, Japan*

Presented by: Shichino, Yuichi

The gene expression profile changes dramatically between mitosis and meiosis in fission yeast. In mitotic cells, the meiosis-inhibitor Mmi1 recognizes the DSR sequence in meiosis-specific mRNAs and eliminates them selectively. Mmi1 is observed as multiple dots in the mitotic nucleus. During meiosis, the master meiotic regulator Mei2 and its binding partner meiRNA, which is non-coding, form a dot structure at the meiRNA gene locus. The Mei2 dot tethers Mmi1 into a single dot and inactivates it. Thus, the meiosis-specific gene expression profile is ensured. meiRNA is thought to support the formation of Mei2 dot, but the function of meiRNA has not been fully understood. To address this problem, we performed functional analysis of meiRNA, which plays an important role in the regulation of meiotic progression.

In the original analysis of meiRNA, the major transcript was thought to be about 0.5 kb in length. However, we realized that the deduced downstream region of the gene carries numerous hexanucleotide motifs that constitute DSR. This led us to re-investigate the role of meiRNA in the mRNA degradation system involving Mmi1. In the mmi1-defective strain, the level of meiRNA expression increased and about 1.2 kb-long transcripts containing a number of DSR motifs (called meiRNA-L) were detected in addition to the 0.5 kb-long transcripts (called meiRNA-S). We then tried to dissect the functional domain of meiRNA-L. We found that the 3' region of meiRNA-L possessed the ability to bind to Mmi1, to promote meiosis, and to generate a dot. In contrast, Mei2 physically interacted with the 5' region of meiRNA-L. Moreover, overexpression of meiRNA-L in mitotic cells increased the number of cells containing a converged Mmi1 dot. Taken together, these results suggest that meiRNA-L tethers Mmi1 and downregulates its function via the 3' region, which abounds in the DSR motifs.

Fine structures of meiosis-specific chromosome and homologous chromosome pairing in *S. pombe*

Ding Daqiao[a], Matsuda Atsushi[a], Haraguchi Tokuko[a,b], Yasushi Hiraoka[b]

[a] *Advanced ICT Research Institute Kobe, NICT, Japan*
[b] *Graduate School of Frontier Biosciences, Osaka University, Japan*

Presented by: Daqiao, Ding

Pairing of homologous chromosome is an essential step in meiosis to ensure effective homologous recombination and reductional segregation in meiotic division. In the fission yeast S. pombe, formation of telomere bouquet and accompanying telomere-led chromosome oscillation align homologous chromosomes to the same direction and effectively promote their contact and pairing. DNA double strand break (DSB) formation is required for stabilizing of the transient interaction between homologous chromosomes and promote further pairing event. In the telomere bouquet nucleus, the meiosis-specific cohesin complex replaces the mitotic cohesin complex and forms an axis on the chromosome. Linear elements, a synaptonemal complex-like structure in fission yeast, form on the cohesin based chromosome axis and are required for DSB formation. We have found that the meiotic cohesin complex modulates chromosome compaction during meiotic prophase. Here we report roles of meiotic cohesin and linear elements in homologous chromosome pairing, and their fine structures observed using 3D structured illumination microscopy (3DSIM) in live cells.

H3K9 methylation, a self propagating mark?

Pauline Audergon, Alexander Kagansky, Alison Pidoux, Robin Allshire

[a] *Wellcome Trust Centre for Cell Biology, University of Edinburgh, UK*
[b]

Presented by: Audergon, Pauline

In fission yeast Schizosaccharomyces pombe, stable heterochromatin is present at three different domains: telomeres, centromeres and mating type. At these three loci, the formation of heterochromatin is targeted by RNAi and is strictly dependent on the histone 3 lysine 9 methyltransferase Clr4. Recent studies have shown that the tethering of Clr4 to an ectopic locus is sufficient to create a domain of heterochromatin structure characterised by the presence of heterochromatin marks, such as H3K9me2, Swi6 deposition and silencing of adjacent genes. In this study, we used an inducible system to tether Clr4 to an ectopic locus and studied the maintenance of the newly formed heterochromatin after removal of the tethered methyltransferase. Our analyses show that in absence of tethered Clr4, the chromatin very rapidly loses its heterochromatic mark indicating that H3K9me2 by itself is not sufficient to maintain the heterochromatic state even in the presence of endogenous Clr4. In addition, the very fast disappearance of H3K9me2 around the tethering site suggests that an active mechanism might be involved in the disassembly of this heterochromatin structure.

Dual roles of fission yeast F-box DNA helicase, Fbh1, during homologous recombination

Yasuhiro Tsutsui[a], Yumiko Kurokawa[b], Yumiko Kawano[a], Hiroshi Iwasaki[a]

[a] *Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan*
[b] *Education Academy of Computational Life Science, Tokyo Institute of Technology, Japan*

Presented by: Tsutsui, Yasuhiro

Homologous recombination is required for repairing DNA double-strand breaks (DSBs), which are induced by exogenous factors such as DNA damaging agents or by endogenous factors such as collapse of DNA replication fork in mitotic cells. If improperly processed, DSBs could lead to chromosome rearrangement, cell death, or tumorigenesis in higher eukaryotes. Fission yeast F-box DNA helicase, Fbh1 is suggested to regulate homologous recombination downstream of SpRad51, which is a homologue of prokaryote RecA recombinase and is essential for strand exchange reaction. F-box motif is conserved among F-box subunits of SCF ubiquitin ligase complex (E3). A mutation of F-box motif in Fbh1 confers DNA damage sensitivity as well as that of helicase motif, suggesting that both helicase and ubiquitin ligase activities play an essential role in Fbh1-mediated DNA repair. However, the precise roles of these activities remain to be elucidated. To examine the Fbh1 function in regulation of homologous recombination biochemically, we purified Fbh1 recombinant protein as a heterodimer together with Skp1. We examined the Rad51-mediated three-strand exchange reaction in the presence of the Fbh1-Skp1 complex. Interestingly, the strand exchange reaction is inhibited by the addition of Fbh1-Skp1 before the reaction starts, whereas it is stimulated by the addition after the reaction starts, suggesting that Fbh1 retains both pro- and anti-recombinase activities. On the other hand, in vitro ubiquitinylation assay revealed that the SCFFbh1 complex promotes ubiquitinylation of Rad51, indicating the possibility that ubiquitin ligase activity of Fbh1 could also regulate homologous recombination directly. Based on our observations obtained so far, a possible molecular mechanism how Fbh1 regulates homologous recombination will be discussed.

Molecular mechanism for the selective removal of meiotic transcripts in fission yeast

Akira Yamashita[a], Tomomi Takayama[b], Yuichi Shichino[a,b], Ryo Iwata[b], Masayuki Yamamoto[a,b]

[a] *Kazusa DNA Research Institute, Japan*
[b] *Dept. Biophys. Biochem., Grad. Sch. Sci., Univ. Tokyo, Japan*

Presented by: Yamashita, Akira

The gene expression profile differs greatly between mitotic and meiotic cells. In the fission yeast Schizosaccharomyces pombe, a large number of meiosis-specific transcripts are eliminated from mitotic cells. This elimination is driven by a YTH-family RNA-binding protein Mmi1. Mmi1 recognizes a specific region, namely, the determinant of selective removal (DSR) on meiotic transcripts and induces nuclear exosome-mediated elimination. During meiosis, the key meiotic regulator Mei2, which is also an RNA-binding protein, turns off the Mmi1-dependent elimination system. Mei2 forms a chromosome-associated dot structure together with non-coding RNA meiRNA. The Mei2 dot sequesters Mmi1 and inhibits its function, so that meiotic transcripts may be readily and stably expressed. Recently, we have found that meiRNA carries DSR and serves as a decoy substrate for Mmi1.

To uncover the detailed molecular mechanisms underlying the Mmi1/DSR elimination system, we sought to identify factors that interact with Mmi1. Consequently, we identified a novel factor involved in the Mmi1/DSR system by regulating the interaction between Mmi1 and another factor in the Mmi1/DSR elimination system.

The role of Stc1 in coupling RNAi and chromatin-modifying enzymes

Francesca Taglini[a], Sreerekha Pillai[b], Chao He[c], Yunyu Shi[c], Elizabeth Bayne[b]

[a] *Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK*
[b] *Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, UK*
[c] *Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, China*

Presented by: Taglini, Francesca

Constitutive heterochromatin is a higher-order chromatin structure fundamental for preserving the genome stability of eukaryotic cells. It ensures the silencing of repetitive sequences such as transposons and telomere and centromere repeats, avoiding chromosome rearrangements, and it also provides the assembly platform for factors involved in essential cellular process such as telomere maintenance and chromosome segregation.

Recently, many studies have shown a role for non-coding RNAs transcribed from repetitive regions in inducing heterochromatin assembly. In S. pombe, pericentromeric repeats are transcribed during S-phase and the resulting transcripts processed by the RNAi pathway into siRNAs. siRNAs are bound by the Ago1-containing RITS complex, and guide it to nascent transcripts based on sequence complementarity. Other factors are also recruited to pericentric chromatin dependent on RNAi, in particular the histone methyltransferase Clr4, a component of the CLRC complex, which is responsible for the deposition of the H3K9 methyl mark promoting the formation and spread of a stable heterochromatic state.

Despite this framework understanding, many steps in the pathway are still enigmatic. A particularly interesting question is how the cross-talk between the RNAi and chromatin modification machinery takes place. Stc1 was recently identified as a novel protein required for pericentric heterochromatin integrity and, specifically, for the establishment of H3K9 methylation via RNAi. Stc1 was shown to mediate the RNAi-dependent recruitment of CLRC to chromatin, apparently acting as a molecular bridge between RITS and CLRC complexes. Our work aims to understand how RNAi and heterochromatin formation are coupled through Stc1. Specifically, I am focussing on characterising the interaction between Stc1 and the CLRC complex.

Involvement of the end-resection machinery in the replication-maintenance functions of homologous recombination

Audrey Costes, Ismail Iraqui, Karine Freon, Sarah Lambert

Institut Curie / CNRS UMR3348, Bat 110, Université Paris-sud XI, 91405 Orsay, France

Presented by: Costes, Audrey

Robust DNA replication is essential to the maintenance of genome stability. During DNA synthesis, the replication fork progression can be compromised by replication fork barriers (RFBs) that slow-down, stall or collapse replication fork. In case of replisome malfunctions, homologous recombination (HR) is an efficient mechanism to restart replication forks. To study the molecular events associated with impediment to forks progression and the mechanisms involved in their restart, we made use of conditional fork obstacles assays that allow a single fork to be blocked at a specific locus in the fission yeast S. pombe. In these assays, halted forks are restarted by HR and we recently reported that fork-restart by HR is error-prone. To get better insight in our understanding of the mechanisms by which HR restarts replication forks, we investigated the roles of DNA helicases known to be involved in replication stress. We have found that Rqh1Sgs1, Fbh1, Rad8Rad5 and Rad54 are dispensable for HR-dependent fork restart. In contrast, the lack of ExoI, a factor of the end-resection machinery, lead to major changes in replication intermediates structures and affect the efficiency of replication fork restart by HR. The involvement of other members of the end-resection pathway will be presented. Our data suggest that the DSB resection machinery might also participate in replication fork reactivation by HR.

The investigation of the role for thiamine in oxidative stress response in *Schizosaccharomyces pombe*

Bedia Palabiyik, Farinaz Jafari Ghods, Ahmet Akçay

İstanbul University, Department of Molecular Biology & Genetics, 34134, Vezneciler, İstanbul, Turkey

Presented by: Palabiyik, Bedia

In this study the relationship between the regulation of thiamine metabolism, glucose repression and oxidative stress response in wild type cells (972h-) and glucose repression resistance mutants, which grown in yeast media with 2-deoxy-D-glucose, was investigated. We determined that the increased expression level of ght3 gene, which have highest affinity among hexose transporter genes, fbp1 gene, as a marker gene for glucose repression, while changed expression level of hxk2 gene in ird mutants compared to wild type. These findings have shown to have lack of glucose repression and the difference of glucose metabolism rates of ird mutants. In microarray data sets, most of genes that are involved in thiamine metabolism were down regulated in ird mutants compared to wild type, while up regulated in ird mutants after exposure to H2O2. In order to understand if thiamine regulation pathway works independently from glucose repression pathway or not, and also to understand its possible effect on oxidative stress response pathway, we will investigate the expression profile of some genes (such as thi2, thi3, thi4, pho4, car1/bsu1, thi9 and ptr2) involved in thiamine metabolism in ird mutants grown in both minimal media with or without thiamine and rich media treated and untreated H2O2, in our ongoing studies. Since, thiamine, as an enzymatic cofactor, regulates carbohydrate metabolism.

The CCR4-NOT complex from *Schizosaccaromyces pombe*

Aleksandra Siwaszek[a], Marta Ukleja[a], Andrzej Dziembowski[a,b]

[a] *Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland*
[b] *Institute of Genetics and Biotechnology, Warsaw University, Warsaw, Poland*

Presented by: Siwaszek, Aleksandra

CCR4-NOT complex is a 1 MDa macromolecular assembly involved in mRNA degradation. In most eukaryotic cells first step of mRNA decay is the removal of poly(A) tail. This process is facilitated by highly conserved CCR4-NOT, complex of nine proteins, two of which, Ccr4 and Pop2 have exonuclease domains. So far the function and biological role of the other subunits remains unknown. The complex is localized in the cytoplasm, nucleus and P-bodies. Apart from deadenylation activity it has been implicated that the complex is involved in many aspects of mRNA metabolism, including initiation and elongation of transcription.

Most studies about this deadenylase were performed in S.cerevisiae in which Pop2 is not fully conserved. Thus, it is important to provide new data about the complex as a whole, its activity, mutant phenotypes and interactions with other cellular components.

Here we present results concerning successful purification of CCR4-NOT from fission yeast strains in which NOT2/POP2 (subunits of the complex) genes were fused to TAP-tag. Purified complex were analysed by in vitro deadenylation assay. As the purification revealed strong interaction between CCR4-NOT and rRNA, the specificity of this interaction was verified by the analysis of protein migration pattern in cell polysomal profiles. Moreover, phenotypes of the deletion mutants of the subunits of the complex, were analysed.

The chromatin architecture of *Schizosaccharomyces pombe*: a role for cohesin.

Shweta Bhardwaj, Nicholas J Proudfoot, Monika Gullerova

Sir William Dunn School of Pathology, University of Oxford, UK

Presented by: Bhardwaj, Shweta

The three-dimensional organization of the genome is important for various processes such as transcription, replication, and repair. In mammals, the sister chromatid cohesion protein, Cohesin and the insulator protein CTCF are critical determinants of chromatin organization. Cohesin is an essential, conserved multi-subunit protein complex, loaded by Mis4/Ssl3 (in fission yeast) and maintained on chromosomes by the antagonistic activities of Eso1 and Wapl. In fission yeast, a major proportion of cohesin is present at sites lacking Mis4, leading us to contemplate a possible role of cohesin at these sites. Using single locus DNA-FISH, we find transient sister chromatid cohesion at such loci (Rad21 only), contrary to stable cohesion at sites co-bound by Mis4/Rad21. Using a combination of chromatin immunoprecipitation-chromosome conformation capture (ChIP-3C) and DNA FISH, we detect colocalization between actively transcribed genes present in regions bound by Rad21/Mis4, extending the possibility of cohesin hubs, analogous to mammalian transcription factories. As expected, such interactions are lost in a rad21ts mutant. Interestingly, inhibition of transcription results in loss of Mis4/Rad21 binding, suggesting an interplay between cohesin and transcription.

