2006
European
Fission Yeast
Meeting

Wellcome Trust Conference
Centre, Hinxton, UK

16-18 March 2006

Organizers:

Jürg Bähler,
Sanger Institute, Hinxton, UK

Valerie Wood,
Sanger Institute, Hinxton, UK

Mitsuhiro Yanagida,
Kyoto University, Japan

Paul Nurse,
Rockefeller University, USA

Cover picture: Photo mosaic of double helix made from various S. pombe pictures (J. Bähler)
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**Schedule Overview**

**Thu 16th March**

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Discussion Session: Resources for the Fission Yeast Community

**Sat 18th March**

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**Fri 17th March**

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Programme

Thu 16th March, 1630-1805

Introduction: Jürg Bähler, Val Wood

Session 1: DNA Metabolism I
Chairs: Tony Carr, Julie Cooper, Paul Russell
Zoi Lygerou
(University of Patras, Greece) T1
A stochastic hybrid model for DNA replication

Chris Norbury
(University of Oxford, UK) T2
Involvement of Cid1 poly(A) polymerase and the sub-telomeric rqh2+ DNA helicase gene in a common pathway following inhibition of DNA replication

Benoit Arcangioli
(Pasteur Institute, France) T3
The essential role of the homologous recombination for replication fork restart

Ken’ichi Mizuno
(Carr lab, University of Sussex, UK) T4
Aberrant chromosome rearrangement induced by replication fork stalling at a palindrome

Osami Niwa
(Kazusa DNA Research Institute, Japan) T5
On the instability of aneuploidy in fission yeast

Paul Russell
(The Scripps Research Institute, USA) T6
Pathways that control the recruitment of Crb2 to sites of DNA damage

Thu 16th March, 2000-2130

Session 2: DNA Metabolism II
Chairs: Tony Carr, Julie Cooper, Paul Russell

Anna Hebdon
(Cooper lab, Cancer Research UK) T7
Surviving DNA damage with dysfunctional telomeres

Edgar Hartsuiker
(University of Sussex, UK) T8
The role of the S. pombe MRN complex in the removal of covalently linked protein from the DNA

Ramsay McFarlane
(University of Wales Bangor, UK) T9
S. pombe meiotic linear elements: the relationship with pre-meiotic DNA replication and meiotic genetic recombination

Discussion Session:
Resources for the Fission Yeast Community
Chairs: Paul Nurse, Mitsuhiro Yanagida

Jacky Hayles
(Cancer Research UK) Status of the S. pombe deletion collection

Mitsuhiro Yanagida
(Kyoto University, Japan) Strain resources in Japan

Val Wood, Jürg Bähler
(Sanger Institute, UK) The future of the S. pombe GeneDB database

Paul Nurse
(Rockefeller University, USA) General discussion Possible future initiatives?

Fri 17th March, 0900-1030

Session 3: Environmental Responses & Signalling
Chairs: Paul Young, Olaf Nielsen

Søren Kjerruff
(Nielsen lab, University of Copenhagen, Denmark) T10 Cdk-phosphorylation of the HMG-domain protein Ste11 controls the switch from mitosis to meiosis in fission yeast

Neil Bone
(Armstrong lab, University of Sussex, UK) T11 Vtc4p: a regulator of vacular size and trafficking in S. pombe

Paul Young
(Queen’s University, Canada) T12 NatB N-acetylation activity is required for Bsu1p pyridoxine transporter function in S. pombe

Clare Lawrence
(Jones lab, Paterson Institute, UK) T13 Regulation of S. pombe Atf1 protein levels by Sty1-mediated phosphorylation and heterodimerisation with Pcr1

Alison Day
(Val lab, University of Newcastle, UK) T14 A conserved cysteine of Sty1 has an important role in oxidative stress resistance

Janni Petersen
(University of Manchester, UK) T15 Control of cell size at division in response to nutrient availability

Fri 17th March, 1100-1230

Session 4: Gene Expression Control: From Chromatin to Proteins
Chairs: Nic Jones, Karl Ekwall

Introduction by Nic Jones
(Paterson Institute, UK)

Daniel Lackner
(Bähler lab, Sanger Institute, UK) T16 Genome-wide translational control in fission yeast

Karl Ekwall
(Karolinska Institute, Sweden) T17 Genome wide roles for histone modifications and RNAI directed chromatin silencing

Anthony Wright
(Karolinska Institute, Sweden) T18 Comparative proteomic analysis of molecular mechanisms that program transcription

Assen Rougoue
(Stewart lab, The University of Technology Dresden, Germany) T19 Chromatin related complexes in S. cerevisiae and S. pombe

José Ayte
(Universitat Pompeu Fabra, Spain) T20 Rem1 expression is regulated at the level of transcription and splicing

Xuefeng Xu
(Gustafsson lab, Karolinska Institute, Sweden) T21 Genome wide occupancy profile of Mediator and the Srb8-11 module reveals interactions with coding regions
Amanda Greenall  
(Whitehall lab, University of Newcastle, UK)  
Hip3 interacts with the HIRA proteins Hip1 and Sim9 and is required for transcriptional silencing and accurate chromosome segregation

Fri 17th March, 1400-1530  
Session 5: Mitotic Spindle  
Chairs: Takashi Toda, Iain Hagan

Anne Kerres  
(Fleig lab, Heinrich-Heine-Universität, Germany)  
The conserved kinetochore component Spc7 regulates microtubule-kinetochore association and is linked to the Sim4-complex

Masamitsu Sato  
(Toda lab, Cancer Research UK)  
Microtubule organisation via regulation of microtubule-associated proteins TACC/Alp7 and TOG/Alp14 throughout the cell cycle

Matylda Szcarielska  
(Hardwick lab, University of Edinburgh, UK)  
Interactions between the spindle checkpoint proteins and the Anaphase Promoting Complex in S. pombe

John Meadows  
(Miller lab, National Institute for Medical Research, UK)  
Dissociation of mitotic spindle position from the timing of anaphase onset in fission yeast

Sylvie Tournier  
(Université Paul Sabatier, France)  
Mechanism controlling perpendicular alignment of the spindle to the axis of cell division in fission yeast

Iva Tolic-Norrelykke (Max Planck Inst. of Molecular Cell Biology and Genetics, Germany)  
Optical tweezers in fission yeast: mechanism of nuclear and septum positioning

Fri 17th March, 1600-1900  
Poster Session  
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Even numbers: 1730-1900

Sat 18th March, 0930-1100  
Session 6: Cytoskeleton and Cell Morphogenesis  
Chairs: Jacky Hayles, Ken Sawin

Introduction by Ken Sawin  
(University of Edinburgh, UK)

Stefania Castagnetti  
(Nurse lab, Cancer Research UK)  
Generating cell form in fission yeast

Dai Hirata  
(Hiroshima University, Japan)  
Control of growth polarity upon perturbed DNA replication

Yolanda Sanchez  
(Universidad de Salamanca, Spain)  
Rg1p is a specific Rho1-GEF that coordinates cell polarization with cell wall biogenesis in fission yeast

Anne Paoletti  
(Curie Institute, France)  
Spatial regulation of mid1p cortical distribution by pom1p kinase

Agnes Grallert  
(Hagan lab, Paterson Institute, UK)  
Characterization of the fission yeast CLASP, Peg1

Itaru Samejima  
(Sawin lab, University of Edinburgh, UK)  
Identification of an MTOC-localisation signal domain in Mto1

Sat 18th March, 1130-1300  
Session 7: Mitotic and Meiotic Cell-Division Cycle  
Chairs: Shelley Sazer, Chris Norbury

Jérome Wuarin  
(University of Dundee, UK)  
Cell growth and cell cycle: Cdc2 meets ribosome biogenesis

Tonje Tvegård  
(Boye lab, Inst. for Cancer Research, Norway)  
A novel checkpoint reveals a coupling between general cell growth and the cell cycle

Hiro Yamano  
(Marie Curie Research Institute, UK)  
Mechanisms of the APC/C-dependent ubiquitination and proteolysis

Koji Nagao  
(Yanagida lab, Kyoto University, Japan)  
The domain of securin required for stabilization and inhibition of separase can have a separate cleavage site by substitutions

Yuko Tonami  
(Murakami lab, Nagoya City University, Japan)  
The mechanism of cell cycle progression through meiosis I by the meiosis-specific forkhead transcription factor Mei4p in fission yeast

Akira Yamashita  
(Yamamoto lab, University of Tokyo, Japan)  
Suppressor mutants of the meiosis I arrest caused by loss of the Mei2p dot

Closing remarks
Talk Abstracts

A stochastic hybrid model for DNA replication.


1. Department of Electrical and Computer Engineering, University of Patras, Greece
2. Department of Medicine, University of Patras, Greece
3. The Rockefeller University, USA

Modelling of biological processes has been a very active research area in recent years, in the hope that mathematical models will provide insight into the underlying biological principles and guide the development of new experiments. Many types of models have been proposed for biological processes in the literature, including purely discrete, graph theoretical models, continuous ODE and PDE models, hybrid and stochastic models. Among these, stochastic hybrid models, which combine continuous dynamics to discrete switches of states and to events determined probabilistically, appear ideally suited to describe biological phenomena.

We develop here a stochastic hybrid model of DNA replication in the cell cycle of the fission yeast. The model captures the interplay between discrete dynamics associated with the firing of origins of replication (pre-replicative, replicating and post-replicative states), continuous dynamics that arise out of the replication process itself (continuous progression of the replication forks along the genome), and stochastic events due to the probabilistic firing process (not all potential origins will fire in each cell and not all will fire at the same time). The model is coded using data recently obtained in fission yeast by some of the authors, and tested by Monte Carlo simulation. The statistics collected through the simulations provide valuable insight into DNA replication in fission yeast and suggest new biological experiments that could be carried out to improve our understanding of the process.

Involvement of Cid1 poly(A) polymerase and the sub-telomeric rqh2’ DNA helicase gene in a common pathway following inhibition of DNA replication


1Sir William Dunn School of Pathology and 2Department of Zoology, University of Oxford and 3Wellcome Trust Sanger Institute, Hinxton, UK.

We previously identified S. pombe cid1’ through a screen for S-phase cell cycle checkpoint genes, and showed that the Cid1 protein is a cytoplasmic non-canonical poly(A) polymerase1,2. Deletion of cid1’ results in a specific subset of checkpoint defects when replicative DNA polymerases are inhibited. To test the hypothesis that Cid1 regulates the polyadenylation of checkpoint gene mRNAs, we have used microarray hybridisation to identify RNA changes that result from cid1’ deletion. Most strikingly, we found cid1’-dependent expression of rqh2’/SPAC212.11 specifically in S phase arrested cdc7’ or DNA pol ã (cdc27) mutants. Rqh2 is the second member of the RecQ DNA helicase family to be described in S. pombe; its closest relative in human cells is the BLM helicase, mutation of which causes the cancer predisposition disorder Bloom’s syndrome. The rqh2 gene appears to be present in four sub-telomeric copies; at least one of these is expressed, following telomere erosion3 or replication stress, to generate an unspliced 7 kb mRNA with a long ORF that includes centromere-related dh repeat sequences upstream from sequences encoding the RecQ helicase domain. Transcription of the dh sequences within rqh2 is important in establishing sub-telomeric heterochromatin via the RNAi-RITS pathway4,5. A highly distinctive pattern of S’ RNA processing indicates that rqh2 transcripts may be direct Cid1 targets. We propose a model in which Cid1-mediated polyadenylation of rqh2 mRNA following replication stress allows Rqh2 translation. Rqh2 may be important for stabilisation and/or resolution of stalled DNA replication forks, particularly telomere-proximal forks, and maintenance of the S-M checkpoint signal.

The essential role of the homologous recombination for replication fork restart.

Laura Roseaulin, Allyson Holmes, Yufuko Akamatsu, Iroshi Iwasaki and Benoit Arcangioli

Unite de la Dynamique du Génome, Structures and Dynamics of the Genomes, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris, Cedex 15, France.

The role of recombination in the recovery of stalled/collapsed replication forks is an essential process in maintaining genomic stability and defects in this pathway cause predisposition to many forms of diseases. In fission yeast, the situation becomes critical when the replication fork collides with the imprint (unrepaired single-strand break or ribonucleotide) at the mat1 locus, and converts it into a one-ended double-strand break. The repair process must restore a fork structure that is suitable to restart replication. We show that a single collapsed replication fork required the homologous recombination (HR) enzymes for viability in fission yeast. Whereas the Rad22, Rhp51 and Rhp54 are essential, Rad50 is not albeit grow very slowly and Swi5 and Rhp57 are interchangeable. Following strand invasion with either the donor loci or the sister chromatid the joint molecule must be resolved to restart the replication fork. We show that Swi10 and Mus81 (XPF nuclease family) participate in two alternative resolution pathways. Swi10 is essential for MT-switching but is dispensable in the absence of donors, conversely Mus81 is dispensable for MT switching, but essential when the sister chromatid is used for repair. The mechanism of choice/exclusion of one or the other nucleases is unknown; however, the bacterial Holliday junction resolvase RusA partially suppress the absence of Mus81. The Rqh1, Srs2 and Fbh1 helicases, intra-S and G2 checkpoints are dispensable. Molecular approaches, in several mutant backgrounds, have been used to detect and follow the fate of the recombination intermediates at mat1. Altogether, these work demonstrate the essential function of the HR machinery in the repair of a single broken replication fork arising during vegetative growth. Since the HR proteins have been shown to be also essential during meiosis. Our results imply a circular relationship between MT switching and meiosis, in which the HR proteins play a central role, extending the question of how genetic recombination works, to why it exists.

Aberrant chromosome rearrangement induced by replication fork stalling at a palindrome

Ken’ichi Mizuno, Johanne Murray and Antony Carr

Genome Damage and Stability Centre, University of Sussex, Brighton, BN1 9RQ, UK.

Genomic rearrangements linked to aberrant recombination are associated with cancer and human genetic diseases. Such recombination has indirectly been linked to replication fork stalling. We have previously established a system, designated RuraR, to induce replication fork stalling (RFS) at a specific site in a controllable manner, using a defined replication fork barrier sequence RTS1 and trans-acting replication fork block protein Rtf1 (Lambert et al, 2005). In the RuraR system, RFS is overcome by homologous recombination between RTS1 sites leading to inversion of the intervening ura4 marker and rarely to site-specific gross chromosome rearrangements.

We have constructed a derivative palindrome system, Rura-arU, by inserting another copy of the ura4 gene in reverse orientation. In contrast to the RuraR system, the viability of the palindrome system is dramatically reduced when replication fork stalling is induced. Southern blot analysis and PFGE show that RFS at the palindrome leads to rapid chromosomal rearrangement and the formation of dentricand acentric sister chromatids. The cells progress through G2 and enter mitosis with normal kinetics suggesting that the rearrangements occur during S phase and the products do not activate the G2/M DNA damage checkpoint. The dentrics align correctly at metaphase with bipolar attachment to the spindle but are then torn apart at anaphase leading to a catastrophic mitosis.