Homologous recombination intermediates leading to chromosomal rearrangements are checkpoint blind

Saed Mohebi, Adam Watson, Antony Carr, Johanne Murray

Genome Damage and Stability Centre, University of Sussex, UK

Presented by: Mohebi, Saed

We have investigated the timing of recombination-dependent replication restart through a single cell cycle using a system which arrests replication forks at a specific locus in the fission yeast genome. In this system replication stalling is brought about by the binding of the transacting factor Rtf1 to the RTS1 polar replication termination sequence. Expression of Rtf1 using the urg1 promoter allows us to induce expression within 30min of the addition of uracil in synchronous cultures. Replication restart at RTS1 is dependent on homologous recombination. However, it occurs at the expense of gross chromosomal rearrangements that occur by either faulty template usage at restart or after the correctly restarted fork U-turns at inverted repeats. Both these mechanisms of chromosomal rearrangement generate acentric and reciprocal dicentric chromosomes. We show by one-dimensional and two-dimensional gel electrophoresis that recombination intermediates become visible in S phase within 15minutes of replication stalling and are maintained into G2 before decreasing, coincident with the accumulation of rearranged products (acentric and dicentric chromosomes) . Chromosome bridges corresponding to the dicentric chromosome are seen in mitosis, which occurs with similar timing to the uninduced cultures. This demonstrates that there is no delay to cell cycle progression. Analysis of Cds1 phosphorylation shows that the inter S phase checkpoint is not activated either in the first or the second cell cycle. Similarly, analysis of Chk1 phosphorylation shows that, consistent with the lack of delay to mitosis, the DNA integrity checkpoint is not activated in the first cell cycle. However, Chk1 activation is seen in the second G2, consistent with breakage of the dicentric chromosome and resection exposing single strand DNA. Thus, recombination-dependent replication restart, recombination intermediates and gross chromosomal rearrangements are checkpoint blind in the first cell cycle.

RNAi coordinates DNA replication and epigenetic modification in heterochromatin through the release of RNA Pol II

Stephane Castel, Jie Ren, Rob Martienssen

Cold Spring Harbor Laboratory, USA

Presented by: Castel, Stephane

One defining characteristic of epigenetic modifications is their heritability. The pericentromeres of the fission yeast Schizosaccharomyces pombe are a perfect model for studying this as they are composed of repressive heterochromatin and repetitive non-coding RNAs whose processing by RNA interference (RNAi) is required for histone modification. We have previously shown that these otherwise silenced non-coding RNAs are expressed specifically during S phase, the same time at which histone marks are deposited and DNA replication occurs. To further study the role of RNAi in regulating transcription, chromatin immunoprecipitation was used to interrogate RNA Pol II (Pol II) status genome wide in wild type and RNAi deficient S. pombe throughout the cell cycle. In the absence of RNAi, Pol II stalling and DNA damage occurs at centromeres during DNA replication and requires repair by homologous recombination to proceed. RNAi is therefore required to coordinate DNA replication and epigenetic modification in centromeric heterochromatin. We propose that transcription of non-coding RNAs during S phase leads to the collision of RNA Pol II with nearby replication forks that is resolved by processing of nascent RNAs by RNAi. This processing releases Pol II and allows DNA replication to proceed, but also functions to localize the repressive histone modifying complex to replicating heterochromatin. The RNAi mediated release of Pol II in the context of replication presents a new paradigm that may operate at euchromatic loci genome wide, and could function analogously in higher organisms. More broadly, these findings and the subsequent model presented provide insight into the inheritance of epigenetic marks, and the observed coupling of histone modification to DNA replication could explain the phenomenon of heterochromatic spreading.

Hydrocortisone effect on *Schizosaccharomyces pombe*'s *rad17+* gene expression

Çağatay Tarhan, Tuğçe Munise Şatir, Merve Yilmazer, Semian Karaer Uzuner

İstanbul University, Department of Molecular Biology & Genetics, 34134, Vezneciler, İstanbul, Turkey

Presented by: Karaer Uzuner, Semian

Hydrocortizone is a glycocortioide that is frequently used in the cure of various diseases. Most commonly used in the suppression of allergic reactions. Some previous studies have observed that oncogenes and p21 gene caused by the increase in their accounts. However, the studies investigating the effect of genes synthesizing proteins that control the rhythm of cell proliferation are quite limited.

The aim of this study is mapping differences of dividing rhythm and transcription differences of rad17+ gene in the precence of hyrocortizon working with wild type Schizosacchoromyces pombe as a model organism. The cells were treated with 0.8 mM hydrocorticon for 5 and 7.5 hours. Then, these treated cells and control cells which were growth in a medium that is not containing hydrocortizon were tested for vitality applying smear and spot cultering. After testing vitality, total RNA isolaton was done for real-time PCR analysis. Real-time PCR analysis was performed with SYBR green.

Significant increasing in the transcription of rad17+ gene was observed. It has been known that rad17+ gene functions in controlling of cell cycle and enabling chromosomal telomere dynamic. According to available data, it can be proposed that hydrocortizon can be damaging DNA and telomere dynamic. For more accurate result, more studies should be done on this subject.

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Distinct stability control of metabolic stress-related sense and antisense long noncoding RNAs

Miki Atsuko[a], Galipon Josephine[a], Oda Arisa[a], Inada Toshifumi[b], Ohta Kunihiro[a]

[a] *Graduate School of Science, the University of Tokyo, Japan*
[b] *Graduate School of Pharmaceutical Sciences, Tohoku University, Japan*

Presented by: Atsuko, Miki

Long non-coding RNA (lncRNA)s are well known as regulators of gene expression. The fbp1+ locus in fission yeast Schizosaccharomyces pombe codes for an essential enzyme in gluconeogenesis. Upon glucose starvation, a cascade of sense lncRNA transcription starting from the far upstream region of fbp1+ is correlated with the opening of chromatin structure and necessary for full induction of fbp1+ mRNA (Hirota et al. 2008). We refer to these lncRNA as "metabolic stress-induced lncRNA" (mlonRNA). Strand-specific RNA-seq analysis revealed an antisense lncRNA (asRNA) overlapping the fbp1+ ORF is transcribed in glucose-rich condition. It quickly disappears upon starvation and is anti-correlated with mlonRNA and mRNA expression (Oda et al., this meeting). In this study, we investigated the fate of these lncRNAs. We found that mlonRNAs and aslncRNAs are 5'-capped, less stable than fbp1+ mRNA, and partially degraded by the nuclear exosome (Galipon et al. 2013 and this study). Interestingly, they are bound by multiple ribosomes in the cytoplasm. Presence of numerous AUGs in these lncRNA suggests degradation by the nonsense-mediated decay (NMD) pathway, however only asRNA is the target of NMD. Moreover, half-life measurements revealed that the stability of asRNA did not significantly change after starvation, suggesting that the disappearance of asRNA is likely due to decreased RNA polymerase II activity, rather than increased instability. These data demonstrate that the stability of two types of fbp1+ lncRNAs is controlled distinct RNA quality control pathways.

We will discuss the possible roles of decapping enzymes, cytoplasmic 5' to 3' exonucleases, and exosome cofactors in the stability control of fbp1+ lncRNAs. We will also argue about the involvement of S. pombe putative factors for the no-go decay (NGD) in the decay of mlonRNA, since they contain many short ORFs and rare codons in their 5' region.

Analysis of *S. pombe* histone post-translational modifications by mass spectrometry

Angevin Thibaut

Genome Damage and Stability Centre, Sussex, UK

Presented by: Thibaut, Angevin

Histones are not only confined to DNA compaction, but are also involved in gene expression. Histones are modified proteins and their patterns of post-translational modifications (PTM) are highly conserved. These PTMs are associated with active/inactive genes and interact with each other. The diversity and cross-talk of histones PTMs have led to the idea these modifications constitute a gene expression 'code'. Also, evidence suggests that these modifications may be epigenetic. Interestingly, a number of histone PTMs and histone variants are known to be involved in the DNA-damage response (DDR). In order for repair proteins to access the site of damage, the histones must be moved aside and modified. Subsequent to repair, histones are restored. It is unknown, however, whether the original pattern of histone modifications and histone variants is restored after successful repair or not, which could serve as a 'memory' of DNA damage.

The aim of the study is to characterize the alteration in histone PTMs associated with the different stages of DDR using Schizosaccharomyces pombe as a model organism. We created a plasmid containing a series of lac operon sequences, allowing efficient purification of the plasmid when transformed into a strain carrying within its genome the lac inhibitor fused to tags. Also, this plasmid contains a HO cleavage site; the HO endonuclease being stably integrated into the strain under the control of an inducible promoter to control its expression. The plasmid can therefore be isolated either before or after the induction of a single targeted DNA double-strand break at the HO site; and histones associated with the different stages of DDR can therefore be purified for subsequent analysis.

Using Mass Spectrometry techniques, we will identify repair-associated modifications. The targeted approach will allow us to obtain chromatin enriched for DDR-associated histone modifications, and to characterize in detail the PTMs pattern before and after repair.

Fission yeast RecQ helicase Rqh1 is required for the maintenance of circular chromosomes

Nambu Tomoko, Takahashi Katsunori, Ueno Masaru

Department of Molecular Biotechnology, Hiroshima University, Higashi-Hiroshima, Japan

Presented by: Tomoko, Nambu

Pot1 binds to single-stranded telomere overhangs and protects chromosome ends from resection. When pot1+ is deleted, only the cells which have circular chromosomes can survive. Rqh1, the RecQ helicase in fission yeast, regulates homologous recombination at multiple stages, including resection, strand displacement, and resolution.

In fission yeast, pot1Δ rqh1Δ double mutants is synthetically lethal, but the mechanism of the lethality is not yet understood [1]. We found that the synthetic lethality of the pot1Δ rqh1Δ double mutants is suppressed by the deletion of rad51+. We also found that the expression of rad51+ in pot1Δ rqh1Δ rad51Δ triple mutant, which has circular chromosomes, leads to the lethality. Moreover, reduction of the expression of Rqh1 protein in pot1Δ cells with circular chromosomes caused chromosome missegregation. These results suggested that Rqh1 is required for maintenance of circular chromosomes.

In fission yeast, deletion of rad57+ decreases high crossover frequency of rqh1Δ cells to 0% [2]. Crossing-over between sister chromatids in circular chromosomes creates circular chromosome dimers, which inhibit proper chromosome segregation. Deletion of rad57+ in pot1Δ rqh1Δ double mutants suppressed the lethality. This suggests that crossing-over between sister chromatids in circular chromosomes caused the lethality of pot1Δ rqh1Δ double mutants.
Taken together, we propose that Rqh1 prevent generation of circular dimers by suppressing crossover between circular monomeric chromosomes [3].

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[2] Hope JC et al., Mol Cell Biol 27,3828-38 (2007)
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Dissecting the role of different domains of the transcription factor Atf1 in the control of transcription initiation at stress genes

Esther Paulo, Patricia Garcia, Jun Gao, Wayne Wahls, Elena Hidalgo

[a] *Oxidative Stress and Cell Cycle Group, Universitat Pompeu Fabra, Barcelona, Spain*
[b] *University of Arkansas for Medical Sciences, USA*

Presented by: Paulo, Esther

In response to hydrogen peroxide (H2O2), Schizosaccharomyces pombe activates different signalling pathways depending on the severity of the stress exerted, the main ones being the Pap1 and Sty1 pathways. The MAP kinase Sty1 pathway becomes activated upon several kinds of stresses: heat shock, stationary phase, osmotic stress and oxidative stress. Upon H2O2 stress, Sty1 is phosphorylated and translocates to the nucleus, where regulates transcription of CESR (core environmental stress response) genes through the transcription factor Atf1. It has been shown that this transcription factor contains several functional regions including a bZIP domain essential for the binding to CRE-like DNA sites at CESR promoters. One of the most important genes in the oxidative stress tolerance response is ctt1 (catalase) which expression is highly up-regulated upon oxidative stress in a Sty1-Atf1-dependent manner. In order to understand better the individual roles of Sty1 and Atf1 on the initial sensing-activating events, we have generated a mutant strain that has a CRE-to-G4BS (GAL4 binding site) substitution at the chromosomal ctt1 promoter. As expected, that strain is very sensitive to H2O2, and ctt1 mRNA does not accumulate upon oxidative stress. Next we constructed several gene chimeras expressing full length Sty1 and Atf1 or a truncated version of Atf1 without the bZIP domain (Gao et al., 2008; Gao et al., 2009) fused to the GAL4 DNA binding domain to dissect the initial sensing-activating events of the Sty1 cascade. In addition, we are also trying to identify a possible auto-inhibition domain of Atf1, since a full length version of Atf1 is not able to suppress the sensitivity to H2O2 in a Δsty1 background while only the bZIP domain is.

Motifs in the Clr2 protein specific for mating-type silencing

Daniel Steinhauf[a], Alejandro Rodriguez[a], Dimitris Vlachakis[b], Gordon Virgo[a], Carolina Kristell[a], Ida Olsson[a], Erik Bongcam-Rudloff[c], Sophia Kossida[b], Pernilla Bjerling[a]

[a] *Dept. of Medical Biochemistry and Microbiology (IMBIM), University of Uppsala Box 582, SE-751 23 Uppsala, Sweden*
[b] *Bioinformatics & Medical Informatics Team, Foundation for Biomedical Research, Academy of Athens, Greece*
[c] *Department of Animal Breeding and Genetics, Swedish University of Agriculture Sciences, Sweden*

Presented by: Steinhauf, Daniel

Schizosaccharomyces pombe is a well-established model for the process of heterochromatin formation. Several components involved in heterochromatin formation have been identified, but the exact sequence of events for initiation remains to be elucidated. Crucial factors involved are RNA transcribed from repeated sequences together with methyltransferase Clr4 and histone deacetylases, for example Clr3, found in the SHREC complex. Clr2 is another crucial factor essential for heterochromatin formation found in the SHREC complex. The exact function of Clr2 has been difficult to pinpoint since no homologous proteins or conserved domains with known functions has been identified in the protein.
Using a bioinformatics approach three conserved motifs in Clr2 were identified, that were named C2SM1-3. C2SM3 corresponds to the previously annotated Clr2 domain (IPR018839). Eight amino acids were mutated in these motifs and integrated at the endogenous clr2 locus. Surprisingly these amino acids were essential for transcriptional repression in the mating-type region, but dispensable for pericentromeric silencing. Several of the mutated strains show unstable silencing in the mating-type region, switching between on and off states, indicative of an establishment or maintenance defect. When tested for silencing in the central core centromere and rDNA the mutant displayed weak effects. In silico modelling suggests that the introduced mutations cause instability to the Clr2 protein. Moreover, a subnuclear localisation of the Clr2 protein is demonstrated. Finally, we show that all the SHREC components are necessary for targeted silencing by tethering of Clr4.
We have identified critical amino acids in the Clr2 protein providing us with the necessary tool to pinpoint the exact function of Clr2 in heterochromatin formation. In addition, these mutations indicate partly separate mechanisms of action for Clr2 in the mating-type as compared to the pericentromeric region.

Impeded DNA replication of telomeric sequences provokes telomeres shortening in fission yeast

Julien Audry[a], Pierre Luciano[a], Toru Nakamura[b], Vincent Géli[a], Stéphane Coulon[a]

[a] *Cancer Research Center of Marseille, Inserm UMR 1068, CNRS UMR 7258 Marseille, France*
[b] *University of Illinois at Chicago, Department of Biochemistry and Molecular Genetics 900 S. Ashland (M/C 669) Chicago, IL 60607,USA*

Presented by: Coulon, Stephane

In fission yeast, the telomeric complex, also named shelterin complex composed by Taz1, Rap1, Rif1, Poz1, Ccq1, Tpz1 and Pot1, ensures the protection of chromosomes ends and contributes to the recruitment of telomerase. Other non-telomeric factors like Rad3 and Tel1 in a less extend, have been shown to participate actively to telomere maintenance. It was established that Rad3 and Tel1 kinases phosphorylate Ccq1 to promote recruitment and activation of telomerase through Ccq1-Est1 interaction when replication is completed. Lately, we have shown that the Replication Proteine A (RPA) participates to telomere maintenance in a telomerase-dependent manner but independently of Rad3 pathway. Our goal is to understand mechanistically how RPA contributes to telomere maintenance.