The generation of the non-equivalent sister chromatids is dependent on the RTS1-Rtf1 system and a subset of recombination and checkpoint proteins. These results lead us to conclude

1) RFS at the palindrome triggers the chromosomal rearrangements through homologous recombination;
2) the generation of non-equivalent sister chromatids is dependent upon the palindromic sequences, which are able to self-pair, forming intrastrand hairpin structures; and
3) the palindrome system provides an inducible system with which to analyse the behaviour of dentric chromosomes.
On the instability of aneuploidy in fission yeast

Osami Niwa, Yoshie Tange, and Atsushi Kurabayashi

Kazusa DNA Research Institute, Japan

In many organisms, like plants and yeasts where ploidy is common, the generation of aneuploids is inevitable; that is, it occurs without erroneous chromosome transmission. Hence, there must be unknown mechanisms that eliminate aneuploids from the population. Growth disadvantage, which is thought to be generally intrinsic to aneuploids, might not sufficiently account for the elimination. Findings from our previous genetic study suggested that at least some types of aneuploids in fission yeast can eventually survive as diploids or haploids after a period of anomalous growth during which unbalanced chromosome compositions must be corrected. In the present study, we reexamined this issue to elucidate the mechanisms underlying aneuploid instability.

We first demonstrated that aneuploids between n and 2n were produced from triplid meioses at the expected ratio; i.e., 75% of the meiotic segregants were aneuploid. Fluorescence microscopy of living cells revealed that abnormal or uneven nuclear divisions occurred in microcolonies from a portion of apparently aneuploid spores. In some cases, haploid or diploid cells emerged from microcolonies that also contained many dying cells. Genetically defined diploid spores did not have such abnormalities.

We suggest that anomalous nuclear divisions occurring in aneuploid cells might, on one hand, lead to the amplification of aneuploidy to enhance cell death, and, on the other hand, lead to the resolution of aneuploids into stable euploids.

To pursue the aneuploid issue more systematically, we constructed a selection system for the disomy of chromosome 1 or 2 using intragenic complementation of ade6-210 and -216 alleles. Each of these alleles was integrated into a site near the respective centromere. This genetic selection system revealed that fission yeast aneuploids can apparently be stabilized through structural changes of chromosomes, including partial duplication, translocation, and circular minichromosomes.

Pathways that control the recruitment of Crb2 to sites of DNA damage

Li-Lin Du1, Toru Nakamura2 and Paul Russell1

1Department of Molecular Biology, The Scripps Research Institute, La Jolla, California, 92037, USA.
2Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL 60607, USA.

Cellular responses to DNA double-strand breaks (DSBs) involve the relocalization of checkpoint proteins to DSBs. The focal assembly of fission yeast checkpoint protein Crb2 at ionizing radiation-induced DSBs requires histone H2A C-terminal phosphorylation and H4-K20 methylation. However, the relevance of Crb2 relocalization remains uncertain because mutants lacking either of these histone modifications lack strong checkpoint defects. I will describe our latest studies on the semi-redundant mechanisms that control the localization of Crb2 at DSBs and explore their functional significance.
Surviving DNA damage with dysfunctional telomeres

Anna K. Hebdon, Kyle M. Miller, Toru Nakamura and Julia P. Cooper

Cancer Research UK, 44 Lincoln’s Inn Fields, London WC2A 3PX, UK

Telomerase maintains telomeres by adding DNA repeats to chromosome ends. Disruption of this enzyme leads to loss of telomeric DNA with successive rounds of DNA replication, and in most cases, senescence. Following loss of Trt1, the catalytic subunit of telomerase in fission yeast, a population of cells survive having lost all telomeric DNA. These cells survive by maintaining each of the three chromosomes as individual circles.

While circular strains are viable, they grow slowly and exhibit DAPI staining patterns suggestive of chromosome segregation problems. Reminiscent of strains lacking the telomere binding protein Taz1, circular strains are hypersensitive to agents that induce DNA double strand breaks. Interestingly, upon chronic treatment with damaging agents, the circular strains are more sensitive than strains lacking the checkpoint protein, Rad3. However, upon acute treatment, rad3 strains display greater sensitivity. The similarities between circular and taz1 strains prompted us to investigate the basis for the defects in these two strains, one missing a telomere binding protein and the other lacking telomeric DNA.

Reintroduction of trt1 to circular strains causes linearization of chromosome III with the addition of telomere repeats. Interestingly, this partially suppresses the drug sensitivity. To further distinguish between the topological issue of chromosome circularity and the presence or absence of telomere sequence, we created strains containing telomere repeats lacking ends, either plasmid based (to allow high copy number) or integrated within the genome. In most cases the presence of telomere repeats did not affect the drug sensitivity. Intriguingly, however, we observed rare survivors with greatly suppressed drug sensitivity upon disruption of telomerase. Analysis of these strains shows that they have survived by a novel mechanism. While they appear to lack the majority of telomeric DNA, they show behaviours distinct from those of typical circular chromosome-containing survivors. Our data suggest that one of these strains survives by amplifying subtelomeric repeat sequences, and the other by amplifying rDNA sequences.

We present our progress in understanding the nature of this strain and the roles of telomeres and topology in the survival of DNA double strand breaks.

The role of the S. pombe MRN complex in the removal of covalently linked protein from the DNA

Edgar Hartsuiker, Kenichi Mizuno, Tony Carr

GDSC, University of Sussex, Brighton, UK

Meiotic recombination is initiated by a DSB which is repaired using the homologous chromosome as a template. This meiosis-specific DSB is created by Rec12 (Spo11 homologue) through a non-reversible topoisomerase-like mechanism. Covalently bound Rec12 has to be removed from the DNA to allow subsequent DSB end resection and meiotic recombination. Here we present evidence that the S. pombe MRN complex (Rad32/Rad50/Nbs1) is directly involved in this removal.

We have created an S. pombe rad50S mutant and characterised its meiotic phenotypes. Using a physical assay, we have shown that the removal of covalently bound Rec12 from the DNA is defective in a rad50S mutation as well as in rad50Δ. Unlike S. cerevisiae, S. pombe rad50S is temperature sensitive for meiotic DSB repair (PFGE) and meiotic spore viability. Furthermore, I found that S. pombe rad50S separates the Rec12 removal defect from downstream meiotic recombination and repair related functions: meiotic recombination, linear element formation and meiosis specific chromatin remodelling at ade6-M26 are not affected in rad50S but strongly defective in rad50Δ. We have found that the rad32-D25A nuclease-dead mutant is also unable to remove Rec12 from the DNA, and that this defect is epistatic with rad50S and rad50Δ mutations, suggesting that the Rad32 (endo) nuclease activity is responsible for Rec12 removal.

Because rad50S is specifically defective in Rec12 removal, but proficient for downstream repair events, we have used it as a tool to study the potential role of the MRN complex in Top1 and Top2 removal in vegetative cells. In line with rad50S not being defective in general DNA repair, we found that the mutant is not sensitive to MMS or γ-radiation (at either permissive or restrictive temperature), whereas rad50Δ is extremely sensitive to these agents. However, the sensitivity of rad50S against campthothecin (locks Top1 on the DNA) and TOP-53 (locks Top2 on the DNA) mimics the temperature sensitive meiotic phenotype: at permissive temperature rad50S is only slightly sensitive to these reagents, but it becomes extremely sensitive at restrictive temperature. The sensitivity of rad50Δ and rad50S to these reagents is epistatic with a rad32-D25A nuclease-dead mutation. These data strongly suggest that the MRN complex is also directly involved in Top1 and Top2 removal.
Schizosaccharomyces pombe meiotic linear elements: the relationship with pre-meiotic DNA replication and meiotic genetic recombination.

Wells, J.L., Dafydd, H. F., Pryce, D.W., Estreicher, A., Loidl, J. McFarlane, R.J.*

North West Cancer Research Fund Institute, University of Wales Bangor, Memorial Building, Deiniol Road, Bangor, Gwynedd, LL57 2UW, United Kingdom.

Department of Chromosome Biology, University of Vienna, A-1030, Vienna, Austria

ramsay@sbs.bangor.ac.uk

The chromosomes of most organisms undergo a process of inter homologue synopsis during meiosis I. In the majority of cases this culminates in the formation of a tripartite proteinaceous structure known as the synaptonemal complex (SC). Linear structures, known as axial elements (AE), are the precursory structures to the fully formed SC. S. pombe does not form detectable SC structures analogous to those of other organisms, but does form proteinaceous thread-like structures, known as linear elements (LinEs), which have many features in common with AEs. Recently it has been demonstrated that Rec10 is a critical component of LinEs and LinEs do not form in mutants with an inactivated rec10 gene. Moreover, Rec10 appears to be the functional counterpart of the Saccharomyces cerevisiae AE protein Red1. The function(s) of Rec10 / LinEs (and AEs) remains unclear, although it has been suggested that they are required to mediate meiotic genetic recombination possible by generating a mechanical context in which crossing over is controlled.

In this study we have explored the relationship of LinEs with DNA replication and meiotic recombination further. We find that LinE structures form in the absence of pre-meiotic DNA replication and that significant levels of meiotic recombination occur in the absence of LinEs, an observation supported by the fact that Rad51 foci are observed in the absence of LinEs. These data suggest that Rec10 has more than one function during meiosis and that at least LinEs function as recombination enhancers. We also expose a more complex relationship between Rec10 and the control of genetic recombination. In a specific rec10 mutant, which is defective in LinE formation, there is a regional difference in control of crossing over and gene conversion events, the implication of these observations are discussed.

Cdk-phosphorylation of the HMG-domain protein Ste11 controls the switch from mitosis to meiosis in fission yeast

Søren Kjærulf, Nicoline Resen Andersen, Mia Trolle Borup and Olaf Nielsen.

Institute of Molecular Biology and Physiology, University of Copenhagen, DK-1353 Copenhagen K, Denmark

At a point in G2 called start in Schizosaccharomyces pombe the cell decides whether to stay in the mitotic cell cycle or to exit the mitotic cell cycle in order to differentiate. When deprived of nutrients S. pombe undergoes sexual differentiation. A prerequisite for this process is induction of the HMG-box transcription factor Ste11 and down regulation of cyclin-dependent kinase (Cdk) activity. Ste11 controls many genes required for this developmental pathway including itself through a positive feed-back loop. We have found that Ste11 function is directly antagonized by Cdk-dependent phosphorylation. Ste11 and mhf2, a Ste11-responsive gene, are periodically expressed through the cell cycle. Ste11 protein and mhf2 transcript begin to accumulate at anaphase, persist in G1 and are down-regulated during S-phase. Ste11 contains a single consensus site for Cdc2 phosphorylation at threonine-82 and mutation of this residue to alanine causes constitutive expression of mhf2 and, hence, loss of its cell-cycle regulated transcription. When threonine-82 is changed to an aspartate, mimicking constant phosphorylation, the level of Ste11 protein and mhf2 transcript is downregulated in mitotically growing cells as well as nitrogen starved cells and sexual differentiation is severely impaired. In vitro, Ste11 can be phosphorylated by either Cig2-Cdc2 or Cdc13-Cdc2 complexes. Cdk phosphorylation of Ste11 does not lead to ubiquitination and degradation, but instead to inhibition of its DNA-binding activity. In G2, when Cdc2-cyclin kinase activity is low, unphosphorylated Ste11 can bind its target sequences and, therefore, activate transcription of the genes involved in differentiation. In the rest of the cell cycle, phosphorylation of Ste11 by Cdc2-cyclin complexes impairs the DNA binding activity of Ste11 and, hence, transcription of Ste11 responsive genes. Thus, specific and timely cell cycle-dependent interactions of Ste11 with Cdc2 may contribute to the periodicity of expression of Ste11 and its target genes, thereby restricting sexual development to G1.
Vtc4p: a regulator of vacuolar size and trafficking in *Schizosaccharomyces pombe*

**N. Bone, P. Nurse* and J. Armstrong**

School of Life Sciences, JMS Building, University of Sussex, Falmer, Brighton, BN1 9QG

*The Rockefeller University, 1250 York Avenue, New York, NY10021, USA

The fission yeast *S. pombe* normally has a complement of 40-80 small vacuoles, the equivalent of lysosomes in higher cells. As well as functioning in storage and protein turnover, we have shown that vacuoles are dynamic structures, involved in response to osmotic stress. In many cases, disruption or mutation of genes involved in membrane traffic pathways leads to altered vacuolar morphology: for example, disruption of *yp17*, involved in the last stage of endocytosis, results in vacuolar fragmentation.

From a collection of temperature sensitive strains, we have examined a strain in which the normal vacuolar morphology is replaced by a single, or a small number, of extremely large vacuoles. Cells containing these aberrant giant vacuoles can have difficulty in dividing, but the trafficking pathways of endocytosis and delivery of most vacuolar enzymes appear normal. The affected gene was identified as *vtc4*, which encodes an 83 kD protein with a hydrophilic C-terminus. The homologous protein in *S. cerevisiae* is a member of a complex, for which several functions have been proposed, on the vacuolar membrane. Expression of a GFP-Vtc4p fusion in *S. pombe* confirms localization to the vacuolar membrane, while the mutant phenotype can be rescued by expression of the un-fused gene. Disruption of the vtc4 gene results in an enlarged phenotype indistinguishable from the original mutant. We propose that Vtc4p has a function in regulating vacuolar size. Although the usual trafficking pathways are normal, we found a requirement for Vtc4p in the Cvt pathway, a route of delivery for two vacuolar proteins which overlaps with the mechanism of autophagy.

Sequencing the vtc4 gene from the mutant revealed no change from the database. A stop codon was, however, found to have been introduced in the 3’ region of the adjacent, convergent ade6 locus: the strain is also ade6. RT PCR shows that the levels of both vtc4 and ade6 transcripts are significantly reduced in the mutant line. We found that this single point mutation confers two distinct phenotypes via two adjacent genes.

**NatB N-acetylation activity is required for Bsu1p pyridoxine transporter function in *S. pombe***

**Nam, A., Chua, G., Owens, B., Freitag, S. and R. G. Young**

Department of Biology, Queen’s University, Kingston, ON K7L 3N6, Canada.

Sensitivity to amiloride, a diuretic drug toxic to the fission yeast, *Schizosaccharomyces pombe*, requires the activity of the plasma membrane vitamin B1 transporter, Bsu1p (formerly Car1p). Amiloride is a competitive inhibitor of pyridoxine uptake and thus affects thiamine metabolism and genes affected by it. bsu1 loss-of-function mutants are amiloride resistant due to a presumed failure to transport amiloride into the cell. The internal target(s) of amiloride toxicity in fission yeast have not yet been well-defined (Jia et al., 1993; Niederberger et al., 1996; Stolz et al., 2005).

We have identified a new locus conferring amiloride resistance, termed *arm1* (for amiloride resistant mutant). Apart from growth on amiloride containing plates, the mutant (and its gene disruption) has a distinct temperature-sensitive cell separation phenotype. At 25°C cells grow well but include a population with multiple septa and cell compartments. At 36°C all cells have misaligned thick septa, multiple cell compartments, and fail to form colonies. By sequence similarity, Arm1p is the auxiliary subunit of the NatB*-acetyltransferase complex. The NatA, B and C complexes in budding yeast are reported to acetylate more than half of all proteins. NatB is thought to acetylate all proteins with M-D- or M-E-amino termini as well as a subset of proteins beginning with M-N- and M-M-. Putative substrates of NatB in fission yeast comprise approximately 500 proteins.