In our study, we took advantage of the RPA1D223Y allele that provokes telomeres shortening. ChIP experiments have shown that D223Y mutation does not impede RPA recruitment at telomeres during replication. Nevertheless, the presence of RPA1D223Y at telomeres is prolonged after replication and prevents the proper recruitment of Pot1. ChIP of replicative polymerases have shown that Polε is recruited at telomeres indifferently in wt and D223Y strains while, surprisingly, we found that Polα primase is sequestered at telomeres after completion of S-phase only in RPA1D223Y mutant. These observations suggest that RPA1D223Y causes defects in replication of telomeric lagging strand. In agreement, 2D-gel analysis showed that replication of telomeric regions is altered in D223Y strains. We believe that incorrect replication of guanine-rich telomeric DNA by lagging strand machinery provokes accumulation of secondary DNA structures like G-quadruplex in RPA1D223Y mutant. Incomplete replication of telomere would not be suitable for telomerase action at chromosome extremities. We are currently testing the hypothesis that RPA might actively recruits helicases to prevent accumulation of DNA secondary structures

Global control of RNA turnover in the fission yeast *Schizosaccharomyces pombe*

Ayesha Hasan, Juan Mata

Department of Biochemistry, University of Cambridge, CB2 1QW, UK

Presented by: Hasan, Ayesha

Gene expression is a highly regulated process that is controlled at several levels, both transcriptional and posttranscriptional. Cellular mRNA levels depend on the rates of both transcription and mRNA decay; therefore, the decay rate of mRNAs is a major determinant of mRNA abundance. Genome-wide studies of RNA decay have shown that all RNAs are degraded at specific rates, which are often adjusted in different conditions. This specificity is thought to be partly conferred by RNA-binding proteins (RBPs) that recognise regulatory sequences in their targets and modulate their interaction with the RNA degradation machinery. We have taken a systematic approach to investigate how cells coordinate RNA degradation pathways through the function of RBPs. A collection of 101 S. pombe strains carrying deletions in non-essential genes encoding RNA binding proteins was profiled using oligonucleotide microarray technology. We found that several RNA binding proteins have clear effects on RNA levels, and used genome-wide measurements of RNA stability to confirm that the effects were due to altered RNA turnover. The lists of affected genes in the RBP deletions were enriched in genes with common properties, suggesting biological significance. However, we also found that the decay rates of a large fraction of mRNAs are not influenced by non-essential RBPs, suggesting that gene-specific mRNA stability is regulated by other means.

Depletion of histone H2A.Z is required for neocentromere accommodation

Yuki Ogiyama, Yuko Ohno, Yoshino Kubota, Kojiro Ishii

Graduate School of Frontier Biosciences, Osaka University, Japan

Presented by: Ogiyama, Yuki

Centromere is epigenetically defined by nucleosomes that contain the histone H3 variant centromere protein A (CENP-A) in most eukaryotes which employ regional centromeres. Specific targeting of the CENP-A-loading chaperone to the centromere is therefore vital for stable centromere propagation; however, the existence of ectopic centromeres (neocentromeres) indicates that this chaperone can function in different chromatin environments. The mechanism responsible for accommodating the CENP-A chaperone at novel chromatin regions is poorly understood. Here, we report the identification of transient, immature neocentromeres in Schizosaccharomyces pombe, which show reduced association with the CENP-A chaperone Scm3 attributable to persistence of the histone H2A variant H2A.Z. Following acquisition of adjacent heterochromatin or relocation of the immature neocentromeres to subtelomeric regions, H2A.Z was depleted and Scm3 was replenished, leading to subsequent stabilization of the neocentromeres. These findings provide novel insights into histone variant-mediated epigenetic control of neocentromere establishment.

Nucleosome dynamics during the expression of the meiotic transcriptional programme in *Schizosaccharomyces pombe*

Ignacio Soriano, Luis Quintales, Francisco Antequera

Instituto de Biología Funcional y Genómica, Consejo Superior de Investigaciones Científicas (CSIC)/Universidad de Salamanca, Campus Miguel de Unamuno, 37007-Salamanca, Spain

Presented by: Soriano, Ignacio

Nucleosomes facilitate the packaging of the eukaryotic genome and modulate the access of regulators proteins to DNA. To determine the extent of chromatin remodelling along a developmental process, we have generated high-resolution nucleosome maps by next generation sequencing that allow us to determine the localization and dynamics of individual nucleosomes during mitosis and meiosis in Schizosaccharomyces pombe. We show that remodelling is limited to approximately 1000 nucleosomes that represent 1.5% of the genome and map to the promoters of a subset of meiosis-specific genes. This fraction contrasts sharply with the remaining 98.5% of the genome, which is organized in a pattern of highly positioned nucleosomes that remains virtually invariable during mitosis and meiosis. This pattern is maintained despite the different processes undergone by the chromosomes, and even across genes that display widely different levels of expression. We also show that nucleosome-depleted regions at the 5' end of genes overlap precisely with clusters of binding sites for transcription factors that are specific for different functional classes of genes. These results suggest the possibility that the sequence-dependent positioning of transcription complexes at promoters could contribute to maintaining a constant nucleosome pattern across the genome.

Characterization of Nrl1 protein in *S. pombe*: a link between RNAi and Pol II?

Lucia Aronica

University of Vienna/Max F. Perutz Laboratories, Austria

Presented by: Aronica, Lucia

Recent evidence indicates the existence of a physical and functional link between RNA-interference (RNAi) and RNA polymerase II (Pol II) throughout the eukaryotic kingdom. However, the role of this complex partnership on the metazoan heterochromatin landscape remains still unexplored. In Caenorhabditis elegans, the NRDE-2 protein connects RNAi and Pol II inhibition, in a process of co-transcriptional gene silencing (CTGS). By in silico analysis, I have identified the ortholog of C. elegans NRDE-2 in S. pombe, and I have named it NRde-2 Like protein 1 or Nrl1. In the project I propose here, I want to investigate whether and how Nrl1 may also represent a link between RNAi and Pol II in S. pombe.

Since Nrl1 has orthologs also in Arabidopsis, Drosophila and human this study may produce important contributions in the field of metazoan RNAi-mediated CTGS, going far beyond the specific yeast system used in this work.

Autophosphorylation of condensin SMC's subunits Cut3 and Cut14; Revelation of a part of the ATP cycle

Yuko Akai, Norihiko Nakazawa, Mitsuhiro Yanagida

Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Presented by: Yanagida, Mitsuhiro

Condensin complex contains two structural maintenance of chromosomes (SMC) subunits and three regulatory non-SMC subunits. It is essential for chromosome condensation and segregation in mitosis, and for DNA damage repair and gene dosage compensation. The terminal domains of SMCs contain the ATPase motifs, and are connected to a helical domain that is interrupted by a central hinge. How the catalytic ATPase motifs affect the role of condensin is poorly understood, however. We here demonstrate that purified SMC subunits, Cut3, Cut14 and the heteromeric complex Cut3-Cut14, are autophosphorylated in vitro by ATP, and this phosphorylation is removed by λ phosphatase. Autophosphorylation requires the ATPase domain of Cut3 and Cut14. Even single Cut3 and Cut14 are phosphorylated dependent on the presence of intact ATPase domain. Curiously, only Cut3 subunit of the complex Cut3-Cut14 is phosphorylated. We investigated this surprising modification reaction of condensin in detail. Phosphorylation neither occurs with ADP nor AMP-PNP, but thio-phosphorylation occurs with ATPγS. This γS thio-phosphorylation is irreversible, as phosphatase fails to remove it, so that evidence is obtained that the in vitro DNA interactions of Cut3-Cut14 are altered upon thio-phosphorylation. We propose the role of autophosphorylation in the context of ATP cycle in condensin by comparing phosphorylation behavior of SMC subunits with that of holocondensin.

Fission yeast Stn1 is essential for telomere stability

Takikawa Masahiro, Tarumoto Yusuke, Iashikawa Fuyuki

Graduate School of Biostudies, Kyoto University, Japan

Presented by: Masahiro, Takikawa

Telomere, a chromosomal domain essential for genetic stability, associates with various proteins. Among them, the CST complex is a phylogenetically conserved protein complex from fission yeast to humans, consisting of Ctc1, Stn1 and Ten1. In fission yeast, the stn1 and ten1 homologue genes have been identified, but the ctc1 homologue remains to be isolated. Both stn1 and ten1 are essential for telomere maintenance: Deletion of stn1 results in deprotected telomeres, leading to formation of self-circularizing chromosomes.

We randomly mutagenized stn1 and isolated a temperature sensitive mutant stn1ts allele. At a permissive temperature, the mutant cells grew vigorously and maintained stable telomeres. When the temperature was shifted from a permissive temperature to a non-permissive temperature, the cells rapidly possessed longer telomeric G-overhang. At the same time, Rad22, a fission yeast Rad52 homologue, accumulated at the telomeres. Continuous cultivation at a non-permissive temperature caused complete telomere loss. We have isolated several genes that suppressed the telomere deprotection upon the inactivation of stn1ts. We are now elucidating the molecular mechanism producing the genetic interactions between stn1 and the suppressor genes.

Fission yeast CSL proteins function as transcription factors

Martina Oravcová, Mikoláš Teska, František Půta, Petr Folk, Martin Převorovský

Department of Cell Biology, Faculty of Science, Charles University in Prague, Viničná 7, 128 43 Prague 2, Czech Republic

Presented by: Oravcová, Martina

Transcription factors of the CSL (CBF1/RBP-Jκ/Su(H)/LAG-1) family belong among key regulators of metazoan development. They are context-dependent activators or repressors of gene expression and function as the effector component of the Notch signalling pathway. We identified two CSL paralogs in the fission yeast, which lacks other Notch pathway components. Cbf11 and Cbf12 exert antagonistic effects on the coordination of cell cycle events and on cell adhesion. However, it is still not clear whether Cbf11/12 are true CSL transcription factors.

We have discovered that the low-complexity N-termini of Cbf11 and Cbf12 are important for their nuclear localisation and DNA binding activity. We have previously shown a sequence-specific DNA binding activity for Cbf11 in vitro. Now we demonstrate that full-length Cbf12 also exerts specific, yet weak, binding to the canonical metazoan CSL response element in vitro.
A point mutation in the murine CSL protein which dramatically reduces the DNA binding activity without negatively affecting protein stability or localization has the same effect in Cbf11/12, suggesting a conserved mechanism of DNA binding.

Both Cbf11 and Cbf12 activate reporter gene expression in S. pombe, although Cbf12 only when overexpressed. Furthermore, Cbf11 can bind the canonical CSL element in the reporter promoter even in vivo.

Our results indicate that the fission yeast CSL proteins are indeed genuine family members capable of functioning as transcription factors, and provide support for the ancient evolutionary origin of this important protein family.

Regulation of non-coding transcription in *Schizosaccharomyces pombe*

Sneha Shah, Sina Wittmann, Cornelia Kilchert, Lidia Vasiljeva

Department of Biochemistry, University of Oxford, UK

Presenter: Shah, Sneha

Elucidating the role of the RNA polymerase II mediated non-coding (nc) transcription in gene regulation has been the focus of a number of recent studies. It has been proposed that nc transcription can regulate expression of the protein-coding genes via transcriptional interference when initiated from the promoter located upstream to mRNA encoding transcription unit. In this scenario, the act of nc transcription *per se* plays an important regulatory role while resulting ncRNA is rapidly destroyed by the nuclear exosome. To ensure that ncRNA is efficiently degraded, in *Saccharomyces cerevisiae* nc RNA 3'end formation is coupled with the exosome recruitment regulated by the Nrd1-Nab3-Sen1 complex. It is not clear how nc transcription is regulated in other organisms and whether Nrd1-dependent mechanism is conserved.

We show that in fission yeast *Schizosaccharomyces pombe*, nc transcription represses expression of a meiotic protein-coding genes via transcriptional interference during mitosis. Interestingly, nuclear exosome is co-transcriptionally recruited via RNA binding protein Mmi1 to nc transcript and appears to be required for transcription termination and degradation of ncRNA. These data implies that coupling between 3' formation and degradation of unstable nc RNAs might be evolutionary conserved. Moreover, in the absence of the exosome, RNAi machinery recruited via ncRNA silences expression of the entire locus. We conclude that multiple redundant silencing pathways cooperate to ensure efficient gene regulation in eukaryotes. Fail safe mechanisms to silence nutrient metabolism genes in fission yeast ensure optimal expression of genes in response to environmental cues.

CLRC organization and Dos1 crystal structure reveal a target-binding site important for heterochromatin silencing

Canan Kuscu[1,2,3,] Thomas Schalch[1,3,5], Mikel Zaratiegui[3,6], Hyun Soo Kim[3], David A. Wah[1,3,4], Robert A. Martienssen[3,4], Leemor Joshua-Tor[1,3,4]

[1] W. M. Keck Structural Biology Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA
[2] PhD program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, NY 11794, USA
[3] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA
[4] Howard Hughes Medical Institute, Cold Spring Harbor, NY 11724, USA
[5] Current address: Department of Molecular Biology, Science III, University of Geneva, CH-1211 Geneva, Switzerland
[6] Current address: Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08854, USA
Authors contributed equally to this work

Presented by: Schalch, Thomas

Repressive histone H3 lysine 9 methylation (H3K9me) and its recognition by HP1 proteins are necessary for pericentromeric heterochromatin formation. In Schizosaccharomyces pombe, H3K9me deposition depends on the RNAi pathway. Clr4, the only known H3K9 methyltransferase in this organism, is a subunit of the CLRC complex, which also contains the cullin Cul4 and its interacting protein Pip1, the adaptor Rik1, a WD repeat protein Dos1, and Dos2. CLRC is an active E3 ligase in vitro, and this activity is necessary for heterochromatin assembly in vivo. The similarity between CLRC and cullin-RING Ligases (CRLs) suggests that the WD repeat protein Dos1 will act to mediate target recognition and substrate specificity for CLRC. Here, we present a pairwise interaction screen that reveals the CLRC subunit arrangement, and the crystal structure of the Dos1 WD repeat domain that has an eight-bladed β-propeller fold. We demonstrate that the surface of Dos1 that does not interact with known CLRC components is required for heterochromatic silencing, underscoring its role as the specificity factor for the ligase.

Wdr70 promotes DNA repair by facilitating homologous recombination

Ming Zeng[1], Laifeng Ren[1], Yuding Wong[1], Xiaobo Wang[1], Haibin Wang[1], Peng Yang[1], Liandi Guo[1], Antony Carr[2], Cong Liu[1]

[1] Laboratory of Genomic-Stability, Development and Stem Cell Institute, The West China Second University Hospital, Sichuan University, Chengdu, 610041, China
[2] MRC/Genome Damage and Stability Centre, University of Sussex, Brighton, BN1 9RQ, UK

Presented by: Liu, Cong

Many DNA lesions are repaired by homologous recombination, a complex process facilitated by a set of regulatory factors and pathways working at levels of DNA and chromatin. CUL4-DDB1-DCAF is a Cullin-ROC-based ubiquitin ligase with multivariate functions in chromatin remodeling and DNA repair. In this study, we employed multicopy genetic screen to identify a novel WD40-repeat protein, Wdr70, as a genetic and physical partner of the Ddb1 in fission yeast and could be a putative substrate-targeting subunit of CUL4-DDB1 E3 ligase. Disruption of Wdr70 gene partially phenocopies hypersensitivity of ddb1-d to DNA damaging reagents. Intriguingly, we show that Pcu4-Ddb1Wdr70 CRL is important for the key molecular events of ssDNA processing and homologous recombination as recruitment of RPA and Rad22 to DSBs is seriously impaired, which consequently leads to reduction of efficiency of homologous recombination and increased rate of mutagenesis. Based on these results, we conclude that CUL4-DDB1Wdr70 is a novel factor participating in the early regulatory events of homologous recombination.

Functional characterization of fission yeast transcription factors by overexpression analysis

Lianne Vachon[a], Justin Wood[a], Eun-Joo Gina Kwon[a], Amy Laderoute[a], Kate Chatfield-Reed[a], Jim Karagiannis[b], Gordon Chua[a]

[a] *University of Calgary, Canada*
[b] *University of Western Ontario, Canada*

Presented by: Vachon, Lianne

In Schizosaccharomyces pombe, over 90% of transcription factor genes are nonessential and the majority do not exhibit significant growth defects under optimal conditions when deleted. These findings complicate the functional characterization of transcription factors and identification of their target genes. To overcome this obstacle, we systematically overexpressed 99 transcription factor genes with the nmt1 promoter. Screening the overexpression array revealed that 64 transcription factor genes exhibited reduced fitness when ectopically expressed. Moreover, cell cycle defects were often observed with these transcription factor mutants. We further investigated three uncharacterized transcription factor genes (toe1+-toe3+) which displayed cell elongation when overexpressed. Ectopic expression of toe2+ and toe3+ also exhibited abnormalities in septum formation and nuclear segregation, respectively. Transcriptome profiling and ChIP-chip analysis of the transcription factor overexpression strains indicated that Toe1 activates genes of the pyrimidine-salvage pathway, while Toe3 regulates target genes involved in polyamine synthesis. We also found that ectopic expression of some putative target genes could recapitulate the cell cycle phenotypes of the transcription factor overexpression strains and these phenotypes could be suppressed by the deletion of certain putative target genes. This study implicates new transcription factors and metabolism genes in cell cycle regulation and demonstrates the potential of systematic overexpression analysis to elucidate the function and target genes of transcription factors in fission yeast.

Predicted fission yeast interaction networks

Vera Pancaldi[a,c], Ömer S. Saraç[b], Charalampos Rallis[c], Janel R. Mc Lean[d], Martin Převorovský[e], Kathleen Gould[d], Andreas Beyer[b], Jürg Bähler[c]

[a] *Spanish National Cancer Research Centre (CNIO), Madrid, Spain*
[b] *Biotechnology Center, Dresden University of Technology (TU Dresden), Germany*
[c] *Department of Genetics, Evolution, and Environment, University College London, UK*
[d] *Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, USA*
[e] *Faculty of Science, Charles University, Viničná 5 128 43 Prague 2, Czech Republic*

Presented by: Pancaldi, Vera

A systems-level understanding of biological processes requires the mapping of cellular component interactions, among which protein-protein interactions are particularly important. For fission yeast, no systematic protein interaction data are available. We exploited gene and protein properties, global genome regulation datasets, and conservation of interactions between budding and fission yeast to predict fission yeast protein interactions in silico.

We have extensively tested our method in three ways: first, by predicting with 70-80% accuracy a selected high-confidence test set; second, by recapitulating interactions between members of the well-characterized SAGA co-activator complex; and third, by verifying predicted interactions of the Cbf11 transcription factor using mass spectrometry of TAP-purified protein complexes. Of the new high-quality interactions that were discovered after we started this work, 73% were found in our predictions.

RNA binding proteins are important players in determining the stability and localization of the mRNAs that they bind to. We have identified significant correlations between features of the proteins and features of their mRNA targets in budding yeast, which allow us to predict these interactions, thus adding a post-transcriptional regulatory layer to the network.

Our predicted protein-protein interactions are freely available through PInt, an online resource on our website (www.bahlerlab.info/PInt). The friendly interface allows the user to visualize the interaction network, identify interactions that are predicted and for which there is already evidence in experimental databases and download all the predictions with confidence scores for further analysis. Combining the predictions with a growing set of experimentally verified interactions, it will be possible to chart a precise description of the extended interactome of fission yeast, giving context to uncharacterised genes and suggesting new biological hypotheses.

Comparative genomic analysis of genetic networks of phenol-stress response in yeasts

Cynthia Selassie[a], Gretchen Edwalds-Gilbert[b], Maria Negritto[a], Kathleen Purvis-Roberts[b], Ruye Wang[c], Zhaohua Irene Tang[b]

[a] *Biology Department, Pomona College, Claremont, CA 91711, USA*
[b] *W.M. Keck Science Center, The Claremont Colleges, Claremont, CA 91711, USA*
[c] *Engineering Department, Harvey Mudd College, Claremont, CA 91711, USA*

Presented by: Tang, Irene

Living organisms are constantly exposed to genetic and non-genetic perturbations; the ability of species to adapt to such environmental stresses is necessary for their survival under natural selection. To understand the stress response mechanisms and the biological effects of some environmental factors, we carried out a comparative genomic study on the genetic networks for phenol stress response in the evolutionary context of fission yeast Schizosaccharomyces pombe and budding yeast Saccharomyces cerevisiae. Phenol derivatives are naturally occurring and synthetic compounds that serve various roles in living organism as environmental factors. The chosen phenol derivatives examined are widely used as food preservatives and high volume industrial chemicals, raising concerns about their impact on global health and ecosystem. Exposure to these compounds has been correlated with cardiovascular diseases, diabetes, and the increased risk for miscarriage. However, little is known about their mode of action and the molecular pathways affected. We screened the genomic deletion libraries of both S. pombe and S. cerevisiae for the growth fitness in the presence of a phenol derivative, bisphenol-A (BPA), butylated hydroxyanisole (BHA), or butylated hydroxytoluene (BHT). The fitness test data of genomic scales were digitalized and quantified to construct profiles of genetic response to each phenol compound. Genes involved in the response networks were thus identified. Clustering analysis was also employed to compare response networks among different phenol derivatives and between the two yeasts. We found that these phenol compounds not only affect cell growth and division but also induce DNA damage in the cells. ER protein sorting and vacuolar trafficking pathways seem important for the stress response based on the sensitivity of the deletion strains observed at a genomic level. The response networks assembled suggest some functional conservation in the two yeasts.

Reproductive isolation in *S. pombe*

Francesc Xavier Marsellach, Daniel Jeffares, Jürg Bähler[a]

Department of Genetics, Evolution and Environment, University College London, UK

Presented by: Marsellach, Xavi

Most researchers use standard S. pombe strains that are all derived from 968 h90, introduced by Leupold [1]. Independent natural isolates of S. pombe have been collected and maintained at different strain collections, but only little information is known about the genetic or phenotypic variation among these isolates [2]. To gain insight into worldwide diversity of S. pombe strains, we have sequenced the genomes of 161 natural wild pombe isolates at high coverage and identified a high quality set of single nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (indels) [3]. These data allowed us to characterize the population structure of S. pombe, which consist of a few well-defined isolated lineages.

It has been suggested that reproductive barriers exist between wild isolates of S. pombe [4], but nothing is known about the molecular mechanisms responsible for such reproductive isolation. We started to characterize the spore survival from diploid S. pombe hybrids obtained from pairwise crosses involving closely and distantly related pairs of S. pombe isolates. We also started to characterize in more detail the molecular and genetic basis of this reproductive isolation, with special emphasis on the role of Bateson-Dobzhansky-Muller genetic incompatibilities in the intraspecific reproductive isolation among S. pombe isolates.

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Bulk segregant analysis reveals the genetic basis of a natural trait variation in fission yeast

Wen Hu[a], Fang Suo, Li-Lin Du

National Institute of Biological Sciences, Beijing, China

Presented by: Hu, Wen

Even though a large number of natural isolates of the fission yeast Schizosaccharomyces pombe are available, genetic and molecular analysis of natural variation in this species is still very limited. An S. pombe strain isolated from grapes in Spain, CBS5557 (formerly known as Schizosaccharomyces malidevorans), cannot utilize maltose as the sole carbon source while most of other isolates, including the lab strain, have this capability. The segregation ratio of this phenotype during outcross suggested that it is determined by a single locus. We applied deep sequencing-based bulk segregant analysis (BSA) to map the causal locus. Initial analysis on a cross between CBS5557 and a lab strain suggested that the locus is situated within a 2.23-Mb chromosome I inversion found in most S. pombe isolates, including CBS5557. To facilitate precise mapping within the inversion region, we created an artificial inversion in a lab strain. Applying BSA on a cross between CBS5557 and the lab strain with artificial inversion led to the identification of the gene agl1, which encodes an extracellular maltase, as the causal locus. The agl1 allele in CBS5557 harbors a 5-bp deletion in the coding sequence near the start codon. The same mutation was found in two other isolates defective in maltose utilization. Our results indicate that this deep sequencing-based method should be generally applicable for uncovering trait-gene relationship in diverse natural isolates of fission yeast.

Deciphering the transcriptional-regulatory network of flocculation in *Schizosaccharomyces pombe*

Gina Kwon[a], Amy Laderoute[a], Kate Chatfield-Reed[a], Lianne Vachon[a], Jim Karagiannis[b], Gordon Chua[a]

[a] *University of Calgary, Canada*
[b] *University of Western, Ontario, Canada*

Presented by: Chua, Gordon

In the fission yeast Schizosaccharomyces pombe, the transcriptional-regulatory network that governs flocculation remains poorly understood. Here, we systematically screened an array of transcription factor deletion and overexpression strains for flocculation and performed microarray expression profiling and ChIP-chip analysis to identify the flocculin target genes. We identified five transcription factors that displayed novel roles in the activation or inhibition of flocculation (Rfl1, Adn2, Adn3, Sre2, and Yox1), in addition to the previously-known Mbx2, Cbf11 and Cbf12 regulators. Overexpression of mbx2+ and deletion of rfl1+ resulted in strong flocculation and transcriptional upregulation of gsf2+/pfl1+ and several other putative flocculin genes (pfl2+-pfl9+). Overexpression of the pfl+ genes singly was sufficient to trigger flocculation, and enhanced flocculation was observed in several combinations of double pfl+ overexpression. Among the pfl+ genes, only loss of gsf2+ abrogated the flocculent phenotype of all the transcription factor mutants and prevented flocculation when cells were grown in inducing medium containing glycerol and ethanol as the carbon source, thereby indicating that Gsf2 is the dominant flocculin. In contrast, the mild flocculation of adn2+ or adn3+ overexpression was likely mediated by the transcriptional activation of cell wall-remodeling genes including gas2+, psu1+, and SPAC4H3.03c. We also discovered that Mbx2 and Cbf12 displayed transcriptional autoregulation, and Rfl1 repressed gsf2+ expression in an inhibitory feed-forward loop involving mbx2+. These results reveal that flocculation in S. pombe is regulated by a complex network of multiple transcription factors and target genes encoding flocculins and cell wall-remodeling enzymes. Moreover, comparisons between the flocculation transcriptional-regulatory networks of S. cerevisiae and S. pombe indicate substantial rewiring of transcription factors and cis-regulatory sequences.

Stress induces remodelling of yeast interaction and co-expression networks

Sonja Lehtinen[a], Francesc Xavier Marsellach[b], Sandra Codlin[b], Alexander Schmidt[c], Clément-Ziza Mathieu[d], Andreas Beyer[d], Jürg Bähler[b], Christine Orengo[e], Vera Pancaldi[f]

[a] *CoMPLEX, Univeristy College London, UK*
[b] *Department of Genetics, Evolution and Environment, University College London, UK*
[c] *Proteomics Core Facility, Biozentrum, University of Basel, Switzerland*
[d] *Cellular Networks and Systems Biology, Biotechnology Center, Technische Universität Dresden, Germany*
[e] *Institute of Structural and Molecular Biology, University College London, UK*
[f] *Structural Biology and BioComputing Program, Spanish National Cancer Research Centre, Mardid, Spain*

Presented by: Lehtinen, Sonja

Network analysis provides a powerful framework for the interpretation of genome-wide data. While static network approaches have proved fruitful, there is increasing interest in the insights gained from the analysis of cellular networks under different conditions. In this work, we study the effect of stress on cellular networks in fission yeast. Stress elicits a sophisticated and large scale cellular response, involving a shift of resources from cell growth and metabolism towards protection and maintenance. Previous work has suggested that these changes can be appreciated at the network level. In this work, we study two types of cellular networks: gene co-regulation networks and weighted protein interaction networks. We show that in response to oxidative stress, the co-regulation networks re-organize towards a more modularised structure: while sets of genes become more tightly co-regulated, co-regulation between these modules is decreased. This shift translates into longer average shortest path length, increased transitivity, and decreased modular overlap in these networks. We also find a similar change in structure in the weighted protein interaction network in response to both oxidative stress and nitrogen starvation, confirming and extending previous findings. These changes in network structure could represent an increase in network robustness and/or the emergence of more specialised functional modules. Additionally, we find stress induces tighter co-regulation of non-coding RNAs, decreased functional importance of splicing factors, as well as changes in the centrality of genes involved in chromatin organization, cytoskeleton organization, cell division, and protein turnover.

Sequence-based, systematic approach to identify factors affecting chronological lifespan in a competitive environment

Theodora Sideri[a], Charalampos Rallis[a], Danny Bitton[a], Sandra Codlin[a], Li-Lin Du[b], Jürg Bähler[a]

[a] *Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK*
[b] *National Institute of Biological Sciences, 7 Science Park Road, Zhongguancun Life Science Park, Beijing, 102206, China*

Presented by: Sideri, Dora

Ageing is the major risk factor for diseases such as diabetes, cancer and neurodegeneration. Hence, better understanding of ageing is important in order to target those pathologies and extend healthy lifespan. Ageing is affected by environmental and genetic factors that are remarkably conserved from yeast to mammals. Examples are such as calorie restriction, which extends lifespan, and the target of rapamycin (TOR) pathway, which shortens lifespan.

We are interested in identifying genetic factors that affect the ageing of cells maintained in a non-dividing, quiescent state (chronological lifespan). The quiescent state is achieved using growth medium which does not contain any nitrogen source. We have exploited a pool of the fission yeast deletion mutants, each identified by 2 unique DNA "barcode" sequences. About 3000 mutants were competitively aged in the same culture, and were maintained without nitrogen. Cell samples were collected at several time points starting when cells entered quiescence (100% survival, reference time point) until complete death of all cells in the pool. DNA was extracted from all time points, the barcode sequences were amplified by PCR and sequenced using next-generation technology. Several novel mutants that increased in abundance during the time course are the focus of our current research, as those mutants exhibit extended chronological lifespan.

Structural comparison between starch and glycogen binding domains in fungi

Sim-Kun Ng[a], Chieh-Ming Yang[a], Ting-Ying Jiang[a], Wei-Yao Chou[b], Tun-Wen Pai[c], Margaret Dah-Tsyr Chang[a]

[a] *Institute of Molecular and Cellular Biology and Department of Medical Science, National Tsing Hua University, Hsinchu, Taiwan, Republic of China*
[b] *Institute of Bioinformatics and Structural Biology and Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan, Republic of China*
[c] *Department of Computer Science and Engineering and Center for Marine Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, Republic of China*

Presented by: Chang, Margaret Dah-Tsyr

Carbohydrate binding modules (CBMs) are defined protein domains capable of binding polysaccharide moieties which are involved in carbohydrate synthesis, metabolism and transport of starch, cellulose, and chitin. To date, within 67 known CBM families, CBM1, CBM43, CBM48 and CBM52 have been identified in *Schizosaccharomyces pombe* 972h-, among which *S. pombe*AMP-activated protein kinase beta subunit homolog (Amk2) belongs to CBM48 family and is defined as a glycogen binding domain (*SpGBD*). Since glycogen and starch are both glucose polymers containing &alpha-1, 4 and &alpha-1, 6 linkages, sequence and structure relationship between fungal starch binding domains (SBDs) and GBDs is *in silico* analyzed. Although primary sequence identities of *Sp*GBD and other CBMs are quite low, our predicted *Sp*GBD structure shows mainly &beta-stranded immunoglobulin-like topology quite conserved in SBDs and GBDs. Interestingly, feature-incorporated alignment (FIA) algorithm revealed two ligand binding sites with a ligand binding clamp in fungal SBDs, while only one ligand binding site with no ligand binding clamp was predicted in *Sp*GBD. Unique and common features in terms of ligand binding sites, ligand binding residues and CBM families in fungal SBDs and GBDs may facilitate further protein design and biotech application.

Proteome-wide search for PP2A substrates in fission yeast

Manuel Bernal[a], Jacob Zhurinsky[a], Ana B. Iglesias[a], Maria A. Sanchez-Romero[b], Antonio J. Perez-Pulido[a], Juan Jimenez[a], Rafael R. Daga[a]

[a] *Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas, Junta de Andalucia, Sevilla, Spain*
[b] *Universidad de Sevilla, Facultad de Biología, Genética, Spain*

Presented by: Bernal, Manuel

PP2A is a major phosphatase in eukaryotic cells that plays an essential role in many processes. PP2A mutations in Schizosaccharomyces pombe result in defects of cell cycle control, cytokinesis and morphogenesis. Which PP2A substrates are responsible for these changes is not known. In this work, we searched for PP2A substrates in S. pombe using two approaches, 2D gel analysis of PP2A complex mutants and identification of PP2A interacting proteins. In both cases, we used mass spectrometry to identify proteins of interest. In the 2D gel experiment, we compared proteomes of wildtype S. pombe, deletion of pta2, the phosphoactivator of the PP2A catalytic subunit, and pab1-4, a mutant of B-type PP2A regulatory subunit. 2174 protein spots were reproducibly resolved by 2D-DIGE and 51 spots demonstrated significant changes between PP2A mutants and the wildtype control. Mass spectrometry analysis of these spots identified 27 proteins that include key regulators of glycerol synthesis, carbon metabolism, amino acid biosyntesis, vitamin production and protein folding. Importantly, we independently identified a subset of these proteins as PP2A binding partners by affinity precipitation, suggesting they may be direct targets of PP2A. Our work contributes to a better understanding of PP2A function and identifies potential PP2A subtrates.