Loss of Arm1p results in a failure of pyridoxine transport as assessed by H-pyridoxine uptake experiments. This occurs both at 25°C where the cells grow well and form colonies and at 36°C. Using Bsu1p-GFP expressed chromosomally from its native promoter as a measure of effective transcription, translation and cellular localization, the Bsu1p pyridoxine transporter is expressed in *arm1* disruptions and localizes appropriately to the plasma membrane. By western blotting the Bsu1p-GFP protein is expressed at similar levels in wild-type and mutant backgrounds. Based on the Bsu1p N-terminal sequence, Bsu1p is not a direct substrate of the NatB complex. A presently unknown NatB substrate presumably requires acetylation for Bsu1p transport activities to occur.

Disruption of *arm1* results in a broad range of other phenotypes many of which, based on data from the *S. cerevisiae* system, are likely the result of failure of tropomyosin-actin interaction. Both actin and tropomyosin are NatB substrates in *S. cerevisiae* and mammalian cells and predicted to be by N-terminal sequence in fission yeast. The *arm1* deletion strain displays a disruption of actin cables, the formation of thick disoriented septa and failure to undergo fluid-phase endocytosis. Disruption of the actin cytoskeleton using the temperature sensitive *cdc8* tropomyosin mutant causes a failure of pyridoxine transport. It is possible that the correct function of the transporter although not its membrane localization is dependent directly or indirectly upon a fully functional actin cytoskeleton.
Regulation of *S. pombe* Atf1 protein levels by Sty1-mediated phosphorylation and heterodimerisation with Pcr1

Clare L. Lawrence, Jessica L. Worthington, Hiromi Maekawa, Wolfgang Reiter, Caroline R.M. Wilkinson and Nic Jones

Cancer Research UK, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX

The Atf1 transcription factor plays a vital role in the ability of *S. pombe* cells to respond appropriately to a variety of different stress conditions including nutritional, osmotic and oxidative stress. It regulates the expression of many genes in a stress-dependent manner and its function is dependent upon the stress-activated MAP kinase, Sty1. Previous studies have demonstrated that Atf1 is directly phosphorylated by Sty1. In this study we have investigated the role of such phosphorylation. The level of Atf1 protein accumulates following stress and this accumulation is lost in a strain defective in the Sty1 signalling pathway. In addition, accumulation of a mutant Atf1 protein that can no longer be phosphorylated is lost. Measurement of the half-life of Atf1 demonstrates that this accumulation is due to changes in Atf1 stability. Atf1 stability is also regulated by Pcr1, a heterodimeric partner of Atf1. Similarly, Pcr1 levels are regulated by Atf1. Thus there exist multiple pathways that ensure that Atf1 levels are appropriately regulated. Surprisingly, loss of Atf1 phosphorylation does not result in a significant loss of stress-activated expression of Atf1 target genes, except in the case of oxidative stress. Accordingly, the phosphorylation mutant strain does not display the same stress sensitivities as the atf1Δ mutant. The significance of these findings will be discussed.

A conserved cysteine of Sty1 has an important role in oxidative stress resistance

Alison M. Day, Stephanie M. Bozont, Brian A Morgan & Elizabeth A. Veal

Institute of Cell and Molecular Biosciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK.

In mammalian cells the JNK and p38 families of stress-activated protein kinases (SAPK) are activated in response to a diverse range of stresses. A SAPK homologue in the fission yeast *Schizosaccharomyces pombe*, Sty1 (also known as Spc1 and Phh1) is also activated by phosphorylation in response to a similar range of stresses including oxidative and osmotic stress. The sensing mechanisms involved in the regulation of these SAPK signalling pathways in response to different stresses are not well understood. 2-cysteine peroxiredoxins are thioredoxin peroxidases that in addition to detoxification of peroxides also have roles in peroxide signalling. Indeed, we have shown that the 2-cys peroxiredoxin in *S. pombe*, Tpx1, regulates the Sty1 SAPK pathway via a mechanism involving a peroxide induced disulphide complex between Tpx1 and Sty1. We have identified conserved cysteines in Tpx1 and Sty1 that are essential for formation of a disulphide bond between Tpx1 and Sty1. Using a serine substitution mutant we have examined further the role of this cysteine in Sty1. Here we will present data revealing the mechanism underlying the role of this cysteine in the oxidative stress response. The conservation of this cysteine in proteins homologous to Sty1 suggests that a similar mechanism may regulate other MAPK.
Control of cell size at division in response to nutrient availability.

Janni Petersen 1,2 and Paul Nurse 1

1The Rockefeller University Box 5 1230 York Avenue, New York NY 10021 USA
2University of Manchester, Michael Smith Building, Oxford Rd, M13 9PT, Manchester U.K.
Janni@petersen@manchester.ac.uk

Cell growth and cell cycle progression are generally tightly coupled, allowing cells to proliferate continuously while maintaining their size. Nutritional shift down experiments has shown that cell size at cell division is controlled by size rather than time (Fantes and Nurse 1977). The critical cell size is modulated by the growth conditions and is reset within a short time after changes in these conditions. In all eukaryotes MAP kinase cascades play an important role in modulating signaling to adapt a range of processes, including transcriptional control with changes in the cell environment. In fission yeast, in addition to responding to stresses, the stress MAP kinase pathway (SRP) has a level of constitutive signaling that is required to control the timing of mitotic commitment in response to nutritional availability (Shiozaki and Russell 1995). The Plo1 kinase links the SRP to this control (Petersen and Hagan 2005), because SRP dependent Phosphorylation on Plo1 Serine 402 (S402) targets it to the SPB, where it modulates mitotic entry. We find that mutation of this site to block phosphorylation, or deletion of the stress MAP kinase Sty1/Spc1, stops cells from responding to changes in available nutrients. Furthermore, Sty1 is activated and Plo1 is phosphorylated on S402 in response to nutritional shifts. Thus nutrient signaling impact on Sty1/Spc1 activity that then impacts on Plo1 phosphorylation in order to promote mitosis.

Fantes and Nurse, Exp Cell Research. 107:377-386

Genome-wide Translational Control in Fission Yeast

Daniel H. Lackner 1,
Traude H. Beilharz 2,
Samuel Marguerat 1,
Thomas Preiss 2 and Jürg Bähler 2

1Wellcome Trust Sanger Institute, Fission Yeast Functional Genomics Group, Hinxton, Cambridge, CB10 1SA, U.K.
2Molecular Genetics Program, Victor Chang Cardiac Research Institute, Darlington (Sydney) NSW 2010, Australia

We are interested in global roles of translational regulation and its coordination with other levels of gene expression control.

To obtain translational profiles for all mRNAs, polysome preparations are separated according to their size using a sucrose gradient and the mRNAs in each fraction (or pools of fractions) are identified and quantified with DNA microarrays. Starting with exponentially growing cells, we analyzed 12 polysome fractions using DNA microarrays containing elements for all known and predicted genes of fission yeast. This approach provided data on average numbers of associated ribosomes for most transcripts. Integration with data on mRNA steady-state levels revealed an interesting bias: the most abundant transcripts seem to be associated with many more ribosomes than less abundant transcripts, although ribosome density seems to only correlate weakly with transcript levels. We also found a strong inverse correlation between ribosome density and gene length: shorter genes tend to be much tighter packed with ribosomes than longer genes.

We are now testing whether these trends depend on other mRNA features such as stability or polyA length. To obtain global data on polyA length, transcripts are fractionated using polyU columns and fractions of different tail lengths are quantified with DNA microarrays. Transcript stability is determined by measuring mRNA levels at different times after blocking transcription.

Integration of these varied data sets will provide insight into global mechanisms of post-transcriptional regulation. Moreover, combining translational and expression profiling of cells in different cell-cycle stages or subjected to various genetic and environmental perturbations will provide a genome-wide view of translational regulation in fission yeast, complementing our expression profiling data.
Genome wide roles for histone modifications and RNAi directed chromatin silencing

Karl Ekwall

Karolinska Institutet, Dept of Biosciences/School of Life Sciences, Univ.
College Sodertorn, Alfred Nobel’s Allé 7, S-141 89, Huddinge, Sweden.
E-mail: karl.ekwall@sh.se.
Telephone: +46 8 6084713, Fax: +46 8 6084510.

Histone deacetylases (HDACs) are evolutionary conserved enzymes which are important for transcription and other chromatin related processes. We have conducted a genome wide investigation into the enzymatic specificity, gene expression profiles, and binding locations of four histone deacetylases (HDACs), representing the three different phylogenetic classes in fission yeast (Schizosaccharomyces pombe). By directly comparing nucleosome density, histone acetylation patterns and HDAC binding in both intergenic and coding regions with gene expression profiles, we found that Sir2 (class III) and Hos2 (class I) have a role in preventing histone loss, sir6 (class I) is the principal enzyme in promoter-localized repression. Hos2 has an unexpected role in promoting high expression of growth-related genes by deacetylating H4K16ac in their open reading frames, sir3 (class I) acts cooperatively with sir2 throughout the genome including the silent regions: rDNA, centromeres, mat2/3 and telomeres. The most significant acetylation sites are H3K14Ac for sir3 and H3K9Ac for sir2 at their genomic targets. sir3 also affects subtelomeric regions which contain clustered stress and meiosis induced genes. Thus, this combined genomic approach has uncovered different roles for fission yeast HDACs at the silent regions in repression and activation of gene expression.


In RNA interference (RNAi) Dicer processes double stranded RNA to produce short siRNAs that act post-transcriptionally to silence gene expression. In several organisms including fission yeast, RNAi also mediates transcriptional silencing via formation of heterochromatin. Fission yeast centromeric repeats are transcribed into siRNA precursors (pre-siRNAs), which are processed by Dicer to direct heterochromatin formation. Recently, Rpb1 and Rpb2 subunits of RNA Pol II were shown to mediate RNAi-directed chromatin modification but did not affect pre-siRNA levels. Here we show that another Pol II subunit, Rpb7 has a specific role in pre-siRNA transcription. We define a centromeric pre-siRNA promoter from which initiation is exclusively sensitive to the rpb7-G150D mutation. In contrast to other Pol II subunits which affect downstream events in the pathway, Rpb7 promotes pre-siRNA transcription required for RNAi-directed chromatin silencing.


Comparative Studies of Molecular Mechanisms that Program Transcription.

Fredrik Fagerström-Billai, Anna Johnsson, Yongtao Xue-Franzén, Maria Lundin and Anthony Wright

School of Life Sciences, Södertörns högskola and Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 89 Huddinge, Sweden.

Differential gene expression is an important mechanism by which the functional diversity of different organisms is generated from their relatively conserved genomes. The flexible mechanism by which activator proteins interact with target proteins makes recruitment of transcriptional co-regulator proteins a potentially evolvable process whereby the conserved gene expression machinery could be adapted to perform new tasks during evolutionary diversification (1-3). Co-regulator proteins would thus represent a class of proteins with conserved structure and molecular mechanism but with diverged physiological function.

We are performing comparative studies of co-regulator proteins using S. pombe as a model system for comparison with S. cerevisiae. We have focused on the Gcn5 co-activator and the Tup1-Ssn6 corepressor, both of which have been extensively studied in S. cerevisiae and are required for adaptation of S. pombe to KCl-mediated stress. In S. cerevisiae DNA-bound repressor proteins have been reported to recruit Ssn6 and thereby Tup1, which subsequently represses transcription via interactions with hypoacetylated histones, histone deacetylases (HDACs) and the RNA Polymerase II mediator complex. Gcn5 is a histone acetyltransferase found within the SAGA complex and other related co-activator complexes.

S. pombe is the only fungus that contains two Tup1-like proteins (Tup11 and Tup12) as a result of gene duplication. While Tup11 and Tup12 have redundant functions at some genes we have shown that Tup12 plays a specific role in regulating genes involved in the KCl-stress response (4). Unlike the Tup11 and Tup12, Ssn6 is an essential protein in S. pombe, suggesting the existence of Ssn6-specific target genes. Using genome-wide expression profiling we have identified classes of target genes that are specific for Ssn6 or Tup11/12 as well as a class that requires both components of the co-repressor. Genome-wide localisation studies show that the location of the three proteins at different parts of the genome is highly correlated. Thus the differential requirements for the different co-repressor subunits for repression of different target genes must represent a post-recruitment facet of the co-repressor. Interestingly, recent experiments using tiling arrays show that the co-repressor is independently located in both the promoters (as expected) and coding regions of target genes.

Gcn5 is specifically required for adaptation to KCl- and CaCl2-mediated stress in both S. pombe and S. cerevisiae. Adaptation to KCl-mediated stress involves regulation of overlapping but distinct sets of response genes in the two organisms. Interestingly, genes that require Gcn5 during adaptation to KCl are highly over-represented in the class of
species-specific response genes. Thus the role of Gcn5 in S. pombe and S. cerevisiae is correlated with gene expression programs that have been established since their divergence in evolution.


Comparative proteomic analysis of chromatin related complexes in S. Cerevisiae and S. Pombe

Assen Rogovê1, Daniel Schacht1,3, Anna Shevchenko5, Rein Aasland2, Andrej Shevchenko2, A. Francis Stewart1

1BIOTEC TU-Dresden, Tatzenberg 47-51, 01307 Dresden, Germany
2present address: The Victor Chang Cardiac Research Institute, Level 6, 384 Victoria Street, Darlinghurst NSW 2010, Australia
3Max Planck Institute for Molecular Cell Biology and Genetics, Pforzheimerstrasse 108, 01307 Dresden, Germany
4Department of Molecular Biology, University of Bergen, HIB, P.O. box 7800, N-5020 Bergen, Norway

We have applied sequential epitope tagging and mass spectrometry (SEAM) approach in Saccharomyces cerevisiae and Schizosaccharomyces pombe to determine and compare the proteomic environments of chromatin related protein complexes.

Initially 29 proteins in S. cerevisiae were selected bioinformatically on the basis of presence of chromatin related domains (SET, SANT, chromo, HDACs and Sir2-homology deacetylase proteins). They were then TAP-tagged and purified. Of the initial 29, 14 proteins were found to exist in multi-protein complexes. Co-immunoprecipitating proteins were identified by mass-spectrometry, TAP-tagged and purified themselves. Through this approach a number of proteins were found to be shared between two or more different protein complexes and were termed ‘proteomic hyperlinks’. Eight of these serve as links in a network of protein complexes including the major yeast histone deacetylase complex (Rpd3C), the major H4 acetyltransferase complex (Esa1/NuA4C) and two chromatin remodeling complexes, one of which (Swr1C) is responsible for incorporation of H2A.Z into chromatin.

A similar approach was then applied then in S. pombe. S. pombe was chosen mainly because of its evolutionary distance from budding yeast and vertebrates, its simplicity and ease of handling. The S. pombe homologs of the proteomic hyperlinks from S. cerevisiae were TAP-tagged, purified and protein complex members identified. While this analysis revealed remarkably conserved proteomic cores of the complexes in the two yeasts, important aspects of the network’s topology differed. The network in S. pombe appears to be more complex and more closely related to partial networks derived from higher organisms. To our knowledge this is the first systematic comparative proteomic attempt between different species. The nature of the proteomic hyperlinks connecting complexes of related but seemingly antagonizing functions raises many questions in regard to the targeting and regulation of such important gene expression machinery especially in the light of the recent findings on the role of Eaf3 in H3-K36Me recognition.
Rem1 expression is regulated at the level of transcription and splicing

Alberto Moldón¹, Jordi Malapeira¹, Gerald R. Smith², Paul Nurse³, Elena Hidalgo³ and José Ayte³

¹Cell Signalling Unit. Universitat Pompeu Fabra. Barcelona, Spain. ²Fred Hutchinson Cancer Research Center. Seattle, USA. ³The Rockefeller University. New York, USA.