The biocatalytic synthesis of levan (β-2, 6-linked fructan) by *Pediococcus acidilactici* by levansucrase from sucrose

Ghada Youssef, Amany Youssef, Shimaa Talha, Samy El-Aassar

Faculty of Science, Alexandria University, Egypt

Presented by: Youssef, Ghada

Microbial fructosyltransferases are polymerases that are involved in microbial fructan (levan, inulin and fructo-oligosaccharide) biosynthesis. Structurally, microbial fructosyltransferase proteins share the catalytic domain of glycoside hydrolases 68 family and are grouped in seven phylogenetically related clusters. The aim of the work was screening the potency of some bacterial isolates to produce extracellular levansucrase activity. Bacteria were isolated from different sources (oral swab, spoiled milk, spoiled whey, and spoiled yoghurt). Pediococcus acidilactici showed the highest levansucrase activity after 2 days fermentation under shacked condition yielding (12.64U/ml), and show high levan production (15.4g/L). Following the optimization of carbon source, nitrogen source, temperature and initial pH of the growth medium in submerged liquid cultures. In fact, sucrose was found to be a good inducer of levansucrase enzymes. The optimal temperature and pH of the levansucrase were 30 °C and 6, respectively. Partial purification of the crude levansucrase of Pediococcus acidilactici carried out by fractional precipitation with ethanol, yielding (41.05%) as a total highest recovered protein. The optimum reaction temperature for semi purified levansucrase was 40° C. the semi purified enzyme showed a maximum activity at a reaction pH of 5.2, and a relatively high activity in a pH range (4.4 to 5.6). Levan has not been utilized, but if developed, could be useful in food and other industrial applications.

Monitoring binding of Meu5 protein by PAR-iCLIP

Cristina Cotobal, Juan Mata

Cambridge University, UK

Presented by: Cotobal, Cristina

The dynamic meiotic expression program is regulated by a combination of transcriptional and posttranscriptional mechanisms. We recently showed that the Meu5 RNA-binding protein stabilizes the transcripts of a set of genes that are induced during the meiotic divisions, thus shaping their meiotic expression profiles. Although we identified the direct RNA targets of Meu5 using genomic methods, we were unable to find a specific Meu5-binding motif on them.

In order to identify Meu5 binding sites with single nucleotide resolution and to find out how Meu5 stabilizes its meiotic RNA targets, we used a method called CLIP (for Cross-Linking and ImmunoPrecipitation. After UV cross-linking, an extract is prepared and treated with RNase in such a way that only RNA fragments protected by bound proteins survive. After that, Meu5p was purified together with associated RNA fragments, and the fragments identified by next-generation sequencing. We used a combination of two published methods: PAR-CLIP (photoactivable analogue ribonucleoside), which uses photoreactive nucleosides to facilitate the crosslink between RNA and proteins, and iCLIP, which allows single-nucleotide identification of cross-linking sites.

We will present preliminary results that demonstrate that the method is working and help identify Meu5 binding sites across the genome.

A transposon-based mutagenesis system in the Sz.pombe model of juvenile CLN3 disease

Rachel Brown[a], Michael Bond[a], Sara Mole[a,b]

[a] *MRC Laboratory for Molecular Cell Biology, University College London, UK*
[b] *Medicine Unit, UCL Institute of Child Health and Department of Genetics, Evolution & Environment, UCL, UK*

Presented by: Brown, Rachel

Juvenile CLN3 disease is caused by mutations in CLN3 and is the most common of the neuronal ceroid lipofuscinoses (NCLs), a group of neurodegenerative lysosomal storage diseases. The disease presents between 3-5 years of age, and is characterised by blindness, seizures, mental and motor deterioration and premature death. As the function of CLN3 remains elusive, there is no treatment and management relies solely on symptomatic and palliative interventions. However, the evolutionary conservation of CLN3 has allowed Sz. pombe to be developed as an experimental platform from which to investigate the molecular mechanisms of juvenile CLN3 disease. This model has contributed to the discovery of a new sub-cellular location of the CLN3 homologue btn1, in addition to suggesting that btn1p is involved in numerous processes since its deletion gives rise to pleiotropic phenotypes. Given this complexity of btn1/CLN3 function, a non-hypothesis based and genome-wide strategy to further identify the pathways btn1p influences may be particularly instructive about the molecular function of btn1p and by extrapolation CLN3. The Sz.pombe model is now being used to facilitate a transposon-based mutagenesis screen to identify genes that when mutated, rescue phenotypes of Sz. pombe in which btn1 is absent (btn1Δ) or harbours a 1kb deletion equivalent to the most common human CLN3 mutation. A focus will be placed on identifying genes that may be beneficial as therapeutic targets. Two different transposon systems, Hermes and piggyBac, have been chosen to develop the necessary mutagenesis libraries. Although this project is currently in its infancy, the inherent benefits of this approach are that the construction of a mutagenesis library can be achieved relatively quickly and the identification of insertion sites can be made with ease. It is hoped that this method will rapidly add to our understanding of btn1/CLN3 function and accelerate drug development for juvenile CLN3 disease.

Analysis of Z-DNA formations in *S. pombe* genome via web-based servers

Ercan Arican

Istanbul University, Science Faculty, Molecular Biology and Genetics Department, Vezneciler-Istanbul, Turkey

Presented by: Arican, Ercan

The structure of chromatin is tremendously dynamic and it plays an effective role in gene expressionand organization of genome. The DNA generally occurs in the right handed B form, alternativestructures such as left handed Z-DNA, DNA triplex, cruciform, and G-quadruplex have beencomprised in transcription and recombination. One of the important examples is the left-handed Z-DNA confermer. The biological role of Z-DNA is an area of active study. Z-DNA formation at apromoter has been shown to correlate with transcriptional activity and also Z-DNA often conducts asan enhancer of gene expression. The purpose of study is definition of Z-DNA formations usingweb-based servers such as non-B DB v2.0 and SIDD/Z-DNA in Saccharomyces pombe genome.Potential 70 Z-DNA formations were found in 1201 screened Conserved Domains (CDS) in S.pombe genome. The goal of the web-based servers are allow scientists to view their own genomicfeature annotations in the context of these predicted motifs and to aid in the formation of hypothesesregarding the potential role of these structures in other cellular processes.

A new rapid method for fission yeast protoplast preparation

Ignacio Flor-Parra[a], Jacob Zhurinsky[a], Manuel Bernal[a], Paola Gallardo[a], Rafael R. Daga[a]

Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide-Consejo Superior de Investigaciones Científicas, Junta de Andalucia, Sevilla, Spain

Presented by: Zhurinsky, Jacob

Protoplasts are yeast cells in which the cell wall has been removed. Genetic manipulation of sterile strains, morphogenesis studies and many other experiments require protoplasts. We developed a new method for fission yeast protoplast preparation. All cells were converted to protoplasts within 10-15 minutes by digesting cell wall with Lallzyme MMX and we have demonstrated their use in plasmid DNA recovery, diploid generation and cell re-polarization experiments. Our rapid protocol should be useful for many applications.

A systems approach linking genotype and environment to phenotype: oxidative stress response mechanisms in fission yeast

Sandra Codlin[a], F. Xavier Marsellach-Castellví[a], Mathieu Clément-Ziza[b],Manos Papadakis[c], Alexander Schmidt[d], Samuel Marguerat[a,e], Ruedi Aebersold[d], Chris Workman[c], Andreas Beyer[b], Jürg Bähler[a]

[a] *Department of Genetics, Evolution & Environment, University College London, UK*
[b] *TU Dresden, Biotechnology Center, Dresden, Germany*
[c] *DTU, Center for Biological Sequence Analysis, Lyngby, Denmark.*
[d] *Institute of Molecular Systems Biology, ETH Zürich, Switzerland*
[e] *MRC Clinical Sciences Centre, Imperial College London, UK*

Presented by: Codlin, Sandra

Cellular protection against oxidative damage is relevant to ageing and numerous complex diseases. For the European PhenOxiGEn project, we are using fission yeast as a model to gain a systems-level understanding of the oxidative stress response and obtain insights into the interplay of variable genotype, phenotype, and environment. We created a genetically and phenotypically diverse library of strains from crosses of three independent parental isolates with distinct stress sensitivities. This strain library will generally be useful for genetic mapping and to relate genotypes to complex phenotypes (linkage analyses, QTL studies). The parental and segregant strains were genotyped and phenotyped (in stressed and unstressed cells) using RNA-seq and proteomics approaches, thus providing a rich basis for genome-wide association studies and mathematical modelling. Genetic, functional genomic, and proteomic approaches, along with computational methods, are applied in parallel to develop protein and gene interaction networks to further support the modelling efforts.

Analysis of the RNA-seq and proteomics data identified expression QTLs (eQTLs) and proteome QTLs under normal growth conditions, and additional QTLs in response to oxidative stress. We present data on a major eQTLs on Chromosome 3, which affects the expression of over 1,000 genes. In particular, this eQTL is also associated with an increase in anti-sense transcription. We identified a frameshift mutation in the swc5 gene within this region, which gave the strongest cis-eQTL. Swc5 is part of the SWR1 complex controlling the insertion of the H2A.Z (Pht1) histone variant into nucleosomes, and plays a role in genome stability, anti-sense transcription and gene-regulation. Our Chip-seq data revealed that although the localisation of H2A.Z remains unchanged, the swc5 frameshift mutation results in a reduction of H2A.Z occupancy over transcription start sites.

Exploring translation in *S. pombe* using ribosomal profiling

Caia Duncan, Juan Mata

Department of Biochemistry, University of Cambridge, Cambridge, CB2 1QW UK

Presented by: Duncan, Caia

Ribosomal profiling is a powerful technique to measure translation genome-wide in growing cells. By comparing ribosome protected fragments to mRNA abundance, translation efficiency can be calculated for every translated mRNA. Schizosaccharomyces pombe has been extensively characterized at the mRNA level; however, much less is known about translational control. Therefore, we have performed ribosomal profiling of a wild type S. pombe strain. Analysis of our data has allowed the identification of translation start sites, uORFs, potential frameshifting events and translation of annotated non-coding RNAs. In addition, we have estimated translational efficiencies and identified subsets of poorly-translated genes that may be subject to translational repression. We are currently performing ribosomal profiling of S. pombe cells during meiotic differentiation and under stress conditions to investigate how translation is regulated in response to environmental and developmental cues.

A configurable tool for community literature curation

Antonia Lock[a], Val Wood[b], Midori Harris[b]

[a] *University College London / PomBase, UK*
[b] *University of Cambridge / PomBase, UK*

Presented by: Lock, Antonia

We have developed a web-based annotation tool, Canto, to support community curation on a large scale. Canto is highly configurable, and can be used with minimal or extensive support from professional curators. It is therefore suitable for use by most research communities, including those not supported by a manual curation team, who want to contribute gene-specific experimental information from their organism of interest to public biological databases.

Canto supports literature-based curation of a wide, and configurable, set of data types, including Gene Ontology (GO) annotations, phenotypes, interactions, and protein modifications.

The tool is fully accessible online, requiring no software download or setup by the end user. Initial feedback from early community users indicates that Canto is easy to use, with an intuitive workflow and integrated help documentation.

Canto was originally developed for community curation by the S. pombe database (PomBase) and its research community, who curate the most extensive set of data types. To date, Canto has also been adopted by the K. pastoris (Pichia) community and for GO annotation workshops at University College London in which researchers and post-graduates are invited to curate their own papers of interest. Ongoing Canto development ensures that feedback from users guides efforts to improve existing features or to implement new ones.

PomBase.org

Mark McDowall[a], Midori Harris[b], Antonia Lock[c], Kim Rutherford[b], Dan Staines[a], Val Wood[b], Jürg Bähler[c], Paul Kersey[a], Steve Oliver[b]

[a] *European Molecular Biology Laboratory - European Bioinformatics Institute, UK*
[b] *University of Cambridge, UK*
[c] *University College London, UK*

Presented by: McDowall, Mark

PomBase (http://www.pombase.org) is a model organism database to support the organization of and access to scientific data for the fission yeast Schizosaccharomyces pombe. PomBase supports genomic sequence and features, genome wide datasets and manual literature curation.

The PomBase interface is built on a Drupal server with the data housed within an Ensembl genome database (http://www.ensemblgenomes.org). Gene summary pages present the data related to a gene, including the gene type, product, sequence features, phenotypes, Gene Ontology annotation, modifications and physical and genetic interactions. A query builder has been, on top of a BioMart server, to allow users to search by multiple feature types. A query history summarises queries and allows queries to be combined and edited. Results pages provide access to gene summary pages.

The Ensembl platform (http://www.ensembl.org) also has a genome browser that can be used to visualise the data. Every gene summary page has direct links to the relevant pages of a customised Ensembl genome browser providing the functionality to store, analyse and visualise a wide variety of datasets mapped to the genome either from sources located on the server or via externally loaded URLs or data files. Examples of supported datasets include whole genome resequencing data, ChIP-chip and ChIP-seq assay, mapping to microarray probes and other high-throughput data types. The Ensembl-style browser also provides views of orthologous regions by comparative analysis of related genomes.

PomBase also provides a community hub for researchers, providing genome statistics, a community curation interface, news, events, documentation FAQs and mailing lists.

Phenotype curation in PomBase

Midori A. Harris[a], Antonia Lock[b], Jürg Bähler[b], Stephen G. Oliver[a], Valerie Wood[a]

[a] *Cambridge Systems Biology Centre and Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK*
[b] *Department of Genetics, Evolution & Environment and UCL Genetics Institute, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK*

Presented by: Harris, Midori

PomBase, the recently established online fission yeast resource, has made the comprehensive and detailed representation of phenotypes a high priority. To support highly specific phenotype annotation, we are actively developing the Fission Yeast Phenotype Ontology (FYPO), a modular ontology that uses several OBO Foundry as building blocks, including the phenotypic quality ontology PATO, the Gene Ontology (GO), and Chemical Entities of Biological Interest (ChEBI).

Phenotype curation is featured in Canto, the PomBase community curation tool. Canto and the Chado database underlying PomBase support annotation of specific alleles, singly or in combinations (e.g. double or triple mutants), to FYPO terms, along with supporting evidence. Relevant experimental conditions can be captured, and targets, expressivity and penetrance can be represented by annotation extensions.

Phenotype annotations are displayed on PomBase gene pages. FYPO terms can be searched by name or ID in the PomBase advanced search. Over 6000 legacy annotations inherited from GeneDB have been converted to FYPO terms, and new annotations are being made in Canto by both PomBase curators and fission yeast community researchers, with over 2200 created to date.

Using Gene Ontology biological process co-annotation for ontology and annotation quality control

Valerie Wood[a], Midori Harris[a], Antonia Lock[b]

[a] *Cambridge University/PomBase, UK*
[b] *University College London/PomBase, UK*

Presented by: Wood, Val

BACKGROUND: Biological processes are accomplished by the coordinated action of sets of gene products. However, some processes are rarely connected to each other because they functionally, temporally or spatially distant. We speculated that we could identify pairs of biological processes which were unlikely to be co-annotated to the same gene products (e.g. amino acid metabolism and cytokinesis), and use any mutually exclusive processes identified to create rules which alert curators to possible annotation errors.

METHODS: Co-annotated terms (annotation intersections) were identified for all pairs of "high level" GO processes for three taxonomically distant organisms (fission yeast, budding yeast, mouse). Intersections where annotations were "null" were used to create rules of the form "x is not usually co-annotated with y". Intersections where annotations were sparse were inspected for spurious annotations, which were either corrected, or, if the annotations were validated the rules were extended to allow these exceptions.

RESULTS: In the rule generation phase of this work, we have identified cases that account for accurate co-annotation as well as several types of error that give rise to incorrect co-annotation. We present descriptions and examples for each type of correct and incorrect case, and indicate how errors have been addressed. The rules will be incorporated into the central GO annotation quality control system, where they can be applied to the entire annotation corpus, allowing further refinement of the rule base as well as identifying, and reducing the occurrence of annotation errors.

Coevolution of RNAi associated proteins in fission yeast

Udita Upadhyaya, Jagmohan Singh

IMTECH, Chandigarh, India

Presented by: Upadhyay, Udita

Tightly controlled establishment and maintenance of heterochromatin is crucial for proper chromosome segregation, transcriptional control and many other chromosome associated processes. In fission yeast, RNA interference (RNAi) is critical for the assembly of heterochromatin at centromeres. Central to this process is the RNA-induced Initiation of Transcriptional gene Silencing (RITS) complex, which physically anchors small non-coding RNAs to chromatin. RITS includes Ago1, the chromodomain protein Chp1, and Tas3, which bridges between Chp1 and Ago1. C-terminal half of Chp1 binds the Tas3 N-terminal domain, revealing Chp1's tight embrace of Tas3. Through Phylogenetic analysis, Synteny mapping and comparative studies, we found that the Chp1-Tas3 association, which provides a platform to recruit both RNAi-dependent and RNAi-independent gene-silencing pathways for locus-specific regulation of heterochromatin, is lost during the course of evolution from S. japonicus and S.pombe to S. octosporus and S. cryophilus through selection. This might suggest that RITS is a redundant mechanism which might have been important to maintain silencing at transposons. Earlier reports suggest that transposons are crucial for centromeric function through maintenance of heterochromatin. An earlier report (Rhind et al, 2011) suggests there is a sequential loss of transposons from S. japonicus through S. pombe to S. octosporus and S. cryophilus. Phylogenetic analysis suggests that Ago1 is retained in both the other species whereas Chp1 and Tas3 are not. We propose that there is Coevolution among RITS components and that RITS components are lost during selection as a result of the sequential loss of transposons in these species of fission yeast or vice versa.

Akiko, Okita
Osaka University
Japan
aokita@bio.sci.osaka-u.ac.jp

Alao, John
University of Gothenburg
Sweden
john.p.alao@cmb.gu.se

Alhazzani, Amal
King Saud University
Saudi Arabia
alhazzni@ksu.edu.sa

Aligué, Rosa
University of Barcelona
Spain
aliguerosa@ub.edu

Allshire, Robin
University of Edinburgh
United Kingdom
robin.allshire@ed.ac.uk

Amelina, Hanna
University College London
United Kingdom
h.amelina@ucl.ac.uk

Andreadis, Christos
University College London
United Kingdom
c.andreadis@ucl.ac.uk

Angevin, Thibaut
University of Sussex
United Kingdom
t.angevin@sussex.ac.uk

Antequera, Francisco
University of Salamanca
Spain
cpg@usal.es

Aoi, Yuki
Kazusa DNA Research Institute
Japan
aoioa@sunny.ocn.ne.jp

Aoki, Keita
National Institute of Genetics
Japan
keaoki@nig.ac.jp

Arata, Mayumi
Kazusa DNA Research Institute
Japan
aratаxxxxxx@gmail.com

Arcangioli, Benoit
Pasteur Institute
France
barcan@pasteur.fr

Arda, Emine Şeküre Nazlı
Istanbul University
Turkey
narda@istanbul.edu.tr

Ari, Sule
Istanbul University
Turkey
sari@istanbul.edu.tr

Arican, Ercan
Istanbul University
Turkey
earican@istanbul.edu.tr

Armstrong, Christine
University College London
United Kingdom
c.armstrong@ucl.ac.uk

Aronica, Lucia
University of Vienna
Austria
lucia.aronica@univie.ac.at

Asakawa, Haruhiko
Osaka University
Japan
askw@fbs.osaka-u.ac.jp

Atkinson, Sophie
University College London
United Kingdom
sophie.atkinson.09@ucl.ac.uk

Audergon, Pauline
University of Edinburgh
United Kingdom
s1060807@sms.ed.ac.uk

Audry, Julien
CRCM: CNRS UMR 7258
France
julien.audry@inserm.fr

Aves, Stephen
University of Exeter
United Kingdom
s.j.aves@exeter.ac.uk

Ayté, José
Universitat Pompeu Fabra
Spain
jose.ayte@upf.edu

Bachand, François
Universite de Sherbrooke
Canada
f.bachand@usherbrooke.ca

Bähler, Jürg
University College London
United Kingdom
j.bahler@ucl.ac.uk

Baidi, Feriel
IGDR UMR6290
France
feriel.baidi@univ-rennes1.fr

Balasubramanian, Mohan
National University of Singapore
Singapore
dbsmkb@nus.edu.sg

Baumann, Peter
Stowers Institute for Medical Research
United States
peb@stowers.org

Bayne, Elizabeth
University of Edinburgh
United Kingdom
elizabeth.bayne@ed.ac.uk

Begnis, Martina
CRUK London Research Institute
United Kingdom
martina.begnis@cancer.org.uk

Bernal Muñoz, Manuel
Universidad Pablo de Olavide-Consejo
Spain
mbermun@upo.es

Bhardwaj, Shweta
University of Oxford
United Kingdom
shweta.bhardwaj@path.ox.ac.uk

Bitton, Danny Asher
University College London
United Kingdom
d.bitton@ucl.ac.uk

Bjerling, Pernilla
Uppsala University
Sweden
pernilla.bjerling@imbim.uu.se

Blaikley, Elizabeth
University of Oxford
United Kingdom
elizabeth.blaikley@oncology.ox.ac.uk

Blum, Sebastian
m2p-labs GmbH
Germany
blum@m2p-labs.com

Boddy, Michael
The Scripps Research Institute
United States
nboddy@scripps.edu

Bond, Michael
University College London
United Kingdom
michael.bond@ucl.ac.uk

Boye, Erik
Oslo University Hospital
Norway
eboye@rr-research.no

Yang, Jay
Singer Instruments Co Ltd
United Kingdom
jack@singerinstruments.com

Bøe, Cathrine Arnason
Oslo University Hospital
Norway
cathrarn@rr-research.no

Braun, Sigurd
Ludwig-Maximilians-University
Germany
sigurd.braun@med.uni-muenchen.de

Brown, Rachel
University College London
United Kingdom
rachel.brown.10@ucl.ac.uk

Brunner, Damian
University of Zurich
Switzerland
damian.brunner@imls.uzh.ch

Bühler, Marc
Friedrich Miescher Institute
Switzerland
marc.buehler@fmi.ch

Cam, Hugh
Boston College
United States
hugh.cam@bc.edu

Cantwell, Helena
University of Sussex
United Kingdom
helena.cantwell@oriel.ox.ac.uk

Carazo Salas, Rafael
University of Cambridge
United Kingdom
cre20@cam.ac.uk

Carr, Antony
University of Sussex
United Kingdom
a.m.carr@sussex.ac.uk

Castel, Stephane
Cold Spring Harbor Laboratory
United States
scastel@cshl.edu

Chacón Rodríguez, Mariola
Max Planck Inst. of Mol. Cell Biol. & Genet.
Germany
mchacon@mpi-cbg.de

Chahwan, Charly
Sussex University
United Kingdom
charly.chahwan@gmail.com

Chan, Kuan Yoow
University of Manchester
United Kingdom
kchan@picr.man.ac.uk

Chang, Ya-Ting
University of Illinois at Chicago
United States
ychang34@uic.edu

Chang, Fred
Columbia University
United States
fc99@columbia.edu

Chang, Margaret Dah-Tsyr
National Tsing Hua University
Taiwan
flyingsim02@hotmail.com

Chatfield-Reed, Kate
University of Calgary
Canada
kchatfieldreed@gmail.com

Chen, Ee Sin
National University of Singapore
Singapore
bchces@nus.edu.sg

Cheng, Tammy
CRUK London Research Institute
United Kingdom
Tammy.Cheng@cancer.org.uk

Chica, Nathalia
University of Salamanca
Spain
nchicab@usal.es

Chmielewska, Aldona
CRUK London Research Institute
United Kingdom
aldona.chmielewska@cancer.org.uk

Chua, Gordon
University of Calgary
Canada
gchua@ucalgary.ca

Cisneros-Barroso, M Eugenia
University of Barcelona
Spain
euge0086@gmail.com

Clifford Hart, Dawn
Grand Valley State University
United States
hartdaw@gvsu.edu

Clines, Becky
Sunrise Science Products
United States
lkylin@sunrisescience.com

Cobley, David
University of Manchester
United Kingdom
david.cobley@postgrad.manchester.ac.uk

Codlin, Sandra
University College London
United Kingdom
s.codlin@ucl.ac.uk

Cohen, Amikam
The Hebrew University Medical School
Israel
amikamc@ekmd.huji.ac.il

Cooper, Julia Promisel
CRUK London Research Institute
United Kingdom
julie.cooper@cancer.org.uk

Costes, Audrey
Institut Curie
France
audrey.costes@curie.fr

Cotobal, Cristina
University of Cambridge
United Kingdom
cc606@cam.ac.uk

Coudreuse, Damien
Rennes Inst. of Genetics and Development
France
damien.coudreuse@univ-rennes1.fr

Coulon, Stephane
CRCM
France
stephane.coulon@inserm.fr

Katie Crawford
Life Technologies
United Kingdom
katie.crawford@lifetech.com

Csikasz-Nagy, Attila
King's College London
United Kingdom
attila.csikasz-nagy@kcl.ac.uk

Curto, Maria Angeles
University of Salamanca
Spain
emecur@usal.es

Daga, Rafael
Universidad Pablo de Olavide-Consejo
Spain
rroddag@upo.es

Dalgaard, Jacob
University of Warwick
United Kingdom
J.Z.Dalgaard@warwick.ac.uk

Davie, Elizabeth
University of Manchester
United Kingdom
elizabeth.davie@postgrad.manchester.ac.uk

de Bruin, Robert
University College London
United Kingdom
r.debruin@ucl.ac.uk

De León, Nagore
University of Salamanca
Spain
nagoret@usal.es

Dehé, Pierre-Marie
Cancer Research Center of Marseille
France
pierredehe@gmail.com

Dempster, Jane
University College London
United Kingdom
j.dempster@ucl.ac.uk

DeWitt, Ashley
Grand Valley State University
United States
dewitash@gvsu.edu

Dhani, Deepsharan
University of Leicester
United Kingdom
dd71@le.ac.uk

Ding, Daqiao
Advanced ICT Research Institute Kobe
Japan
dingdaqiao@gmail.com

Dodgson, James
University of Cambridge
United Kingdom
jd535@cam.ac.uk

Dowdle, Adam
Singer Instrument Co Ltd
United Kingdom
adam@singerinstruments.com

Du, Li-Lin
National Institute of Biological Sciences
China
dulilin@nibs.ac.cn

Du, Wei
University of Manchester
United Kingdom
wei.du@manchester.ac.uk

Duarte, Paulo
Instituto Gulbenkian de Ciência
Portugal
pduarte@igc.gulbenkian.pt

D'Urso, Gennaro
University of Miami School of Medicine
United States
GDUrso@med.miami.edu

Duncan, Caia
University of Cambridge
United Kingdom
cdsd3@cam.ac.uk

Dziadkowiec, Dorota
Wroclaw University
Poland
dorota.dziadkowiec@uni.wroc.pl

Ebrahimi, Hani
Cancer Research UK
United Kingdom
hani.ebrahimi@cancer.org.uk

Eckert, Daniela
TU Brunswick
Germany
d.eckert@tu-bs.de

Ekwall, Karl
Karolinska Institutet
Sweden
karl.ekwall@ki.se

Ellis, David
University College London
United Kingdom
david.ellis.11@ucl.ac.uk

Endesfelder, Ulrike
Johann Wolfgang Goethe-University
Germany
endesfelder@chemie.uni-frankfurt.de

Espenshade, Peter
Johns Hopkins University
United States
peter.espenshade@jhmi.edu

Estravís, Miguel
University of Salamanca
Spain
estravis@usal.es

Escandell Planells, Jose
Instituto Gulbenkian de Ciência
Portugal
jplanells@igc.gulbenkian.pt

Farmer, Sarah
University College London
United Kingdom
sarah.farmer@ucl.ac.uk

Fennell, Alex
CRUK London Research Institute
United Kingdom
alex.fennell@cancer.org.uk

Fernandez-Alvarez, Alfonso
CRUK London Research Institute
United Kingdom
Alfonso.Fernandez-Alvarez@cancer.org.uk

Finegan, Tara
University of Cambridge
United Kingdom
tmf32@cam.ac.uk

Fischer, Tamas
Heidelberg University
Germany
tamas.fischer@bzh.uni-heidelberg.de

Fleig, Ursula
University of Düsseldorf
Germany
fleigu@uni-duesseldorf.de

Flor-Parra, Ignacio
Columbia University
United States
if2195@columbia.edu

Formstecher, Etienne
Hybrigenics Services
France
eformstecher@hybrigenics.com

Forsburg, Susan
University of Southern California
United States
forsburg@usc.edu

Gallardo Palomo, Paola
Universidad Pablo de Olavide-Consejo
Spain
handstock@gmail.com

Gangloff, Serge
UMR3525 CNRS-Pasteur Institute
France
serge.gangloff@pasteur.fr

Garcia, Patricia
Universitat Pompeu Fabra
Spain
patricia.garcia@upf.edu

Geymonat, Marco
Cambridge University
United Kingdom
mg604@cam.ac.uk

Giménez-Zaragoza, David
University of Barcelona
Spain
davidgimenez@ub.edu

Glatz, Attila
Bio. Res. Ctr/Hung. Acad. Sci
Hungary
attila@brc.hu

Gould, Kathy
Vanderbilt University
United States
kathy.gould@vanderbilt.edu

Grallert, Beata
Oslo University Hospital
Norway
beata.grallert@rr-research.no

Grallert, Agnes
University of Manchester
United Kingdom
agrallert@picr.man.ac.uk

Greenwood, Jessica
CRUK London Research Institute
United Kingdom
jessica.greenwood@cancer.org.uk

Gregan, Juraj
University of Vienna
Austria
juraj.gregan@univie.ac.at

Grewal, Shiv
National Institutes of Health
United States
grewals@mail.nih.gov

Gu, Ying
Temasek Life Sciences Laboratory
Singapore
guying@tll.org.sg

Gullerova, Monika
University of Oxford
United Kingdom
monika.gullerova@path.ox.ac.uk

Guring, Resham Lal
University of Sussex
United Kingdom
RG299@SUSSEX.AC.UK

Gutierres, Pilar
CRUK London Research Institute
United Kingdom
Pilar.Gutierrez-Escribano@cancer.org.uk

Habib, Ahmed
Hiroshima University
Japan
ahmed.gamalbiopharma@yahoo.com

Hachet, Olivier
University of Lausanne
Switzerland
olivier.hachet@unil.ch

Haering, Christian
EMBL Heidelberg
Germany
christian.haering@embl.de

Hagan, Iain
University of Manchester
United Kingdom
ihagan@picr.man.ac.uk

Halic, Mario
Genzentrum / LMU Munich
Germany
halic@genzentrum.lmu.de

Halova, Lenka
University of Manchester
United Kingdom
lenka.pribylova@manchester.ac.uk

Harada, Masayuki
Kyoto University
Japan
mharada.m08@lif.kyoto-u.ac.jp

Harris, Midori
PomBase / University of Cambridge
United Kingdom
mah79@cam.ac.uk

Hartmann-Petersen, Rasmus
University of Copenhagen
Denmark
rhpetersen@bio.ku.dk

Hartsuiker, Edgar
Bangor University
United Kingdom
e.hartsuiker@bangor.ac.uk

Hasan, Ayesha
Cambridge University
United Kingdom
ah649@cam.ac.uk

Hatimy, Abubakar
University of Sussex
United Kingdom
a.hatimy@sussex.ac.uk

Hauf, Silke
Friedrich Miescher Laboratory
Germany
silke.hauf@tuebingen.mpg.de

Hayashi, Takeshi
Okinawa Inst. of Science and Technology
Japan
hayashi@oist.jp

Hayles, Jacqueline
CRUK London Research Institute
United Kingdom
jacqueline.hayles@cancer.org.uk