Gene expression controls at the level of RNA are crucial for meiosis. In the last few years, splicing regulation is emerging as an important key step for regulating different cellular processes, including differentiation program. Here we show that a fission yeast cyclin, Rem1, is present only during meiosis. When rem1 is expressed during mitotic cycle, even at very low level, induces mitotic catastrophes. rem1 expression is regulated at the level of both transcription and splicing. rem1 transcription during meiosis is regulated by forkhead transcription factors, being induced during pre-meiotic S phase and with a peak in transcription before the onset of meiosis I. On the other hand, splicing is regulated independently of transcription, with Mei4 as a positive and Cig2 a negative factor of this process. Furthermore, there are elements in the promoter of rem1 that control the meiotic-specific splicing of this gene.

Genome wide occupancy profile of Mediator and the Srb8-11 module reveals interactions with coding regions

Xuefeng Zhu¹, Marianna Wirén², Indranil Sinha², Nina N. Rasmussen², Tomas Linder², Steen Holmberg², Karl Ekwall², Claes M. Gustafsson²

¹Department of Laboratory Medicine. Karolinska Institute, Novum, S-141 86 Huddinge, Sweden. ²Department of Biosciences/Dept of Natural Sciences, Univ. College Sodertorn, Alfred Nobel's Allé 7, S-141 89, Huddinge, ³Department of Genetics, Institute of Molecular Biology, Oester Farimagsgade 2A, DK-1353 Copenhagen K, Denmark

The Mediator complex supposedly functions as a bridge between gene specific transcription factors and the general transcription machinery at the promoter. Together, CDK8, CycC, Med11, and Med12, are parts of a repressive module (the Srb8-11 module), which prevents RNA polymerase II interactions with core Mediator and that is displaced upon activation of transcription. The Srb8-11 containing Mediator represses basal transcription in vitro and genetic analysis also indicates that the Srb8-11 module is involved in the negative regulation of genes in vivo.

Here we use chromatin immunoprecipitation and DNA microarrays to investigate the genome wide localization of Mediator and the Srb8-11 module in fission yeast. Mediator and the Srb8-11 module display very similar binding patterns, and interactions with promoters and upstream activating sequences correlates with increased transcription activity. Unexpectedly, Mediator also interacts with the downstream coding region of many genes. These interactions display a negative bias for positions closer to the 5’ ends of the open reading frame and appear functionally important, since down regulation of transcription in a temperature sensitive med17 mutant strain, correlates with changes in Mediator binding in the coding region. We propose that Mediator coordinates transcription initiation with transcriptional events in the coding region of eukaryotic genes.
Hip3 interacts with the HIRA proteins Hip1 and Slm9 and is required for transcriptional silencing and accurate chromosome segregation

Amanda Greenall
and Simon Whitehall

Institute of Cell and Molecular Biosciences University of Newcastle, Newcastle upon Tyne, NE2 4HH

HIRA proteins are members of an evolutionarily conserved family of histone chaperones that mediate nucleosome assembly. We have previously demonstrated that the S. pombe HIRA proteins Hip1 and Slm9 are required for the propagation of pericentric heterochromatin and thus accurate chromosome segregation. HIRA proteins are known to function as components of large protein complexes but the composition of these complexes has not been defined. Therefore we have used single-step affinity purification and MALDI-TOF mass spectrometry to identify factors that interact with both Hip1 and Slm9. This analysis identified Hip3, a previously uncharacterised 187 kDa protein, with similarity to S. cerevisiae Hir3. Consistent with this, cells disrupted for hip3” exhibit a range of growth defects that are similar to those associated with loss of Hip1 and Slm9. These include temperature sensitivity, a cell cycle delay and synthetic lethality with cdc25-22. Furthermore, genetic analysis also indicates that disruption of hip3” is epistatic with mutation of hip1” and sim9”. Mutation of hip3” alleviates transcriptional silencing at several heterochromatic loci including in the outer (otr) centromeric repeats, indicating that Hip3 is required for the integrity of pericentric heterochromatin. As a result loss of Hip3 function leads to high levels of minichromosome loss and an increased frequency of lagging chromosomes during mitosis. Importantly, the function of Hip1, Slm9 and Hip3 is not restricted to constitutive heterochromatic loci as these proteins also repress the expression of a number of euchromatic genes.

The conserved kinetochore component Spc7 regulates microtubule-kinetochore association and is linked to the Sim4-complex

Anne Kerres and Ursula Fleig

Lehrstuhl für funktionelle Genomforschung der Mikroorganismen, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany
email: Anne.Kerres@uni-duesseldorf.de

A critical aspect of mitosis is the capture and correct attachment of the kinetochores by the dynamic microtubules (MTs) of the spindle. Attachment is carried out between the plus-ends of MTs and the kinetochore, a proteinaceous structure assembled on the centromeric DNA. A large number of proteins are known to be required for the complex process of association between the kinetochore and the plus-ends of spindle MTs. However the precise interaction partners at the MT-kinetochore interface are poorly understood.

We previously identified the essential kinetochore protein Spc7 as a suppressor of the MT-plus-end localized protein Mal3, which belongs to the highly conserved EB1 family. Spc7 is a conserved protein as homologues were identified from yeast to human (Cheeseman et al., 2004, Genes Dev, 18:2255-68; Kerres et al., 2004, Mol Biol Cell, 15:5255-67; Nekrasov et al., 2003, Mol Biol Cell, 14:4931-46; Obuse et al., 2004, Nat Cell Biol, 6:1135-41).

We showed that Spc7 and Mal3 interact genetically as well as physically and that expression of a dominant-negative variant of Spc7 results in severe defects in the association of kinetochores with MTs. Spc7 associates constitutively with the inner centromeric region in S. pombe but plays no role in the transcriptional silencing of genes placed within this region. The Spc7 protein was found to interact with two conserved kinetochore complexes, namely the Ndc80 and the Mis12 complex; an interaction also conserved in higher eukaryotes (Cheeseman et al., 2004; Obuse et al., 2004; Kerres et al., 2004; Liu et al., 2005, EMBO J. 24:2919-30).

We have recently shown that Spc7 also plays a role in the function of another kinetochore complex, the 12 component Sim4-Mal2-Mis6 complex (Liu et al., 2005). One of the components of the Sim4-Mal2-Mis6 complex was isolated as a suppressor of the non-growth-phenotype of a spc7 temperature sensitive mutant. Furthermore these two proteins can be co-immuno-precipitated. In addition the correct localisation of at least one member of the Sim4-Mal2-Mis6 complex is dependent on the presence of functional Spc7. We therefore propose that Spc7 has a central role in serving as a connecting element between various kinetochore complexes.

What is the role of Spc7 at the MT-kinetochore interface? The Mad2 and Mph1 branches of the spindle checkpoint are both activated in spc7 mutant strains indicating that Spc7 is required for the attachment of kinetochores with MTs as well as bipolar attachment of sister kinetochores to the spindle. Further analysis of various spc7 mutants indicates that the formation and function of the mitotic spindle is severely affected in these strains. Interestingly some of the spc7 mutant phenotypes can be suppressed partially by expression of extra tubulin possibly pointing to a role of Spc7 in MT dynamics. Full-length Spc7 interacts with the plus-end MT protein Mal3, while a truncated version of Spc7 appears to associate with the mitotic spindle in a Mal3 independent way.
Microtubule organisation via regulation of microtubule-associated proteins
TACC/Alp7 and TOG/Alp14 throughout the cell cycle

Masamitsu Sato and Takashi Toda
Laboratory of Cell Regulation, Cancer Research UK, 44 Lincoln’s Inn Fields, London, WC2A 3PX, United Kingdom

Centrosomal protein TACC (transforming acidic coiled-coil protein) and TOG (tumor overexpressed gene) are conserved MAPs that regulate microtubule dynamics in many aspects of cellular phenomena. TACC and TOG form a complex and the localisation of TOG to the centrosome is dependent upon TACC.

In fission yeast, the orthologs of TACC (Alp7) and TOG (Alp14) function throughout the cell cycle. During interphase, Alp7 and Alp14 are required to organise cytoplasmic microtubule structure, and in mitosis, they play key roles in formation of bipolar spindle and establishment of amphitelic microtubule-kinetochore attachment in the nucleus.

Therefore, localisation of Alp7-Alp14 complex must be regulated in order to execute multiple functions at each location. Since yeast cells undergo closed mitosis in which the nuclear envelope does not break down, Alp7-Alp14 complex needs to translocate from the cytoplasm to the nucleus upon mitotic entry and vice versa upon mitotic exit.

Here we show that Alp7/TACC possesses an ability to actively transport Alp7-Alp14 complex into the nucleus. Surprisingly, nuclear import of Alp7-Alp14 occurs not only during mitosis but also even during interphase, in which Alp7-Alp14 complex is exported to the cytoplasm. During mitosis, cells are utilising molecular schemes which accumulate Alp7-Alp14 in the nucleus effectively. Nucleocytoplasmic shuttling of microtubule-associated proteins TACC-TOG via the Ran GTPase system is an efficient way to regulate both interphase and mitotic microtubules in a spatial and temporal manner.

Regulatory circuit of TACC-TOG as a possible cargo of Ran might be conserved as a mechanism underlying microtubule dynamics and spindle formation.

Interactions between the spindle checkpoint proteins and the Anaphase Promoting Complex in Schizosaccharomyces pombe

Matylda Szaniecka and Kevin Hardwick
Wellcome Centre for Cell Biology, University of Edinburgh, Kings Buildings, EH9 3J

The spindle checkpoint monitors the metaphase – anaphase transition by ensuring correct microtubule – kinetochore attachment. In the case of an unattached kinetochore or if the tension between microtubule and kinetochore is not sufficient, the kinetochore emits a signal which is recognised by the components of the spindle checkpoint. This results in a series of interactions between the checkpoint proteins (Mad1, Mad2, Mad3, Bub1, Bub3, Mph1) and several complexes are formed. This, in turn leads to the inhibition of the Anaphase Promoting Complex (APC), accumulation of mitotic proteins and anaphase delay. APC is a multisubunit ubiquitin ligase, which acts as an E3 enzyme in the ubiquitin pathway. By attaching polyubiquitin chains onto its substrates it targets them for degradation by the 26S proteasome. In mitosis APC is responsible for targeting for degradation anaphase inhibitors: securin and cyclin B.

It is not clear how the checkpoint proteins inhibit APC from degrading its substrates. One possibility is that they bind directly to the APC and block its active sites, either by inducing a conformational change or by competing with its substrates.

There are however many other possible mechanisms, such as binding and blocking of the APC activator Cdc20 which has been shown to form a complex with checkpoint proteins such as Mad2 and Mad3. Changes in the status of Cdc20/APC phosphorylation may also be important.

We are currently looking at one of those mechanisms: the direct binding of Mad and Bub proteins to the APC in fission yeast Schizosaccharomyces pombe. We have found that Mad2 and Mad3 proteins coimmunoprecipitate with Lid1-TAP subunit of the APC in a mitotic arrest. This interaction is partially dependent on the APC activator protein – Slp1 (Cdc20 homologue). Moreover it seems to be dependent on specific motifs found in Mad3 protein, the KEN-boxes.

KEN-boxes are known to serve as recognition signals for APC – mediated degradation, similar to D-boxes. We are investigating the possible role of these motifs in mediating the inhibitory protein binding. Our results suggest that one of the Mad3 KEN-boxes is not only important for the interaction of the spindle checkpoint proteins with the APC, but it is also required to form the mitotic checkpoint complex (MCC), which is necessary for proper checkpoint function. In fact, both of the KEN motifs in Mad3 seem to be required for inducing a checkpoint – dependent metaphase arrest.

Further studies in this area will lead to a better understanding of the ways in which this important mechanism (the spindle checkpoint) prevents cells from missegregating their chromosomes.
Dissociation of mitotic spindle position from the timing of anaphase onset in fission yeast

John C. Meadows
and Jonathan B.A. Millar

Division of Yeast Genetics,
National Institute for Medical Research,
The Ridgeway, Mill Hill,
London NW7 1AA, UK

It has previously been proposed that in fission yeast mitotic spindle position is monitored by a checkpoint that controls the timing of anaphase onset. This checkpoint is activated by treatment of cells with Latrunculin A, an inhibitor of actin polymerisation, and requires a subset of spindle assembly checkpoint proteins including Bub1, Bub3, Mad3 and Mph1. We find that cells lacking Mto1, a centrosomin-like protein, have mis-positioned spindles but are not delayed in the timing of sister chromatid separation. Instead we find that Latrunculin A delays the onset of anaphase and causes mitotic spindles to collapse. This effect is exacerbated in cells lacking Ase1, a microtubule associated protein (MAP), which stabilises anti-parallel spindle midzone microtubules. These results suggest that Latrunculin A delays the onset of anaphase by destabilising formation of a bipolar mitotic spindle rather than by causing spindle mis-orientation.

Mechanism Controlling Perpendicular Alignment of the Spindle to the Axis of Cell Division in Fission Yeast

Gachet Y', Reyes C', Goldstone S', Hyams J', Tournier S'.

'LBMC-CNRS UMR5088,
Institut d'Exploration Fonctionnelle des Géonomes (IFR109), Université Paul Sabatier, 118 route de Narbonne,
31062 Toulouse, France

Institute of Molecular Biosciences,
Massey University, Private Bag 11 222,
Palmerston North, New Zealand.

In animal cells, the mitotic spindle is aligned perpendicular to the axis of cell division. This ensures that sister chromatids are separated to opposite sides of the cytokinetic actomyosin ring (CAR). We show that, in fission yeast, spindle rotation is dependent on the interaction of astral microtubules with the cortical actin cytoskeleton. Interaction initially occurs with a region surrounding the nucleus, which we term the astral microtubule interaction zone (AMIz). Simultaneous contact of astral microtubules from both poles with the AMIz directs spindle rotation and this requires both actin and two type V myosins, Myo51 and Myo52. Finally, we show that disruption of the actin cytoskeleton is monitored by a checkpoint that regulates the timing of sister chromatid separation. We find that whereas sister kinetochore pairs normally congress to the spindle midzone before anaphase onset, this congression is disrupted when the actin cytoskeleton is disturbed. By analyzing the timing of kinetochore separation, we find that this anaphase delay requires the Bub3, Mad3, and Bub1 but not the Mad1 or Mad2 spindle assembly checkpoint proteins. In agreement with this, we find that Bub1 remains associated with kinetochores when actin is disrupted. These data indicate that, in fission yeast, the integrity of the actin cytoskeleton is monitored by a subset of spindle assembly checkpoint proteins.
Optical tweezers in fission yeast: Mechanism of nuclear and septum positioning

Isabel Raabe¹, Leonardo Sacconi², Francesco Pavone³, Iva Tolic-Nerelykke⁴

¹Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany
²European Laboratory for Non-linear Spectroscopy, Via Nello Carrara 1, 50019 Sesto Fiorentino (Florence), Italy

Cells of Schizosaccharomyces pombe have a centrally placed nucleus and divide by fission at the cell center. Microtubules are required for the central position of the nucleus. Genetic studies suggested that the position of the nucleus may determine the position of the septum. Alternatively, the septum may be positioned by the spindle, or by morphogen gradients or reaction diffusion mechanisms. We investigated the role of microtubules in nuclear positioning, as well as the role of the nucleus in septum positioning, by displacing the nucleus with optical tweezers. A displaced nucleus returned to the cell center by the pushing force exerted by microtubules against the cell tips. Nuclear displacement during interphase or early prophase resulted in asymmetric cell division, whereas displacement during prometaphase resulted in symmetric division as in unmanipulated cells. These results suggest that the division plane is specified by the pre-dividing nucleus. Since the yeast nucleus is centered by microtubules during interphase but not in mitosis, we propose that the establishment of the division plane at the beginning of mitosis is an optimal mechanism for accurate symmetric division in these cells.