Heimlicher, Maria
University of Zurich
Switzerland
maria.heimlicher@imls.uzh.ch

Helmlinger, Dom
CRBM
France
dhelmlinger@crbm.cnrs.fr

Hermand, Damien
FUNDP-NARC
Belgium
Damien.Hermand@fundp.ac.be

Hetzel, Christian
m2p-labs GmbH
Germany
hetzel@m2p-labs.com

Hidalgo, Elena
Universitat Pompeu Fabra
Spain
elena.hidalgo@upf.edu

Higa, Mari
Kinki University
Japan
ototo520@ybb.ne.jp

Higgins, Andrew
University College London
United Kingdom
andrew.higgins.11@ucl.ac.uk

Highfield, Roger
Science Museum Group
United Kingdom
Roger.Highfield@ScienceMuseum.ac.uk

Hiraoka, Yasushi
Osaka University
Japan
hiraoka@fbs.osaka-u.ac.jp

Hodge, Ian
Formedium Ltd
United Kingdom
ianhodge@formedium.com

Hoffman, Charles
Boston College
United States
hoffmacs@bc.edu

Holm, Laerke
Copenhagen University
Denmark
lrholm@bio.ku.dk

Hoya, Marta
University of Salamanca
Spain
martahg@usal.es

Hu, Wen
National Institute of Biological Sciences
China
huwen@nibs.ac.cn

Huang, Ying
Nanjing Normal University
China
yhuang@njnu.edu.cn

Huang, Yinyi
Mechanobiology Institute
Singapore
mbihyy@nus.edu.sg

Huisman, Stephen
University of Zürich
Switzerland
Stephen.huisman@imls.uzh.ch

Humphrey, Timothy
University of Oxford
United Kingdom
timothy.humphrey@oncology.ox.ac.uk

Hunt, Tim
CRUK London Research Institute
United Kingdom
Tim.Hunt@cancer.org.uk

Iglesias Romero, Ana Belén
Universidad Pablo de Olavide-Consejo
Spain
ana_belen_1988@hotmail.com

Irvine, Danielle
Murdoch Childrens Research Institute
Australia
danielle.irvine@mcri.edu.au

Ito, Yuna
Kinki University
Japan
yuna_hana_nina@yahoo.co.jp

Iwasaki, Hiroshi
Tokyo Institute of Technology
Japan
hiwasaki@bio.titech.ac.jp

Iyer, Divya
University of Massachusetts Medical School
United States
DivyaRamalingam.Iyer@umassmed.edu

Jackson, Lauren P.
University of Cambridge
United Kingdom
lpj21@cam.ac.uk

Jafari Ghods, Farinaz
Istanbul University
Turkey
Farinaz_1358@yahoo.com

Ruchi Jain
Harvard Medical School
United States
Ruchi_Jain@hms.harvard.edu

Jeffares, Daniel
University College London
United Kingdom
d.jeffares@ucl.ac.uk

Jeffery, Linda
CRUK London Research Institute
United Kingdom
linda.jeffery@cancer.org.uk

Ji, Liang
Institut Curie
France
lji@curie.fr

Jimenez, Juan
Universidad Pablo de Olavide de Sevilla
Spain
jjimmar@upo.es

Jin, Quan-wen
Xiamen University
China
jinquanwen@xmu.edu.cn

Jones, Steve
University College London
United Kingdom
j.s.jones@ucl.ac.uk

Jourdain, Isabelle
The Biotechnology Centre of Oslo
Norway
i.m.m.jourdain@biotek.uio.no

Jørgensen, Maria
University of Copenhagen
Denmark
mjoergensen@bio.ku.dk

Kakui, Yasutaka
CRUK London Research Institute
United Kingdom
yasu.kakui@cancer.org.uk

Kanoh, Junko
Osaka University
Japan
jkanoh@protein.osaka-u.ac.jp

Karaer Uzuner, Semian
Istanbul University
Turkey
semka@istanbul.edu.tr

Karnataki, Nahush
Life Technologies
United Kingdom
nahush.karnataki@lifetech.com

Kashiwazaki, Jun
Gakushuin University
Japan
jun.kashiwazaki@gakushuin.ac.jp

Käufer, Norbert F.
Technische Universität Braunschweig
Germany
n.kaeufer@tu-bs.de

Kawamukai, Makoto
Shimane University
Japan
kawamuka@life.shimane-u.ac.jp

Kawashima, Shigehiro
University of Tokyo
Japan
skawashima@mol.f.u-tokyo.ac.jp

Kaykov, Atanas
The Rockefeller University
United States
kaykova@rockefeller.edu

Kearsey, Stephen
University of Oxford
United Kingdom
stephen.kearsey@zoo.ox.ac.uk

Kersey, Paul
EMBL-EBI / PomBase
United Kingdom
pkersey@ebi.ac.uk

Keifenheim, Daniel
University of Massachusetts Medical School
United States
daniel.keifenheim@umassmed.edu

Kelkar, Manasi
University of Lausanne
Switzerland
manasi.kelkar@unil.ch

Kelsall, Emma
University of Leicester
United Kingdom
ek117@le.ac.uk

Kilchert, Cornelia
University of Oxford
United Kingdom
cornelia.kilchert@bioch.ox.ac.uk

Kitamura, Kenji
Hiroshima University
Japan
kkita@hiroshima-u.ac.jp

Klar, Amar
NCI, USA
United States
klara@mail.nih.gov

Klinger, Franzisca
TU Brunswick
Germany
f.klinger@tu-braunschweig.de

Klutstein, Michael
CRUK London Research Institute
United Kingdom
Michael.Klutstein@cancer.org.uk

Knutsen, Jon Halvor Jonsrud
Oslo University Hospital
Norway
jon.halvor.knutsen@rr-research.no

Kong, Daochun
Peking University
China
kongdc@pku.edu.cn

Kovacikova, Ines
University of Vienna
Austria
ines.kovacikova@univie.ac.at

Kowalczyk, Katarzyna
University of Manchester
United Kingdom
katarzyna.kowalczyk@postgrad.manchester.ac.uk

Koyano, Takayuki
Hiroshima University
Japan
koyano119@hiroshima-u.ac.jp

Køhler, Julie Bonne
University of Copenhagen
Denmark
jbkoehler@bio.ku.dk

Kramarz, Karol
University of Wrocław
Poland
kramarz.karol@gmail.com

Kumar, Deepak
Indraprastha University
India
deepakkumar2588@gmail.com

Kume, Kazunori
CRUK London Research Institute
United Kingdom
Kazunori.Kume@cancer.org.uk

Labbe, SimonLiu,
Universite de Sherbrooke
Canada
Simon.Labbe@USherbrooke.ca

Laboucarie, Thomas
CRBM
France
tlaboucarie@crbm.cnrs.fr

Lages, Bruno
University College London
United Kingdom
bruno.lages.09@ucl.ac.uk

Lambert, Sarah
Institut Curie/CNRS
France
sarah.lambert@curie.fr

Lawson, Jonathan
University of Cambridge
United Kingdom
jtdl2@cam.ac.uk

Lehtinen, Sonja
University College London
United Kingdom
sonja.lehtinen.10@ucl.ac.uk

Leong, Hui Sun
University of Manchester
United Kingdom
hsleong@picr.man.ac.uk

Levin, Henry
NIH
United States
henry_levin@nih.gov

Li, Cuifang
Kinki University
Japan
licuifang2010@yahoo.co.jp

Li, Fei
New York University
United States
fl43@nyu.edu

Cong Liu,
Sichuan University
China
lcmicro@yahoo.com

Liu, Victor
University of Massachusetts Medical School
United States
victor.liu@umassmed.edu

Liu, Qi
Peking University
China
liuqi458@pku.edu.cn

Lopez-Aviles, Sandra
University of Oslo
Norway
sandra.lopez-aviles@biotek.uio.no

Lygerou, Zoi
University of Patras
Greece
lygerou@upatras.gr

Ma, Yan
Kobe University
Japan
mayan@med.kobe-u.ac.jp

Mabuchi, Issei
Gakushuin University
Japan
issei.mabuchi@gakushuin.ac.jp

Madrid, Marisa
University of Murcia
Spain
marisa@um.es

Malecki, Michal
University College London
United Kingdom
m.malecki@ucl.ac.uk

Mana-Capelli, Sebastian
University of Massachusetts Medical School
United States
sebastian.mana-capelli@umassmed.edu

Mandeville, Ryoko
CRUK London Research Institute
United Kingdom
Ryoko.Mandeville@cancer.org.uk

Manjón, Elvira
University of Salamanca
Spain
elvira87@usal.es

Marasovic, Mirela
Gene Center Munich
Germany
mirela.marasovic@gmail.com

Marguerat, Samuel
Imperial College London
United Kingdom
samuel.marguerat@csc.mrc.ac.uk

Markie, Michael
Faculty of 1000
United Kingdom
michael.markie@f1000.com

Marsellach-Castellví, Francesc Xavier
University College London
United Kingdom
f.marsellach-castellvi@ucl.ac.uk

Martienssen, Robert
Cold Spring Harbor Laboratory
United States
martiens@cshl.edu

Martin, Sophie
University of Lausanne
Switzerland
sophie.martin@unil.ch

Martin, Rebeca
University of Salamanca
Spain
rebemg@usal.es

Martín, Ruth
University of Oslo
Norway
r.m.martin@biotek.uio.no

Masai, Hisao
Tokyo Metropolitan Inst. of Medical Science
Japan
masai-hs@igakuken.or.jp

Masuda, Hirohisa
CRUK London Research Institute
United Kingdom
hiro.masuda@cancer.org.uk

Masukata, Hisao
Osaka Univsersity
Japan
masukata@bio.sci.osaka-u.ac.jp

Mata, Juan
University of Cambridge
United Kingdom
jm593@cam.ac.uk

Mathiassen, Søs Grønbæk
University of Copenhagen
Denmark
SMathiassen@bio.ku.dk

Matsumoto, Seiji
Tokyo Metropolitan Inst. of Medical Science
Japan
matsumoto-sj@igakuken.or.jp

Matsuo, Yuzy
CRUK London Research Institute
United Kingdom
yuzy.matsuo@cancer.org.uk

McCollum, Dannel
University of Mass. Medical School
United States
dannel.mccollum@umassmed.edu

Mcdowall, Mark
EMBL-EBI / PomBase
United Kingdom
mcdowall@ebi.ac.uk

McFarlane, Ramsay
Bangor University
United Kingdom
r.macfarlane@bangor.ac.uk

Meadows, John
University of Warwick
United Kingdom
j.c.meadows@warwick.ac.uk

Merlini, Laura
University of Lausanne
Switzerland
laura.merlini@unil.ch

Miki, Atsuko
University of Tokyo
Japan
astro02mickey@gmail.com

Millar, Jonathan
University of Warwick
United Kingdom
J.Millar@warwick.ac.uk

Miller, Crispin
University of Manchester
United Kingdom
cmiller@picr.man.ac.uk

Millington, Christopher
Inst. of Genetics and Development, Rennes
France
christopher.millington@univ-rennes1.fr

Minc, Nicolas
Insitut Jacques Monod
France
minc@ijm.univ-paris-diderot.fr

Mishra, Mithilesh
Temasek Life Sciences Laboratory
Singapore
mishra@tll.org.sg

Miyamoto, Masaaki
Kobe University
Japan
miya@kobe-u.ac.jp

Mizuno, Kenichi
University of Sussex
United Kingdom
k.mizuno@sussex.ac.uk

Moazed, Danesh
Harvard Medical School
United States
danesh@hms.harvard.edu

Mohebi, Saed
University of Sussex
United Kingdom
sm413@sussex.ac.uk

Moiseeva, Vera
University College London
United Kingdom
v.moiseeva@ucl.ac.uk

Mole, Sara
University College London
United Kingdom
s.mole@ucl.ac.uk

Moreno, Sergio
University of Salamanca
Spain
smo@usal.es

Mori, Risa
CRUK London Research Institute
United Kingdom
risa.mori@cancer.org.uk

Motamedi, Mo
Harvard Medical School
United States
mmotamedi@hms.harvard.edu

Mulvihill, Dan
University of Kent
United Kingdom
d.p.mulvihill@kent.ac.uk

Muñoz Félix, Sofía
University of Salamanca
Spain
sofiamf@usal.es

Murakami, Yota
Hokkaido University
Japan
yota@sci.hokudai.ac.jp

Murakami, Hiroshi
Chuo University
Japan
hmura03@yahoo.co.jp

Murray, Jo
University of Sussex
United Kingdom
j.m.murray@sussex.ac.uk

Musgrove, Nick
Infors UK Ltd
United Kindgom
n.musgrove@infors-ht.com

Nakagawa, Takuro
Osaka University
Japan
takuro4@bio.sci.osaka-u.ac.jp

Nakamura, Toru
University of Illinois at Chicago
United States
nakamut@uic.edu

Nakazawa, Norihiko
Okinawa Inst. of Science and Technology
Japan
nakazawa@oist.jp

Nambu, Tomoko
Hiroshima University
Japan
1990tmk0816@gmail.com

Naruse, Hajime
Kinki University
Japan
s1593d2416_reward@yahoo.co.jp

Navarro, Francisco
CRUK London Research Institute
United Kingdom
Francisco.Navarro@cancer.org.uk

Nielsen, Helena B. N.
University of Copenhagen
Denmark
HBN@bio.ku.dk

Niki, Hironori
National Institute of Genetics
Japan
hniki@nig.ac.jp

Nilsson Lock, Antonia
University College London / PomBase
United Kingdom
a.lock@ucl.ac.uk

Nishimura, Shinichi
Kyoto University
Japan
nshin@pharm.kyoto-u.ac.jp

Noguchi, Eishi
Drexel University College of Medicine
United States
enoguchi@drexelmed.edu

Norbury, Chris
University of Oxford
United Kingdom
chris.norbury@path.ox.ac.uk

Novak, Bela
University of Oxford
United Kingdom
bela.novak@bioch.ox.ac.uk

Nozaki, Shingo
National Institute of Genetics
Japan
snozaki@nig.ac.jp

Nurse, Paul
The Royal Society
United Kingdom
Paul.Nurse@royalsociety.org

Oda, Arisa
University of Tokyo
Japan
1969613806@mail.ecc.u-tokyo.ac.jp

Ogiyama, Yuki
Osaka University
Japan
ogiyama@fbs.osaka-u.ac.jp

Ohta, Kunihiro
The University of Tokyo
Japan
kohta@bio.c.u-tokyo.ac.jp

Okada, Naoyuki
University of Tokyo
Japan
0197423425@mail.ecc.u-tokyo.ac.jp

Okamoto, Sho
National Institute of Genetics
Japan
sokamoto@nig.ac.jp

Oliferenko, Snezhana
TLL
Singapore
snejana@tll.org.sg

Olmsted, Zachary
Univ. at Albany - State Univ. of New York
United States
zolmsted@albany.edu

Önay Uçar, Evren
Istanbul University
Turkey
evrenonay@istanbul.edu.tr

Oravcova, Martina
Charles University in Prague
Czech Republic
martina.nema@seznam.cz

Örs Gevrekci, Aslıhan
Bilecik Şeyh Edebali Üniversitesi
Turkey
aslihanors@yahoo.co.uk

Pai, Chen-Chun
Oxford University
United Kingdom
chen-chun.pai@oncology.ox.ac.uk

Palabiyik, Bedia
Istanbul University
Turkey
bediag@istanbul.edu.tr

Pancaldi, Vera
Spanish National Cancer Centre
Spain
vpancaldi@cnio.es

Partridge, Janet
St. Jude Children's Research Hospital
United States
janet.partridge@stjude.org

Partridge, Linda
University College London and Max Planck Institute for Biology of Ageing
United Kingdom / Germany
Partridge@age.mpg.de

Patterson, James
CRUK London Research Institute
United Kingdom
jop23@cam.ac.uk

Paulo, Esther
Universitat Pompeu Fabra
Spain
esther.paulo@upf.edu

Pérez-Hidalgo, Livia
University of Salamanca
Spain
liviap@usal.es

Perrot, Anthony
IGDR
France
anthony.perrot@univ-rennes1.fr

Pesti, Miklós
University of Pécs
Hungary
pmp@gamma.ttk.pte.hu

Petersen, Janni
University of Manchester
United Kingdom
janni.petersen@manchester.ac.uk

Petrini, Edoardo
Babraham Institute
United Kingdom
edoardo.petrini@babraham.ac.uk

Pidoux, Alison
University of Edinburgh
United Kingdom
alison.pidoux@ed.ac.uk

Pillai, Sreerekha
University of Edinburgh
United Kingdom
Sreerekha.Pillai@ed.ac.uk

Pluskal, Tomáš
Okinawa Inst. of Science and Technology
Japan
pluskal@oist.jp

Portaniter, Marina
Centro Biologia Molecular "Severo Ochoa".
Spain
mportantier@cbm.uam.es

Prasad, Punit
Karolinska Institutet
Sweden
punitprasad@gmail.com

Převorovský, Martin
Charles University in Prague
Czech Republic
prevorov@natur.cuni.cz

Rallis, Charalampos (Babis)
University College London
United Kingdom
c.rallis@ucl.ac.uk

Ramírez, Manuel
University of Patras
Greece
manuelrg@upatras.gr

Ramos, Mariona
University of Salamanca
Spain
mrv@usal.es

Ren, Jie
Cold Spring Harbor Laboratory
United States
jren@cshl.edu

Reuter, Max
University College London
United Kingdom
m.reuter@ucl.ac.uk

Revilla, María Teresa
University of Salamanca
Spain
newe_neo85@hotmail.com

Reyes, Félix
Centro Andaluz de Biología del Desarrollo
Spain
felixreyes90@gmail.com

Rhind, Nick
UMass Med School
United States
nick.rhind@umassmed.edu

Riehlman, Tim
University at Albany
United States
triehlman@albany.edu

Rincon, Sergio
Institut Curie
France
sergio.rincon@curie.fr

Roche, Benjamin
Cold Spring Harbor Laboratory
United States
broche@cshl.edu

Rodriguez, Alejandro
Uppsala University
Sweden
alejandro.rodriguez@imbim.uu.se

Rodriguez, Maria
University College London
United Kingdom
m.rodriguezlopez@ucl.ac.uk

Rodriguez-Gabriel, Miguel A.
Centro Biologia Molecular "Severo Ochoa".
Spain
marodriguez@cbm.uam.es

Rokeach, Luis
Université de Montréal
Canada
luis.rokeach@umontreal.ca

Rothe, Christiane
Oslo University Hospital
Norway
christiane.rothe@rr-research.no

Ruan, Kun
Osaka University
Japan
kunruan@fbs.osaka-u.ac.jp

Ruchman, David
University of Vienna
Austria
david.ruchman@univie.ac.at

Russell, Paul
The Scripps Research Institute
United States
prussell@scripps.edu

Sabouri, Nasim
Umeå University
Sweden
nasim.sabouri@medchem.umu.se

Sadaie, Mahito
Kyoto University
Japan
msadaie@lif.kyoto-u.ac.jp

Saitoh, Shigeaki
Kurume University
Japan
saitou_shigeaki@kurume-u.ac.jp

Sakuno, Takeshi
University of Tokyo
Japan
sakuno-t@iam.u-tokyo.ac.jp

Salguero-Corbacho, Israel
University of Cambridge
United Kingdom
i.salguero@gurdon.cam.ac.uk

Sansó, Miriam
Mount Sinai School of Medicine
United States
miriam.sanso@mssm.edu

Santosa, Venny
Kwansei Gakuin University
Japan
liebe_uc@yahoo.co.id

Sato, Hiroshi
Kurume University
Japan
satou_hiroshi@kurume-u.ac.jp

Sato, Masamitsu
Waseda University
Japan
masasato@waseda.jp

Sawin, Kenneth
University of Edinburgh
United Kingdom
ken.sawin@ed.ac.uk

Sazer, Shelley
Baylor College of Medicine
United States
ssazer@bcm.edu

Schalch, Thomas
University of Geneva
Switzerland
thomas.schalch@unige.ch

Schiklenk, Christoph
EMBL Heidelberg
Germany
schiklen@embl.de

Schwer, Beate
Weill Cornell Medical College
United States
bschwer@med.cornell.edu

Seike, Taisuke
Osaka City University
Japan
saysay@sci.osaka-cu.ac.jp

Shah, Sneha
University of Oxford
United Kingdom
sneha.shah@bioch.ox.ac.uk

Shaikh, Nadeem
CRUK London Research Institute
United Kingdom
nadeem.shaikh@cancer.org.uk

Shichino, Yuichi
Kazusa DNA Research Institute
Japan
yshichino@gmail.com

Shiozaki, Kaz
Nara Institute of Science and Technology
Japan
kaz@bs.naist.jp

Sideri, Theodora
University College London
United Kingdom
t.sideri@ucl.ac.uk

Simanis, Viesturs
EPFL Lausanne
Switzerland
viesturs.simanis@epfl.ch

Singer, Jan
Singer Instrument Co Ltd
United Kingdom
jan@singerinstruments.com

Singer, Harry
Singer Instrument Co Ltd
United Kingdom
harry@singerinstruments.com

Siwaszek-Wojcicka, Aleksandra
Inst. of Biochemistry and Biophysics, PAS
Poland
asiwaszek@ibb.waw.pl

J. Sjölander, Johanna
University of Gothenburg
Sweden
johanna.johansson.sjolander@cmb.gu.se

Skouteri, Meliti
University of Sussex
United Kingdom
ms441@sussex.ac.uk

Smith, Gerry
Fred Hutchinson Cancer Research Center
United States
gsmith@fhcrc.org

Soriano, Ignacio
University of Salamanca
Spain
isoriano@usal.es

Steinhauf, Daniel
Uppsala University
Sweden
daniel.steinhauf@imbim.uu.se

Styers, Melanie
Birmingham-Southern College
United States
mstyers@bsc.edu

Suarez, M Belen
University of Salamanca
Spain
belensu@usal.es

Suarez, Illyce
Univ. of Miami Miller School of Medicine
United States
isuarez@med.miami.edu

Subramanian, Lakxmi
University of Edinburgh
United Kingdom
lakxmi.subramanian@ed.ac.uk

Sugiura, Reiko
Kinki University
Japan
sugiurar@phar.kindai.ac.jp

Sugiyama, Tomo
University of Tsukuba
Japan
sugiyamt@biol.tsukuba.ac.jp

Sunnerhagen, Per
University of Gothenburg
Sweden
Per.Sunnerhagen@cmb.gu.se

Sutani, Takashi
University of Tokyo
Japan
tsutani@iam.u-tokyo.ac.jp

Svetina, Saša
University of Ljubljana
Slovenia
sasa.svetina@mf.uni-lj.si

Swaffer, Matthew
CRUK London Research Institute
United Kingdom
matthew.swaffer@cancer.org.uk

Sweet, Steve
University of Sussex
United Kingdom
s.m.sweet@sussex.ac.uk

Szijgyarto, Zsolt
University of Leicester
United Kingdom
zs72@le.ac.uk

Szilagyi, Zsolt
University of Gothenburg
Sweden
zsolt.szilagyi@medkem.gu.se

Taglini, Francesca
University of Dundee
United Kingdom
f.z.taglini@dundee.ac.uk

Takikawa, Masahiro
Kyoto University
Japan
mtakikawa.m09@lif.kyoto-u.ac.jp

Tallada, Victor
Universidad Pablo de Olavide
Spain
valvtal@upo.es

Tanaka, Katsunori
Kwansei Gakuin University
Japan
katsunori@kwansei.ac.jp

Tanaka, Kayoko
University of Leicester
United Kingdom
kt96@le.ac.uk

Tang, Ngang Heok
CRUK London Research Institute
United Kingdom
ngang-heok.tang@cancer.org.uk

Tang, Zhaohua Irene
Claremont Colleges
United States
ztang@kecksci.claremont.edu

Tange, Yoshie
Osaka University
Japan
tange@fbs.osaka-u.ac.jp

Tanny, Jason
McGill University
Canada
jason.tanny@mcgill.ca

Tao, Evelyn Yaqiong
National University of Singapore
Singapore
a0068183@nus.edu.sg

Tarhan, Cagatay
Istanbul University
Turkey
cagataytarhan@yahoo.com

Tarumoto, Yusuke
Kyoto University
Japan
ytarumot@lif.kyoto-u.ac.jp

Tay, Ye Dee
University of Manchester
United Kingdom
ydtay@picr.man.ac.uk

Testori, Marian
Grand Valley State University
United States
testorim@mail.gvsu.edu

Thon, Geneviève
University of Copenhagen
Denmark
gen@bio.ku.dk

Toda, Takashi
CRUK London Research Institute
United Kingdom
takashi.toda@cancer.org.uk

Togashi, Naoyuki
Kazusa DNA Research Institute
Japan
naoyuki-togashi@hotmail.co.jp

Tolic-Norrelykke, Iva
MPI-CBG
Germany
tolic@mpi-cbg.de

Tomita, Kazunori
University College London
United Kingdom
k.tomita@ucl.ac.uk

Tormos-Pérez, Marta
University of Salamanca
Spain
mtor@usal.es

Tran, Phong
Institut Curie
France
phong.tran@curie.fr

Tsukamoto, Yuta
Kobe University
Japan
103s310s@stu.kobe-u.ac.jp

Tsutsui, Yasuhiro
Tokyo Institute of Technology
Japan
ytsutsui@bio.titech.ac.jp

Tvaruzkova, Jarmila
Charles University in Prague
Czech Republic
jarmila.tvaruzkova@natur.cuni.cz

Ueno, Masaru
Hiroshima University
Japan
scmueno@hiroshima-u.ac.jp

Upadhyay, Udita
Institute of Microbial Technology
India
udita@imtech.res.in

Uyeda, Ei-ichi
Gakushuin University
Japan
eiichiueda@yahoo.co.jp

Vachon, Lianne
University of Calgary
Canada
liannevachon@gmail.com

van Trigt, Laurent
University College London
United Kingdom
ucgalva@ucl.ac.uk

Vasiljeva, Lidia
University of Oxford
United Kingdom
lidia.vasilieva@bioch.ox.ac.uk

Vavylonis, Dimitrios
Lehigh University
United States
vavylonis@lehigh.edu

Veal, Elizabeth
Newcastle University
United Kingdom
e.a.veal@ncl.ac.uk

Verde, Fulvia
University of Miami School of Medicine
United States
fverde@miami.edu

Verdel, André
CNRS/INSERM
France
andre.verdel@ujf-grenoble.fr

Villalobos Quesada, Maria Jose
University of Manchester
United Kingdom
mvillalobos@picr.man.ac.uk

Walker, Carol
Oxford University
United Kingdom
carol.walker@oncology.ox.ac.uk

Wang, Hai-Tao
National Institue of Biological Sciences
China
wanghaitao@nibs.ac.cn

Wang, Shao-Win
National Health Research Institutes
Taiwan
shaowinwang@nhri.org.tw

Wang, Yamei
Xiamen University
China
wangyamei@xmu.edu.cn

Wang, Ruye
Harvey Mudd College
United States
rwang@hmc.edu

Wardlaw, Christopher
University of Sussex
United Kingdom
cpw24@sussex.ac.uk

Watanabe, Yoshinori
Institute of molecular Cellular Biosciences
Japan
ywatanab@iam.u-tokyo.ac.jp

James Watson
Life Technologies
United Kingdom
james.watson@lifetech.com

Watts, Felicity
University of Sussex
United Kingdom
f.z.watts@sussex.ac.uk

Weisman, Ronit
Open University of Israel
Israel
ronitwe@openu.ac.il

Weston, Louise
CRUK London Research Institute
United Kingdom
louise.weston@cancer.org.uk

Whitby, Matthew
University of Oxford
United Kingdom
matthew.whitby@bioch.ox.ac.uk

Whitehall, Simon
Newcastle University
United Kingdom
simon.whitehall@ncl.ac.uk

Williamson, Fiona
University College London
United Kingdom
f.williamson@ucl.ac.uk

Wolf, Dieter
Sanford-Burnham Medical Research Inst.
United States
dwolf@sanfordburnham.org

Wood, Elizabeth
CRUK London Research Institute
United Kingdom
elizabeth.wood@cancer.org.uk

Wood, Valerie
PomBase / Cambidge University
United Kingdom
vw253@cam.ac.uk

Wu, Jianqiu
The Ohio State University
United States
wu.620@osu.edu

Wu, Pei-Yun Jenny
Inst. of Genetics and Development, Rennes
France
pei-yun.wu@univ-rennes1.fr

Wysocki, Robert
University of Wroclaw
Poland
robert.wysocki@biol.uni.wroc.pl

Yagupsky, Pablo
Ben-Gurion University of the Negev
Israel
yagupsky@bgu.ac.il

Yamamoto, Masayuki
Kazusa DNA Research Institute
Japan
myamamoto@kazusa.or.jp

Yamamoto, Ayumu
Shizuoka University
Japan
sayamam@ipc.shizuoka.ac.jp

Yamano, Hiro
University College London
United Kingdom
h.yamano@ucl.ac.uk

Yamashita, Akira
Kazusa DNA Research Institute
Japan
ymst@kazusa.or.jp

Yanagida, Mitsuhiro
Okinawa Inst. of Science and Technology
Japan
myanagid@gmail.com

Yance Chávez, Tula del Carmen
University of Barcelona
Spain
tyance@hotmail.com

Yonekura, Toshiya
Nara Institute of Science and Technology
Japan
t-yonekura@bs.naist.jp

Young, Paul
Queen's University
Canada
Paul.Young@queensu.ca

Youssef, Ghada
Alexandria University
Egypt
amin_ghada@yahoo.com

Yu, Haochen
ETH Zurich
Switzerland
haochen.yu@bc.biol.ethz.ch

Yukawa, Masashi
CRUK London Research Institute
Kingdom
Masashi.Yukawa@cancer.org.uk

Zaaijer, Sophie
CRUK London Research Institute
United Kingdom
sophie.zaaijer@cancer.org.uk

Zaratiegui, Mikel
Rutgers University
United States
zaratiegui@dls.rutgers.edu

Zhang, Jing
Oxford University
United Kingdom
jing.zhang@stx.ox.ac.uk

Zhang, Jing
Oxford University
United Kingdom
jing.zhang@dpag.ox.ac.uk

Zhao, Yuan
CRUK London Research Institute
United Kingdom
yuan.zhao@cancer.org.uk

Zhurinsky, Jacob
Univ. Pablo de Olavide
Spain
izhu@upo.es

Zocco, Manuel
Ludwig Maximilians Universität
Germany
manu.zocco@yahoo.it

Zofall, Martin
NCI/NIH
United States
zofallm@mail.nih.gov

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Six Previous International Fission Yeast Meetings

1. Edinburgh, UK (Sep 1999), organized by S. MacNeill
2. Kyoto, Japan (Mar 2002), organized by M. Yanagida/P. Nurse
3. San Diego, USA (Aug 2004), organized by P. Russell
4. Copenhagen, Denmark (Jun 2007), organized by O. Nielsen
5. Tokyo, Japan (Oct 2009), organized by Y. Watanabe/M. Yamamoto
6. Boston, USA (Jun 2011), organized by N. Rhind

EMERGENCY INFORMATION

If you find a fire: Set off the alarm via a red 'break-glass' box then leave the building by following the green fire exit route signs in the corridors and stairs. Once you have left the building, stay outside – well clear of the exits - until informed that it is safe to re-enter

The building has an automatic fire detection system and the alarm will sound continuously if this is activated.

EVACUATION PROCEDURE

On hearing the continuous alarm;

1. **Proceed quickly & calmly to the nearest exit.**
   Green signs mark escapes routes/Fire exits.
   Do not stop to collect personal belongings.

2. **Proceed to the assembly point in your area.**
   Do not congregate in the road or outside the entrances, as emergency vehicles will require access to the building.

3. **Persons with restricted mobility should** wait at refuge points or contact Reception on Ext 8133.

FIRE ALARM WEEKLY TEST

Fire alarms are tested on Thursday mornings at 9.30am. There is no need to evacuate unless the alarm sounds for more than approx 50 seconds.

FIRST AID & EMERGENCY SERVICES
Call 8133 providing details of incident and any injuries.

For info of nearest exits
Beveridge Hall – Malet Street side exit
Macmillan Hall & Ground floor meeting rooms – Montague Place exit (Under ceremonial stairs)
1st, 2nd & 3rd floor meeting rooms – Staircase 1, Malet Street main exit
Stewart House – Follow signs for nearest exits.

Conference Office
Estates & Facilities Department

More notes