Generating cell form in fission yeast

Stefania Castagnetti¹, Béla Novák¹,² and Paul Nurse⁴

¹Cancer Research UK, 44 Lincoln’s Inn Fields, London, WC2A 3PX, UK
²Molecular Network Dynamics Research Group of Hungarian Academy of Sciences and Budapest University of Technology and Economics, 1111 Budapest, Gellert ter 4, Hungary
³Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Understanding how cellular form is generated and maintained requires the identification of mechanisms acting beyond direct intermolecular interactions. We have investigated the activation of a new growth zone in the middle of a normally rod-shaped fission yeast cell. The positioning of growth zones is regulated by the polarity marker Tea1 delivered by microtubules to cell ends. In the absence of microtubules, a new growth zone is activated near the nucleus in the middle of the cell. This only occurs when the nucleus is a sufficient distance from growing ends because these ends exert lateral inhibition over new growth zone activation. A two component morphogenetic mechanism for activating a new growth zone is proposed: a Tea1-microtubular delivery component that positions a second spontaneous symmetry breaking module which includes the lateral inhibitory component acting nearby existing sites of growth.
Control of growth polarity upon perturbed DNA replication

Muneyoshi Kanai1, Takashi Toda1 and Dai Hirata1

1Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi Hiroshima 739-8530, JAPAN
2Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, PO Box 123, 44 Lincoln’s Inn Fields, London WC2A 3PX, UK

In fission yeast, at a specific point in the G2 phase, growth polarity switches drastically from monopolar to bipolar. This phenomenon is called NETO (New End Take Off). For NETO to take place, two requirements have to be fulfilled, a critical cell size and completion of DNA replication.

However, the mechanism underlying NETO remains to be understood. Here we show that S-phase checkpoint pathway is required for the maintenance of the monopolar growth in the pol1/mon7 mutant. Indeed, the over-expression of either Rad3 or Cds1 kinase was able to inhibit the switch to bipolar under the normal growth condition. Further, the activity of the Dyrk family Pom1, essential for the switch to bipolar, in the pol1 mutant was higher than that in the G1-arrested cdc10 mutant with monopolar growing manner. These results suggest that Rad3-Cds1 kinases coordinate NETO with completion of DNA replication by regulating a molecule(s) that acts downstream of Pom1.

Rgf1p is a specific Rho1-GEF that coordinates cell polarization with cell wall biogenesis in fission yeast.

Patricia García, Virginia Tajadura and Yolanda Sánchez

Instituto de Microbiología Bioquímica, CSIC/Universidad de Salamanca and Departamento de Microbiología y Genética, Universidad de Salamanca. Campus Miguel de Unamuno. 37007, Salamanca, Spain. E-mail: ysm@usal.es

In order to maintain intracellular osmolarity and to produce cell shapes other than spheres, cell wall expansion must be focused on particular regions. Fission yeast is a useful model system for studying cell wall biosynthesis and how this fits in the complex morphogenetic processes required for the cell shape to be attained.

Schizosaccharomyces pombe cell wall is an extracellular matrix consisting of an outer layer of glycoproteins and an inner layer of carbohydrate polymers. It has been strongly suggested that (1,3)-β-glucan polymer is synthesized first, followed by the addition of other components. The enzymatic system that catalyzes the synthesis of this polysaccharide is β(1,3)-glucan synthase (GS). GS is composed of at least two fractions: the catalytic moiety of the enzyme (encoded by the bgs family of genes) and the regulatory component (Rhō1p). Rhō1p acts as a binary switch by cycling between an inactive GDP-bound and an active GTP-bound conformational state and stimulates GS in its GTP-bound prenylated form. Rhō1p regulates cell integrity by controlling the actin cytoskeleton and cell wall synthesis.

We identified a new GEF (Guanine nucleotide Exchange Factor), named Rgf1p, specifically regulating Rhō1p during polarized growth.

rgf1Δ cells are defective in cell integrity and lyse with a phenotype similar to cells devoid of Rhō1 or Pck1/2 activity. In addition, cells deleted for rgf1Δ display a defect in actin organization during bipolar growth.

Overexpression of rho1Δ suppressed the rgf1Δ phenotypes while deletion of rgf1Δ suppressed the severe growth defect in a Rhō1-GAP null mutant (rgf1Δ). Rgf1p interacts functionally with, and acts as a positive regulator of Rhō1 increasing the amount of GTP-bound Rhō1p and the β-1,3-glucan synthase activity. Rgf1p localized to the growing ends and the septum, where Rhō1p is known to function. Our results suggest that Rgf1p probably activates the Rhō functions necessary for coordinating actin deposition with cell wall biosynthesis during bipolar growth, allowing the cells to remodel their wall without risk of rupture.
Spatial regulation of mid1p cortical distribution by pom1 kinase

Séverine Morizur* and Anne Paoletti*

*UMR144 du CNRS, Institut Curie, 26 rue d’Ulm 75248 PARIS Cedex 05 France.
paoletti@curie.fr

In fission yeast, a major determinant for the division plane placement is mid1p, which defines a medial cortical compartment in interphase and recruits myosin II heavy chain myo2p at the onset of mitosis. This event initiates contractile ring assembly in a medial position and ensures the production of equally sized daughter cells after cytokinesis. How mid1p anchoring is restricted to the medial cortex is not understood. We report that in pom1 mutants, mid1p is not restricted to the medial cortex but redistributes towards one pole of the cell. Coupling between mid1p localization and nuclear position is abolished. The asymmetric cortical distribution of mid1p negatively correlates with the monopolar growth pattern of pom1 cells. It accounts for the displacement of the contractile ring towards the non-growing tip during mitosis, while mid1p dissociation from the contractile ring and region of septum formation at mitosis exit is not perturbed. Similar defects in mid1p distribution were not found in other NETO mutants like tea1Δ, tea3Δ or bud6Δ. We conclude that pom1 kinase has a specific role in restricting mid1p distribution from the non-growing cell tips. Our data also identify a pom1-independent mechanism that prevents mid1p association with the growing tips.

Characterization of the fission yeast CLASP, Peg1

Agnes Grallert, Christoph Beuter*, Inga Karig*, Deepti Wilks, Steve Bagley*, Rachel Craven*, Ursula Fleig*, Iain M. Hagan

Cell Division, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, *Heinrich-Heine-Universität; Lehrstuhl für funktionelle Genomforschung der Mikroorganismen, Universitätsstrasse 1, 40225 Düsseldorf, Germany, *Advanced imaging facility, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX

The +TIPs CLIP170, CLASP and EB1 accumulate at the plus end of microtubules where they control microtubule dynamics and mediate interactions with specific targets. We identified fission yeast CLASP in a screen for mutations that compromised spindle formation and another for molecules that antagonised EB1 function. As expected Peg1 associated with kinetochores, however, analysis of the interphase function of Peg1 with the conditional mutant, peg1.1, identified some novel and important properties. First Peg1 flipped from stabilizing mitotic microtubules to de-stabilising interphase microtubules. Second, Peg1 was required to slow the polymerization of interphase microtubules that had established end on contact with the cortex at cell tips. Third, S. pombe CLASP antagonized CLIP170 (Tip1) and EB1 (Mal3) because microtubule bundles were longer and brighter in peg1.1 and peg1Δ cells, while they are unusually short and unstable in cells that lack Mal3 or Tip1 function. Fourth, although Peg1 resembled higher eukaryotic CLASPs by physically associating with both Mal3 and Tip1, neither of these +TIPs were required for Peg1 to de-stabilise interphase microtubules. Nor did Peg1 require Mal3 or Tip1 to bind microtubules. Further, Mal3 and Tip1 do not require Peg1 to associate with microtubules or cell tips. Consistently, while deletion of either mal3 or tip1 disrupts the linear cell growth, ablation peg1 has no effect. We are currently investigating the mechanism by which Peg1 affects interphase microtubules.
Identification of an MTOC-localisation signal domain in Mto1

Itaru Samejima, Sergio Rincon*, and Ken Sawin

Wellcome Trust Centre for Cell Biology, University of Edinburgh, United Kingdom

*visiting from Instituto de Microbiologia Bioquímica, Universidad de Salamanca, Spain

The microtubule cytoskeleton is a dynamic system that creates various types of microtubule arrays under different cellular states. One of the strategies employed to define a particular shape of microtubule cytoskeleton is to restrict microtubule nucleation to specific intracellular sites. mto1Δ (microtubule organizer 1) is central to all cytoplasmic microtubule nucleation, from both spindle pole body (SPB) and non-SPB sites. mto1Δ has been shown to recruit gamma tubulin complex to these sites, and new cytoplasmic microtubules are no longer created in the mto1Δ mutant. We have now identified a small domain that is necessary to target Mto1 to potential sites for microtubule organising centres (MTOCs). Unlike the wild-type protein, which is present at all MTOCs, a mutant lacking the domain was absent from the equatorial MTOC (eMTOC) and the SPB. Fine mapping of the domain suggests that the localisation signals to the eMTOC and SPB involve two distinct but adjacent subdomains, and that different mechanisms operate during interphase and mitosis to establish SPB localisation. Imaging of GFP-tubulin and wild-type and mutant Mto1-GFP revealed a strong correlation between mto1p (mis)localisation and sites available for microtubule nucleation. It is known that many proteins at the SPB constitute the septation initiation network (SIN), a signal transduction pathway that organizes events necessary to complete cell division. We will discuss regulation of Mto1 localisation in relation to the SIN pathway, which is also required for eMTOC formation.

Cell growth and cell cycle: Cdc2 meets ribosome biogenesis.

Peter Stansfield, Margaret Harley, Fredrik Berklund, Jerome Wuarin

Biomedical Research Centre, Ninewells Hospital, University of Dundee, Dundee DD1 9SY

In a growing population of cells, cell growth and cell cycle have to be coordinated to guarantee the maintenance of cell size. How this coordination is regulated is still not clear. Recent discoveries have indicated that ribosome biosynthesis plays a critical role in establishing critical cell size thresholds at which cell cycle transitions can occur.

The synthesis of ribosomes uses up vast amounts of the resources in rapidly growing cells, and is emerging as a key control point for the regulation of cell growth and division, in yeast and human cells.

In all organisms, during ribosome biosynthesis, a polycistronic pre-ribosomal RNA (pre-rRNA) transcript is spliced to the mature 18S rRNA component of the 40S subunit and to the 5.8S and 25S rRNA component of the 60S subunit. This pathway turns out to be very complex in eukaryotic cells and requires large number of trans-acting factors. In the budding yeast *Saccharomyces cerevisiae*, around 170 proteins and around 70 small nucleolar RNAs have been shown to participate in the post-transcriptional steps of ribosome subunit synthesis, in addition to the 80 ribosomal proteins and the rRNAs themselves.

We have isolated and analysed complexes containing the major CDK in fission yeast, Cdc2. We have identified the proteins associated with cdc2 using a new proteomics technology termed iTRAQ. We identified cdc2 and associated cyclins, the ORC complex and also many proteins controlling ribosome biogenesis. Mutations in some of these proteins cause cell cycle defect, indicating that in addition to controlling ribosome biogenesis, these proteins play a critical role in cell cycle regulation. We propose that cdc2 interaction with ribosome biogenesis proteins is a key control point in the coordination of cell growth and cell cycle.
A novel checkpoint reveals a coupling between general cell growth and the cell cycle


Department of Cell Biology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet HF, Montebello, 0310 Oslo, Norway and Department of Zoology, University of Oxford, UK.

We have shown that fission yeast cells delay entry into S phase after UV irradiation in G1 (1). The G1 delay does not depend on any of the classical checkpoint proteins Rad3, Cds1 and Chk1. Consistently, and in contrast to other classical checkpoints, Cdc2 is not phosphorylated during the course of the delay. Our recent data suggest that the G1 delay is caused by a novel checkpoint mechanism linking translation and cell cycle progression. We report here that UV irradiation activates the kinase Gcn2, which phosphorylates eIF2α, a key protein in the regulation of translation. When Gcn2 phosphorylates eIF2α, translation is inhibited and preparation for DNA replication (formation of the pre-replication complex) is delayed. The checkpoint is totally dependent on Gcn2. Surprisingly, all components required for formation of the pre-replication complex are synthesized on time even in UV-irradiated cells, suggesting that an active mechanism is inhibiting entry into S phase. In a gcn2 mutant, there is no reduction in the translation rate and the UV-induced cell cycle delay is abolished. Possibly, phosphorylation of eIF2α, and not necessarily the reduced translation, is required for the UV-induced G1 delay. In a non-phosphorylatable eIF2α mutant, the formation of pre-RC is not delayed. However, UV irradiation does repress translation in this mutant, suggesting an eIF2α-independent pathway for Gcn2-dependent regulation of translation. We are investigating further how the translation machinery generates a signal to regulate cell cycle progression.


Mechanisms of the APC/C-dependent ubiquitylation and proteolysis

Hiro Yamano, Michelle Trickey, Margaret Hanwell and Yuu Kimata

Marie Curie Research Institute, The Chart, Oxted, Surrey, RH8 0TL, U.K.

The ubiquitin pathway is an ATP-dependent tagging system for protein degradation. The anaphase-promoting complex/cyclosome (APC/C) is an essential E3 ubiquitin ligase that ubiquitylates numerous proteins at specific times in the cell cycle. Although the broad outlines of the APC/C’s regulation are understood, many important questions about this large ubiquitin ligase complex remain unanswered. We are studying the molecular mechanisms by which the APC/C recognises substrates and ubiquitylates them for degradation. We have set up APC/C-Fizzy-dependent and APC-Fizzy-related dependent destruction assays in frog egg extracts and using them we have identified several new APC/C substrates in fission yeast. To investigate receptor(s) that specifically bind to the substrates, we use a site-specific photo-crosslinking technique. We show that the APC/C or Fizzy family protein binds to the substrate, but the receptor is not exclusive to either the APC/C or Fizzy, suggesting that cells use different receptors depending on the substrates. We will discuss how the APC/C recognises different substrates with latest data.
The Domain of Securin Required for Stabilization and Inhibition of Separase Can Have a Separase Cleavage Site by Substitutions

Koji Nagao and Mitsuhiro Yanagida

Initial Research Project, Okinawa Institute of Science and Technology, and Graduate School of Biostudies, Kyoto University, Japan

Securin-separase complex is required for sister chromatid separation. Securin degrades in an APC/cyclosome dependent manner at anaphase. Separase is activated upon the destruction of securin and cleaves cohesin. Fission yeast securin/Cut2 required for proper separase localization has the motifs for destruction and separase-binding at the N- and C-termini, respectively. Here we show the third essential domain, which becomes toxic when the 76-amino acid fragment (81-156) in the middle of securin/Cut2 is overproduced.

The fragment inhibits separase, while separase is recruited normally and securin is destructed. It may interfere the separase activation after securin destruction. If the 177-DIE 179 stretch in Cut2 is substituted to AIA, the fragment toxicity and the full-length function are abolished. The Cut2 fragment is physically interacted with separase. Interestingly, Cut2 is cleaved in a separase dependent manner if the cleavage consensus by separase is introduced following the DIE sequence. These finding is consistent with our proposed model that the DIE region in Cut2 may mimic the cleavage site of separase and inhibit the activation of separase as a pseudo substrate. A temperature sensitive mutation cut1-K73/separase isolated by its specific resistance to the fragment toxicity resides in the superhelical region of securase, suggesting that the catalytic site and the helical region in securase may cooperate for activation.

The mechanism of cell cycle progression through meiosis I by the meiosis-specific forkhead transcription factor Mei4p in fission yeast


Department of Biochemistry and Cell biology
Nagoya City University Graduate School of Medicine

In most eukaryotes, the Cdc2p kinase complexed with the B-type cyclin plays a central role in the control of entry and progression through mitosis and meiosis. The Cdc2p protein kinase is fully activated at the onset of mitosis and meiosis by dephosphorylation of Cdc2p tyrosine-15. Phosphorylation of Cdc2p on tyrosine-15 is catalysed mainly by the Wee1p kinase, and dephosphorylation is carried out mainly by the Cdc25p phosphatase. The meiosis-specific forkhead-type transcription factor Mei4p is required for expression of many genes which are necessary for recombination and sporulation. Mei4p can bind heptamer sequence (GAAYAYA) designed FLEX. mei4 deleted cells completely arrest before meiosis I after meiotic DNA replication. The mechanism of cell cycle arrest in mei4 cells is unknown at present. We have recently found that Cdc2p on tyrosine-15 phosphorylation was maintained in mei4 cells.

We show here that dephosphorylation of Cdc2p tyrosine-15 by overexpression of cdc25+ or inactivation of wee1+ induced meiosis I in mei4 deleted cells. The level of cdc25+ mRNA was high during meiosis I whereas it was low in mei4 cells. In contrast, the level of wee1+ mRNA was low during meiosis I, whereas it was maintained in mei4 cells. There are four FLEXs around cdc25+, while there are five FLEXs near wee1+. Electro mobility shift assays showed that Mei4p bound all of FLEX around cdc25+ and wee1+ in vivo. Chromatin immunoprecipitation assays also showed that Mei4p bound most of the FLEXs around cdc25+ and wee1+ in vitro. Finally, in cells deleted four FLEXs around cdc25+, entry into the meiotic nuclear division was severely delayed and cdc25+ mRNA level was low. In addition, overexpression of mei4+ induced meiotic nuclear division earlier by inducing cdc25+ mRNA and reducing wee1+ mRNA.

These results suggest that Mei4p is a rate-limiting regulator of meiosis I by activating cdc25+ and inhibiting wee1+ transcriptions.
Suppressor mutants of the meiosis I arrest caused by loss of the Mei2p dot

Akira Yamashita¹, Ryo Iwata¹
and Masayuki Yamamoto¹²

¹Molecular Genetics Research Laboratory, ²Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan

In fission yeast, an RNA-binding protein Mei2p is crucial for induction and promotion of the meiotic cell cycle. Mei2p forms a dot structure in the horse-tail nucleus during meiotic prophase. The Mei2p dot is located at the sme2 locus on chromosome II. The sme2 gene encodes a non-coding RNA, meiRNA, which binds to Mei2p and is essential for progression of meiosis. If cells lack meiRNA, they cannot form the Mei2 dot and arrest prior to meiosis I. Recently, we have found that this dot structure, composed of Mei2p and meiRNA, sequesters another RNA-binding protein named Mmi1p. Mmi1p binds to a group of meiosis-specific transcripts and renders them unstable during the mitotic cell cycle. The meiotic arrest due to the loss of Mei2p dot can be rescued by a reduction in Mmi1p activity (Harigaya, Y. et al., submitted). However, the molecular mechanism of mRNA destabilization by Mmi1p remains elusive. To isolate factors that may function in the same regulatory pathway as Mmi1p, we set out to screen for suppressor mutants of the meiotic arrest of the sme2Δ mutant, which cannot form the Mei2p dot. We obtained several candidates, cloned the responsible genes, and identified factors implicated in the regulation of poly(A) tail generation.

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Poster Abstracts

Bioinformatics, Genomics and Tools

Phenotype and Gene Ontology in Fission Yeast

Tim Beck and John Armstrong

School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK.

Traditionally, most biological data is not recorded within any particular framework or ontology. An ontology is a structured vocabulary consisting of a hierarchy of terms where the terms are precisely defined and relate to each other in meaningful ways. The most prevalent biological ontology is the Gene Ontology (GO). GO has been utilized to unify the terminology used between model organism databases in three domains of knowledge: molecular function, biological process and cellular component. The University of Sussex is host to FYSSION, a community resource that makes available Schizosaccharomyces pombe mutant libraries. At present mutant phenotypes are classified by free-text keywords. This hinders database data entry and querying since the terminology used is ambiguous. There is therefore the need to develop an ontology where phenotypes can be precisely described without the chance of misinterpretation. Work is underway at Sussex to develop an ontology that is specific to mutant yeast phenotypes, so allowing the capture of more granular data and thus allowing more specific database searches. During the ongoing process of phenotype classification it has been decided to draw a distinction between phenotypes observable at the colony level and those at the cellular level. This has tentatively formed the basis for two separate ontologies that are used to describe structural phenotypes within each domain. Colony phenotypes contain high-level categories relating to differentiation events (e.g. ability to mate or form hyphae) and sensitivity events (e.g. growth after exposure to differing environmental conditions). Cellular and organelle morphologies form the highest order categories for cellular phenotypes. The process of defining lower level terms and relationships is in progress using phenotype data available from FYSSION and from published work. It is expected that during the compilation of the phenotype ontology there will be an overlap with terms defined in GO. It is intended that where possible the GO terms will be incorporated to avoid terminology redundancy.

Bioinformatic prediction of functional linkage applied to fission yeast proteins

Daniel Barker*, Valerie Wood¹, Andrew Meade¹ and Mark Pagel¹

¹Sir Harold Mitchell Building, School of Biology, University of St Andrews, St Andrews, Fife, KY16 9TH, U.K.
²The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, U.K.
³AMS Building, School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, U.K.

*Corresponding author, email db6@st-andrews.ac.uk

We describe a recent bioinformatic method to predict functional linkage among proteins, using patterns of gene presence and absence across several species’ genomes (Barker & Pagel 2005). The method detects independent instances of the correlated gain or loss of pairs of genes. Once one gene coding for a component of a functional unit (e.g. structural complex or biochemical pathway) has been lost from a genome, we assume the remaining components of the unit may be under reduced selective pressure and may also be rapidly lost during evolution. The method achieves 100% specificity (i.e. zero false positives) at stringent cut-offs (Barker & Pagel 2005).

The method requires a summary of gene presence and absence in the genomes of several species (‘phylogenetic profiles’ for the genes in the sense of Pellegrini et al. 1999), and a phylogenetic tree relating the species (e.g. derived from nucleotide or protein multiple alignments). For fully sequenced genomes, gene presence/absence data may be obtained bioinformatically, for example by sequence similarity searches on genome-wide peptide sets. Maximum likelihood models of trait evolution (Pagel 1994) are then fitted to each pair of genes, to detect whether there is statistically significant correlation in gene gain/loss on the species’ phylogenetic tree.

Existing functional annotation is not required by the method, which derives pairs of functionally associated genes on the basis of gene presence/absence and the phylogenetic tree only. Thus the method is complementary to bioinformatic approaches that use sequence analysis or phylogenetics to share functional annotation of a known protein among its homologues or orthologues. The method holds promise for improved functional annotation of the Schizosaccharomyces pombe genome. We present a case study using S. pombe, 14 other ascomycetes, three other fungi and three animals, in which a novel functional association is predicted between the products of S. pombe gene sen34 (coding for probable tRNA-splicing endonuclease subunit Swiss-Prot O60156, containing PFAM domain PF01974) and an S. pombe gene of unknown function (protein O13890, containing domain of unknown function PF02696). The strong correlation in losses of these two genes (p < 0.005) is strong evidence of functional linkage between their products, and more generally between protein domains PF01974 and PF02696.

Does your species show in GO? Gene Ontology annotation outreach.

Jennifer I. Clark and Midori Harris

GO, EMBL-EBI, WT Genome Campus, Hinxton, CB10 1SD, United Kingdom

The best known goal of the Gene Ontology (GO) project is the development of controlled vocabularies for the description of attributes --molecular function, biological process, and cellular component -- of genes and gene products. The usage of GO terms in database annotations is an indispensable aspect of the project; GO annotations are now available for over 30 genomes, with recent additions including chicken and several prokaryotes. To improve annotation coverage further, the GO Consortium has recently begun an effort to actively support new groups seeking to use GO for gene product annotation, and to make the resulting data available to the public. GO Consortium members offer annotation tutorials at Consortium-wide courses ('Annotation Camps'), courses run by individual member databases, and conferences; several databases make annotation tools publicly available. Individual database curators may also learn directly from ‘mentors’ with extensive experience using the GO system. In the future, the GO Consortium plans to develop tools and communication routes to enable individual researchers to contribute annotations for their area of expertise.

Saccharomyces pombe 70-mer Genome Set

Malcolm Cook, Madelaine Marchin, and Chris Seidel

Stowers Institute for Medical Research
1000 E 50th St
Kansas City, Mo 64110
corresponding author:
cws@stowers-institute.org

Abstract: We have designed a publicly available genomic reagent for producing DNA microarrays to measure gene expression in S. pombe. The set currently contains 8785 probes and consists of sequence optimized 70-mer oligonucleotides which can be synthesized and printed on glass slides using conventional techniques. While S. pombe has approximately 5000 genes, our approach to probe design was to consider exons within 1000 bases of the 3' end of each gene as primary design targets. If exons were not available, or if they were shorter than 80 bases, then the terminal 1 kb of the gene was used as a design target. The result is 6918 longmer oligonucleotides for detecting coding sequences or exons, in which many genes are assayed by more than one probe. The purpose of the 3' bias is to compensate for the inefficiency of reverse transcriptase labeling of cDNA. In addition, the set contains probes to detect 333 3' UTRs and 1521 introns. The sequences are publicly available. The genome set will make genomic investigations into S. pombe gene expression more accessible to the community.
Imaging and Functional Analysis of 4,100 Schizosaccharomyces pombe Temperature Sensitive Mutants

James Dodgson and John Armstrong

Department of Biochemistry, University of Sussex, Brighton, Sussex, BN1 9QG

The FYSSION resource incorporates two libraries of Schizosaccharomyces pombe mutant strains, one affecting temperature-sensitive essential genes the other non-essential genes. A microscopy platform was developed for the imaging of 4,100 temperature-sensitive strains stained with the vacuolar stain CDCFDA and the endocytic marker FM 4-64. A range of morphological mutants were identified including strains affected in vacuole biogenesis and in cell shape; in addition a strain that forms invasive hyphae on rich medium was discovered. Further functional analysis of the morphological mutants has centred on describing the mutant phenotypes in greater detail and in complementing the strains with a plasmid based genomic library. A classical genetic approach involving mapping mutations of interest to markers is underway.

ProbeExplorer: a transcriptomic web-tool that includes S. pombe together with other genomes and the mapping of microarrays oligo probes to transcripts

Alberto De Luis and Javier De Las Rivas

Bioinformatics and Functional Genomics Research Group, Cancer Research Center (CIC, USAL-CSIC), E37007 Salamanca, Spain. (jrlivas@usal.es, aldeluis@usal.es)

ProbeExplorer is an open access web-based bioinformatic tool designed to show the association between Affymetrixmicroarray oligonucleotide probes and transcripts in a genomic context, but flexible enough to serve also as a simplified genome and transcriptome browser. Coordinates and sequences of the genomic entities (loci, exons, transcripts), including vector graphics outputs, are provided for fifteen metazoa organisms and for two yeasts (S. cerevisiae and S. pombe). Alignment tools are used to built the associations between the Affymetrixmicroarray probe sequences and the transcriptomes for human, mouse, rat and yeasts. Search by keywords is available and user can also do searches and alignments on the genomes introducing any DNA or protein sequence query using an incorporated BLAST2 tool. ProbeExplorer uses ENSEMBL (v.36) data for the metazoa genomes, GeneDB data for the S. pombe genome and SGD data for the S. cerevisiae genome. In this way the tool integrates in a single database the fission yeast genomic and transcriptomic data to allow search and comparative analysis with the other genomes. Also specific to yeasts, ProbeExplorer includes the mapping of all probe sequences from YEAST2 and Ygs08 Affymetrixexpression GeneChips on S. cerevisiae and S. pombe loci.

See Web page: http://probeexplorer.cicancer.org/
Comparing haploinsufficient mutants between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* through Gene Ontology (GO) functional analysis

Sangjo Han, Minho Lee and Dongsup Kim*

* To whom correspondence should be addressed at: Department of BioSystems, Korea Advanced Institute of Science and Technology, 373-1, Guseong-dong, Yuseong-gu, Daejeon, 305-701, Republic of Korea. E-mail: kds@kaist.ac.kr

Haploinsufficiency is defined as a dominant phenotype in diploid organisms that are heterozygous for a loss-of-function allele. Using complete set of *Saccharomyces cerevisiae* heterozygous deletion strains, Adam et al. revealed that haploinsufficient (HI) mutants are functionally enriched for metabolic processes carried out by molecular complexes such as ribosome, and mostly due to insufficient protein production[1]. Recently, our collaborating group at Korea Research Institute of Bioscience & Biotechnology (KRIIB) has constructed genome-wide heterozygous mutants in *Schizosaccharomyces pombe*. For the comparative genomics study between *S. pombe* and *S. cerevisiae*, they performed the time series experiments of 96 well plate-based O.D measurements for ~4,000 strains. We applied logistic growth model to their data and estimated growth rates of each strains in each experiment. Then, using nested ANOVA model considering the effects among experiments and plates, we selected HI mutants which seemed to show distinctively slow growth rates when tested at the significance level of 0.2. Despite the preliminary nature of the experimental data, we performed the GO functional analysis on the selected HI mutants. We will discuss the comparative study of two important models in terms of haploinsufficiency based on GO functional analysis


Where do we GO next? Involving biologists in Gene Ontology development

Midori Harris

GO, EMBL-EBI, WT Genome Campus, Hinxton, CB10 1SD, United Kingdom

The Gene Ontology (GO) project http://www.geneontology.org/ constructs and uses ontologies to facilitate the biologically meaningful annotation of genes and their products in a wide variety of organisms. The GO ontologies describe gene product attributes in three key biological domains: molecular function, biological process, and cellular component. Within GO, terms and relationships can be added, refined, and reorganized as biological knowledge advances. Curators who use GO terms for gene product annotation play a key role in the development of GO. To complement their input, the GO Consortium strives to involve members of the biological research community in the ontology development process. Several novel mechanisms of soliciting and incorporating community input have guided the most recent changes in the ontology content. To promote communication among contributors and ensure consistency within the ontology, the GO Consortium has established Curator Interest Groups, which are formed of Consortium members and community experts, and focus on specific areas within the ontologies. In addition, GO curators and biologists come together to consider specific biological topics at meetings devoted to ontology content. Many recent improvements in GO stem from the first content meeting, in which members of the GO group and domain experts in plant pathogens, the cell cycle, and metabolism participated.
Analysis of systematic genome wide deletions of the fission yeast

Schizosaccharomyces pombe

Hayles, J.1, Hoe, K-L.1, Kim, DU.1, Park, H.1, Won, M.2, Yoo, H-S.1, Palmer, G.1, Duhig, T., Peat, N.1, Mandeville, R.2, Wexler, S.2, and Nurse, P.3

1 Cell Cycle Laboratory, Cancer Research UK, The London Research Institute, 44, Lincoln’s Inn Fields, London WC2A 3PX, UK
2 The Rockefeller University, 1230 York Avenue, New York, New York 10021 USA
3 Korea Research Institute of Bioscience and Biotechnology (KRIBB), Laboratory of Human Genomics P.O. Box115, Yusong, Daejon 305-333 Korea
4 Bioneer Corporation 49-3 Munpyeong-dong, Daedeok-gu, Daejeon 306-220, South Korea

We are using a genome wide set of deletion mutants to identify the major sets of cell cycle and cell morphology genes in fission yeast. Fission yeast has an estimated 4973 protein coding genes. KanR heterozygous deletion diploids have been constructed from a h+/h+ ade6-M210/ade6-M216, leu-32/leu1-32, ura4-D18/ura4-D18 strain and these diploids used to generate haploid spores, by a high throughput transformation of pON177 containing Mat1-M. The subsequent spores have been germinated and screened for cdc and cut phenotypes, cell shape defects and for cell viability. We have found that about 28% of fission yeast genes are essential compared to about 18-20% in budding yeast and a comparison between fission yeast and budding yeast suggests that only about 60% of essential genes in fission yeast are also essential in budding yeast. The current estimate is that there will be 180 new cell cycle genes and 166 new morphology genes from a total set of 322 cell cycle genes and 244 morphology genes.

The UniProt Knowledgebase, the Fungal Proteome Annotation Program (FPAP) and the status of the

Schizosaccharomyces pombe genome data within this framework

Vivien Junker1, Kati K. M. Laiho2, Marc Feuermann1, Ivo Pedruzzi1, Rolf Apweiler1, Amos Bairoch1

1 Swiss Institute of Bioinformatics, CMU, 1 rue Michel-Servet, CH-1211 Geneva 4, Switzerland
2 The EMBL Outstation - The European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, United Kingdom

The UniProt Knowledgebase (UniProtKB) provides a central database of protein sequence and function data. UniProtKB consists of two separate datasets, namely TrEMBL and Swiss-Prot. TrEMBL contains translations of coding sequences in the EMBL/DDBJ/GenBank nucleotide databases and directly determined protein sequences extracted from the literature, submitted directly to UniProtKB and sequences of Protein Data Bank (PDB) structures. Swiss-Prot is the manually annotated section of UniProtKB. It strives to provide complete, high quality annotation of protein sequences via reading and analysing corresponding articles published about the particular sequence. There is also minimal redundancy and extensive cross-referencing to other databases.

For example, there are cross-references to the initial data source when derived from translation of coding sequences (EMBL). There are also cross-references to PDB when sequence and 3D-structure information are available. Sequence pattern and profile matches are cross-referenced to InterPro. InterPro is a database of protein families, domains and functional sites in which identifiable features found in known proteins can be applied to unknown protein sequences. It does not create signatures itself but amalgamates the efforts of the member databases. These currently include PROSITE, Pfam, PRINTS, ProDom, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER. Interaction data and biological information, when available, are given via IntAct and GO cross-references, respectively. There can also be cross-references to organism-specific databases.

The Fungal Proteome Annotation Program (FPAP) was set up to deal with the increasing amount of sequence data, resulting from complete sequencing of fungal genomes. Schizosaccharomyces pombe is one of the model yeasts within FPAP. The current status of S. pombe entries as of 24-JAN-2006 in UniProt release 6.9, which consists of Swiss-Prot release 48.9 and TrEMBL release 31.9 is 2944 and 2251, respectively.
The More the Merrier: Comparative Analysis of Microarray Studies on Cell Cycle-Regulated Genes in Fission Yeast

Samuel Marguerat1, Thomas S. Jensen2, Ulrik de Lichtenberg1, Brian T. Wilhelm1, Lars J. Jensen1 and Jürg Bähler1

1 Cancer Research UK Fission Yeast Functional Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK
2 Center for Biological Sequence Analysis, BioCentrum-DTU, Technical University of Denmark, DK-2800 Lyngby, Denmark

* These authors equally contributed to this study

The last two years saw the publication of three genome-wide gene expression studies of the fission yeast cell cycle. While these microarray papers largely agree on the main patterns of cell cycle-regulated transcription and its control, there are discrepancies with regard to the identity and numbers of periodically expressed genes. We present benchmark and reproducibility analyses showing that the main discrepancies do not reflect differences in the data themselves, microarray or synchronization methods seem to lead only to minor biases, but rather in the interpretation of the data. Our reanalysis of the three data sets reveals that combining all independent information leads to an improved identification of periodically expressed genes. These evaluations suggest that the available microarray data do not allow reliable identification of more than about 500 cell cycle-regulated genes. The temporal expression pattern of the top-500 periodically expressed genes is generally consistent across experiments, and the three studies together with our integrated analysis provide a coherent and rich source of information on cell cycle-regulated gene expression in S. pombe. We hope that this report will resolve the apparent discrepancies between the previous studies and be useful both for wet-lab biologists and for theoretical scientists who wish to take advantage of the data for follow-up work.

Comparative tandem repeat analysis of the S. pombe genome

Ann-Marie Patch and Stephen J. Aves

School of Biosciences, University of Exeter, Exeter EX4 4QD, UK
S.J.Aves@exeter.ac.uk

Tandem repeats are approximate or identical copies of contiguous DNA sequences. They are often polymorphic, undergo copy number mutation, are commonly exploited as genetic markers, have been identified as causal agents for several human diseases, and increasing evidence suggests that they play a variety of regulatory and evolutionary roles. We have performed a comprehensive and comparative analysis of tandem repeats in the genomes of Schizosaccharomyces pombe, Saccharomyces cerevisiae and a representative human chromosome. Tandem repeats are widespread in S. pombe, despite its small genome size. Excluding centromere, telomere and dispersed repeat sequences we detected 819 non-overlapping tandem repeats in S. pombe and 1615 in S. cerevisiae, representing 0.61% and 0.77% of these genomes respectively; this compares with 2.08% for human chromosome 21. Tandem repeats are widely dispersed in a non-random, clustered distribution, frequently occurring in protein coding as well as non-coding sequences. Tandem repeats in non-coding sequences typically have a range of repeat unit lengths up to 35 bp, whereas those in protein coding regions have repeat unit lengths that are predominantly multiples of three and include many greater than 100 bp. Despite the long evolutionary distances that separate the three species, tandem repeat characteristics are conserved, but the patterns of the most commonly identified consensus sequences are species-specific. Tandem repeats occur more frequently than expected in genes encoding proteins involved in the biogenesis and organisation of cell organelles, the cytoskeleton and ribosomes. Analysis of yeast orthologous genes showed over half (53.3%) of S. pombe tandem repeat-containing genes have a similar sequence in a conserved position in the S. cerevisiae orthologue. Tandem repeats within protein coding regions of S. pombe non-orthologues have patterns that point to expansion and contraction of encoded alpha helix regions. A DNA fingerprinting analysis of six independent S. pombe wild-type isolates using selected tandem repeats identified polymorphic loci for 11 out of 15 tandem repeats within protein coding sequences and 10 out of 11 in non-coding sequences. Two loci have been identified as polymorphic in laboratory strains.
A large-scale screen in S. pombe identifies seven novel genes required for critical meiotic events

Cristina Martin-Castellanos1, Miguel Blanco1, Ana E. Rozalén1, Lívia Pérez-Hidalgo1, Ana I. García1, Francisco Conde1, Juan Mata1, Chad Ellermeier2, Luther Davis2, Pedro San-Segundo2, Gerald R. Smith2, and Sergio Moreno1

1Instituto de Biología Molecular y Celular del Cáncer, CSIC/Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain
2The Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK

Meiosis is a specialized form of cell division by which sexually reproducing diploid organisms generate haploid gametes. During a long prophase, telomeres cluster into the bouquet configuration to aid chromosome pairing, and DNA replication is followed by high levels of recombination between homologous chromosomes (homologs). This recombination is important for the reductional segregation of homologs at the first meiotic division; without further replication, a second meiotic division yields haploid nuclei. In the fission yeast Schizosaccharomyces pombe, we have deleted 175 meiotically upregulated genes and found seven genes not previously reported to be critical for meiotic events. Three mutants (rec24, rec25, and rec27) had strongly reduced meiosis-specific DNA double-strand breakage and recombination. One mutant (htt2) was deficient in karyogamy, and two (bqt1 and bqt2) were deficient in telomere clustering, explaining their defect in recombination and segregation. The moa1 mutant was delayed in premeiotic S phase progression and nuclear divisions. Further analysis of these mutants will help elucidate the complex machinery governing the special behavior of meiotic chromosomes.

Analysis of 5' and 3' Untranslated Regions in S. pombe cDNAs.

Nigel Peat*, Trevor Duhig*, Aengus Stewart*, Jacky Hayles* and Paul Nurse*

*Cell Cycle Laboratory, Cancer Research UK, London UK.
*Bioinformatics & Biostatistics, Cancer Research UK, London UK.
*Rockefeller University, New York, USA.

Using a cDNA library we are sequencing clones at random to analyse the 5' and 3' un-translated regions (UTRs). We are also using this data to look for the existence of true inter-genic regions in S.pombe. From the genomic sequencing project (Wood et al. Nature, 415, 871-880, 2002) it is known that the distances between tandem, convergent and divergent genes in S.pombe are greater than those in S.cerevisiae. There are several possible explanations for these results; 5' mRNA regions may be systematically longer in S.pombe; Promoter regions may be of greater complexity in S.pombe and therefore require longer 5' UTRs; S.pombe may have genuine intergenic regions. So far we have sequenced over 2,790 cDNAs and have identified over 500 unique genes; results from this study are presented here.
YOGY: a web-based integrated database to retrieve protein orthologs from fission and budding yeast

Christopher J. Penkett, James A. Morris, Valerie Wood, and Jürg Bähler

Cancer Research UK Fission Yeast Functional Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK

We present YOGY (Yeast OrtholoGY), a web based resource for orthologous proteins from the two yeasts Schizosaccharomyces pombe and Saccharomyces cerevisiae. Using a yeast gene or protein as a query, this database provides rich, combined information on orthologs in various other species using data from four independent resources: KOGs, Inparanoid, Homologene, and a table of curated yeast orthologs. Associated Gene Ontology (GO) terms of orthologs can be retrieved for functional inference. Integrating these different and complementary datasets provides a straightforward resource to identify known and predicted orthologs of yeast proteins.

YOGY is accessible online at: http://www.sanger.ac.uk/cgi-bin/PostGenomics/S_pombe/YOGY/yogy-search.pl

Pfam and the Pombe Proteome: Comparisons and Complexes

Benjamin Schuster-Böckler, Jaina Mistry, John Tate, Valerie Wood, Alex Bateman, Rob Finn

Wellcome Trust Sanger Institute, Hinxton, UK

Pfam is a protein family database. Each family is represented by curated multiple sequence alignments, profile Hidden Markov Models (HMMs) and annotation. Pfam has over 75% coverage of the S. pombe proteome, and thus provides a rich resource for understanding the domain repertoire in S. pombe. Similar levels of coverage have been achieved for other organisms making Pfam an excellent tool for comparative proteomics. The Pfam website provides simple tools for analysing the distribution of a domain within a single proteome and between proteomes. Tools for comparing domain repertoire between species are also available.

Recently, Pfam has introduced a hierarchical classification of protein families, termed 'clans'. Members of a clan (protein families) have been identified using a variety of methods, including sequence, structure and profile-profile comparisons. We have made tools available for understanding the relationship of the families within a clan. Clans have enabled the association of many more families to a known structure and enriched annotation. For example, several domains of unknown function (DUFs) can be assigned a putative function.

In addition to domain annotation, Pfam allows the exploration of protein interactions at the domain and sequence levels using iPfam. iPfam is a database of interacting Pfam domains. These interactions are identified by mapping Pfam domains on to known 3D structures and calculating the physical bonds that form the interaction. From these calculations, multiple sequence alignments with markups of contacting residues, graphs showing the domain interaction networks and details of residue-residue bonding are available via a series of web tools and downloadable files.
Regulatable promoter with short induction time

*Stephen Watt, Juan Mata, Gavin Burns, and Jürg Bähler

Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK.
*email: sbw@sanger.ac.uk; tel: +44 (0)1223-494862

A regulatable promoter that can be switched on and off within short times would be a useful tool for the fission yeast community. Microarrays are ideal to screen for genes that are strongly regulated under selected conditions, which otherwise have little effect on global expression profiles. We identified three genes whose expression levels are uracil-dependent. The gene regulated the most strongly in response to uracil is SPAC1002.19. After uracil addition, it is induced ~20-fold within 15 minutes and ~100-fold within 60 minutes. The basal expression level seems low (background signal level). After uracil wash out, the gene is ~8-fold repressed within 30 minutes and ~100-fold within 120 minutes. We cloned a 675bp fragment taken from the promoter region of SPAC1002.19 into the KanMX6 and NatMX6 cassettes of PCR targeting plasmids, enabling the control of transcription of a selected gene at its normal chromosomal locus. We demonstrate that this system allows alternating transcription of the *pom1* gene within 15 minutes. Comparisons with different *ntt1* constructs suggest that the amount of regulation is between *ntt41* and *ntt81* and the overexpression is much weaker than with the strongest *ntt1* promoter. The new system may be more suitable to provide a pulse of transcription within a short time (e.g., during a particular cell-cycle stage) rather than completely switching off gene expression. More experience with other genes is required to know how useful this new regulatable promoter will ultimately be.

Fission yeast, the canonical eukaryote

Valerie Wood

The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA

One of the broader aims of biology is to provide a complete description of an eukaryotic cell and ultimately to dissect completely the molecular mechanisms which form the basis of eukaryotic life. Many core eukaryotic processes are conserved and it is generally accepted that these are often carried out by orthologous proteins (Chervitz et al 1998). This conservation has allowed the description of numerous "canonical" parts and mechanisms to describe the consensus of shared sequence, parts, features and functions. These consensuses can be merged and extended into the concept of a 'canonical eukaryote' to describe universal parts and mechanisms necessary to define an eukaryotic cell. The yeasts *S. cerevisiae* and *S. pombe* are both making major contributions to our fundamental understanding of the biology of a 'canonical eukaryote'. This is primarily because the majority of the gene products in *S. cerevisiae* and *S. pombe* have some functional similarities with genes present in higher eukaryotes, making them both ideal models for eukaryotic processes (Wood et al 2002). The budding yeast *S. cerevisiae* is universally considered likely to be the first eukaryotic organism to be fully understood in terms of its basic biology (where we know, or can infer, something about the molecular function, biological process and cellular component of each and every protein coding gene) because of its long distinguished history and intensity of study (Hughes et al 2004, Fields and Johnston 2005). However, the fission yeast displays a number of biological features and characteristics (including a higher level of conservation and closer correspondence of many biological processes with higher eukaryotes, lower redundancy, and fewer genes) which are making it a strong contender in this particular race. Moreover, recent reviews suggest that many *S. cerevisiae* genes have produced no data in large scale functional genomics experiments, and that previously known genes are highly overrepresented in these datasets (Hughes et al 2004). Functional genomics is therefore not providing the expected clues to the function of these experimentally intractable genes. The rate at which novel genes are characterised, and current annotation statistics suggest that it is not inconceivable that the fission yeast will be the first organism to achieve the landmark status where we have some information about the basic molecular function, biological process and cellular component of every protein coding gene product. This is a prerequisite step towards a more complete understanding of a eukaryotic cell. Here I present the current view of the proteome based on annotation to gene ontology (GO) terms, from both literature curation and annotation transfer from orthologous proteins, and a comparison with *S. cerevisiae*. I will also present the current level of coverage based on membership of identified protein families and a comparison with *S. cerevisiae*.

Current data indicates:

i) *S. pombe* has a lower percentage, and absolute number, of genes which are not assigned to a single GO term.

ii) *S. pombe* has fewer orphans (sequences with no identified similarity and no published data).

iii) *S. pombe* has a higher percentage of proteins covered by protein families in
the Pfam protein family database, and a higher proportion of these families are shared with metazoans.

iv) *S. pombe* has fewer conserved genes where nothing is known about the biological process or molecular function, and a greater number of these ‘unknown conserved genes’ are shared with higher eukaryotes.

I will also present data which identifies the members of the set of ‘core’ eukaryotic genes conserved from yeast to metazoa, for which experimentation has provided no clues to molecular function or biological process in any organism studied so far, and are therefore ideal targets for intensive study.

**Environmental Responses and Signalling**

**Hyphal Growth in S. Pombe**

Evelyn Amoah-Buahin, Klara Enczi, Monica Pacurar and *John Armstrong*

Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, UK

*Presenting author.*

Email: J.Armstrong@sussex.ac.uk

*S. pombe* grows in a single-celled form, or can mate and undergo meiosis and sporulation. We found that wild-type *S. pombe* can also differentiate to form elaborated branched hyphae which invade deep into solid medium.

Nitrogen deprivation in the presence of an abundant carbon source appears to be the main stimulus for hyphal growth. No MAP kinase is necessary for the response, but components of the cyclic AMP signalling pathway are required, in a reciprocal fashion to their requirement in mating. Deletions of those components not required gives a ‘hyperhyphal’ phenotype in which hyphal growth is more efficient than normal.

The large amount of information already available on cell growth and signalling in *S. pombe* provides an excellent opportunity to investigate this process of differentiation and morphogenesis at the molecular level. We are investigating the differences in RNA and protein composition between single cells and hyphae. By growing the cells in microscope chambers it is possible to image GFP fusion proteins within hyphae.

Cisplatin-induced stress response of human drug-sensitive and-resistant tumor cells

Giovanni Luca Beretta, Laura Gatti, Elisabetta Corna, Franco Zunino, Jürg Bähler and Paola Perego

Instituto Nazionale Tumori, via Venezian 1, 20133 Milan ITALY
Welcome Trust Ranger Institute, Hinxton, Cambridge, CB10 1SA, UK

Global gene expression studies in fission yeast have shown that cisplatin activates a stress response, including glutathione-S-transferase, heat shock, and repair genes. Transcriptional response could explain increased ability to repair or tolerate DNA damage in resistant cells. In the present study, we investigated whether human genes, orthologous to those found in yeast, were modulated by cisplatin in pairs of cisplatin-sensitive and -resistant human tumor cell. Molecular and biochemical approaches were used to assess the cell response to cisplatin. Glutathione-S-transferase activity was increased upon drug exposure both in some sensitive and resistant cells. Cisplatin up-regulated Hsp70, Hsp90, Rad51 and the alpha proteasome subunit in some of the resistant cells. cDNA array studies in cisplatin-sensitive and -resistant cervix squamous cell carcinoma cells indicated that the expression of components of the recombinational repair pathway was activated in sensitive cells, whereas resistant cells up-regulated DNA damage recognition/repair proteins. Modulation of genes involved in mitotic progression was shown in resistant cells, similarly to what observed in resistant cells. Our results indicate that some pathways activated by cisplatin exposure are conserved between yeast and human cells, thereby supporting the interest of yeast as a model system to study selected aspects of cellular response to platinum compounds.

Ish1p-Ish1p interactions in vivo by FRET

Finan, K., Rajagopalan, R., Theis, S. and P. G. Young.

Dept. of Biology, Queen's University, Kingston, ON K7L 3N6, Canada
Current address: Sir William Dunn School of Pathology, Oxford University, Oxford, OX1 3RE, U.K.

Ish1p is a protein of largely unknown function with orthologues in many other fungal systems. It is expressed strongly in a MAPK dependent fashion during stress response and localizes to membranes of the nuclear envelope and cell periphery. By two hybrid analysis we have shown that Ish1p can bind to itself and presumably it forms dimers or oligomers in vivo. The interaction domain has been partially defined. Strong Ish1p overexpression is lethal.

By two hybrid and co-immunoprecipitation, we have shown that Ish1p interacts with Bisp. Bisp binds to the same Ish1p domain that is involved in Ish1p-Ish1p dimerization. Bisp is highly conserved in all eukaryotes (except budding yeast), localizes to the nucleus and does not have a definitive function associated with it. Bisp overexpression causes a cell cycle delay but the cells will still form colonies.

We have investigated Ish1p dimerization/oligomerization in vivo using CFP/YFP FRET. We have shown that Ish1-CFP and Ish1-YFP produce a FRET signal in all of the membrane systems to which they are localized during log phase, heat shock and osmotic stress. We do not detect differences in the relative amount of apparent dimers under any condition tested. We have succeeded in partially titrating out the Ish1p FRET signal in the nucleus by overexpressing the Ish1p-Ish1p interaction domain targeted to the nucleus.

Although we also investigated the Ish1p-Bisp1p using CFP/YFP FRET, we were unable to detect a FRET signal between Ish1-CFP and Bisp1-YFP, even during strong overexpression of both proteins. We also found that overexpression of Bisp1p had no effect on the fraction of Ish1p forming dimers, suggesting that Bisp1p may bind to an Ish1p dimer/oligomer.
Homocysteine and Cysteine mediated growth defect is not associated with induction of oxidative stress response genes in yeast.

Arun Kumar, Liho John, Md. Mahmood Alam, Ankit Gupta, Gayatri Sharma, Beena Pillai*, and Shantanu Sengupta*
From Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India

Intracellular thiols like cysteine, homocysteine and glutathione play a critical role in the regulation of important cellular processes. Alteration of intracellular thiol concentration results in many diseased states, for instance, elevated levels of homocysteine is considered to be an independent risk factor for cardiovascular disease. Budding Yeast (Saccharomyces cerevisiae) has proved to be an excellent model system for studying many human diseases since it carries homologues of nearly 40% of human disease genes and many fundamental pathways are highly conserved between the two organisms. Here we demonstrate that cysteine and homocysteine, but not glutathione, inhibits yeast growth in a concentration dependent manner. Using deletion strains (str2Δ and str4Δ) we show that cysteine and homocysteine independently inhibit yeast growth.

Transcriptional profiling of yeast exposed to cysteine and homocysteine revealed that genes coding for antioxidant enzymes like glutathione peroxidase, catalase and superoxide dismutase were down-regulated. Furthermore, transcriptional response to homocysteine did not show any similarity to the response to hydrogen peroxide. We also failed to detect induction of reactive oxygen species in homocysteine and cysteine treated cells, using fluorogenic probes. These results indicate that homocysteine and cysteine induced growth defect is not due to the oxidative stress. However, we found an increase in the expression of KAR2 gene, a well known marker of endoplasmic reticulum (ER) stress and also observed HAC1 cleavage in homocysteine and cysteine treated cells which indicates that homocysteine and cysteine mediated growth defect may probably be attributed to ER stress. Transcriptional profiling also revealed that genes involved in one-carbon metabolism, glycolysis and serine biosynthesis were up-regulated on exogenous addition of cysteine and homocysteine suggesting that cells try to reduce the intracellular concentration of thiols by upregulating the genes involved in their metabolism.

Global transcriptional responses of fission yeast entering stationary phase

Christopher J. Penkett, Luis López-Maury, and Jürg Bähler
Cancer Research UK Fission Yeast Functional Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK

Cell growth and proliferation are controlled by the availability of nutrients. When cells exhaust available nutrients, they enter a quiescent stationary phase characterized by cell cycle arrest and distinct physiological, biochemical, and morphological changes. We are studying genome-wide gene expression of fission yeast in response to limiting nutrients and after refeeding. We have followed cells over a period of 11 days as they went from exponential growth to stationary phase in EMM medium. Most of the transcriptional and physiological changes occur during a short physiological window of 2-3 hours at the end of exponential growth (ODs between 1.5 and 2.5).

Preliminary data on this ongoing project will be presented.
Mechanism of action of chromium compounds on Schizosaccharomyces pombe cells

M. Pesti,1 Z. Gazdag,1 G. Papp,1 J. Antal,1 T. Stromář-Rácz,1 Zs. Koosz,1 K. Takács,1 I. Pócsi,1 J. Belágyi,1 P. Raspò2

1University of Pécs, Faculty of Sciences, Department of General and Environmental Microbiology, Hungary, 2University of Debrecen, Faculty of Sciences, Department of Microbiology and Biotechnology, Hungary, 3University of Ljubljana, Food Science and Technology Department, Slovenia

Stable chromium(VI)-sensitive and -tolerant mutants were obtained by induced mutagenesis of fission yeast heterothallic strains 4chr'lys-1-131h and 6chr'leu-332h. The segregation of tetrads of selected tolerant mutants, 4chr-67T, 4chr-14T, indicated that tolerance was determined by single mutation of non allelic origin. Both chromium(III) and (IV) induced decreases in the phase transition temperatures of the 5- and 14-doxy sterol acid and 3-doxybutyric spin probes labelled plasma membranes. Cr(III) treatment caused a strong fluidizing effect and the loss of metabolites adsorbtion at 260 nm which process might be the main cause of growth inhibition and cell killing by these impermeable ions. The changes in local structure produced by Cr(VI) and its unstable intermediates, the presence of Cr(III) in the outer and inner headgroup regions of the plasma membrane, and the changes in the electric charge of the cell surface could all affect the physiological function of the cells.BBA 1421:175-182.1999; FEMS Microbiol. Lett. 182:375-380. 2000; BBA 1611:217-222. 2003). Fast transport of 109CrO4 was detected in a sensitive mutant, 6chr-57S, while the tolerant mutant chr1-66T and the parental strain 6chr- exhibited significantly lower uptake. (Folia Microbiol. 49, 31-36.2004). Fast transport of 109CrO4 and increased bioaccumulation was detected in a sensitive mutant, chr-515, in contrast to the tolerant one, 4chr-67T (FEMS Microbiol. Lett. 178:109-115. 1999).

Cr(VI) tolerance and the oxidative stress sensitivity of 4chr-67T mutant were attributed to a decreased specific glutathione reductase (GR) and mitochondrial MnSOD activity. (J. Basic Microbiol. 43:96-103. 2003). Cr(VI) sensitivity of the 6chr-57S mutant accompanied with significantly increased GR and glucose-6-phosphate dehydrogenase activities and high intracellular superoxide and peroxide concentrations in spite of its decreased glutathione (GSH) content. These data strongly suggested that instead of GSH, the NAPDH/GR system was the major one-electron Cr(VI) reductant in vivo (J. Basic Microbiol. 42:410-421. 2002). However, transformation of chr-1-67T mutants with vector carrying GR gene did not alter the Cr(VI) sensitivity. But sequence analysis of the transcription factor Papi/Chrl/Ca3 (Mai.Gen. Genomics 271:161-170. 2004) proved that Papi1 plays a central role in the determination of chromium (VI) resistance via down-regulation of GR enzyme (unpublished).

Pro-oxidative vs. antioxidative properties of ascorbic acid in chromium(VI)-induced damage was studied. Ascorbic acid influenced Cr(VI) toxicity both as a reducing agent, by decreasing Cr(V) persistence, and as an antioxidant, by decreasing intracellular superoxide anion and hydrogen peroxide formation and by quenching free radicals formed during Cr(VI) to Cr(V) reduction (J. Appl. Toxicol. 25: 2005. in press).

Corresponding author:
micro@ttk.pte.hu

Metazoan RBP-J/Suppressor of Hairless homologs in S. pombe

Martin Plevorovský, Jan Ryneš, Tomáš Grouší, Petr Folk, František Půta

Department of Physiology and Developmental Biology, Faculty of Science, Charles University in Prague, Viniceň 7, Prague 2, 128 00, Czech Republic

The CSL (CBF1/Su(H)/LAG-1) proteins are highly evolutionarily conserved transcription factors that mediate the effector step of the transmembrane receptor Notch signal transduction pathway. Upon activation and proteolytic processing the intracellular part of Notch enters the nucleus and binds to a CSL protein, converting it from a repressor to a potent activator of the responsive genes. This pathway is essential for metazoan development and its misregulation results in severe abnormalities, lethality or cancer (1, 2).

We have identified and cloned two putative CSL family member genes in S. pombe, provisionally designated cbf1-1 and cbf1-2. Their protein products share 35% and 23% similarity to human CBF1, respectively. Manual inspection of their sequences revealed that all amino acid residues required for DNA binding are conserved in the S. pombe proteins (3). In vitro gel-shift assays proved that both recombinant and endogenous Cbf1-1p recognize and bind to DNA probes. Both Cbf1-1p and Cbf1-2p fusions with EGFP localize to the nucleus of living S. pombe cells. Single deletions of each gene appear to be viable (4, 5).

Our data strongly suggest that there are bona fide homologs of the metazoan CSL transcription factors in S. pombe. We are working on detailed characterization of the cbf1-1 and cbf1-2 genes and we hope to solve the mystery of proteins regulating multicellular development appearing in a unicellular organism.

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References
Investigating the mechanism of Int6-induced drug resistance

Emma Rawson, Caroline Jenkins, Chris Norbury

Sir William Dunn School of Pathology, University of Oxford, UK

The translation initiation factor component Int6 (eIF3e) was previously identified in an overexpression screen through its ability to cause multidrug resistance in fission yeast. Further investigation showed this resistance to be dependent on Int6-mediated activation of the AP-1 transcription factor Pap1, but not on previously described protein-protein interactions of endogenous Int6. Serial truncations of Int6 have identified a 70 amino acid fragment sufficient to induce the drug resistance phenotype. Mutation to alanine of a single conserved phenylalanine residue within this region abrogated resistance. His-tag fusions of this 70aa minimal fragment and the inactive mutant form have been constructed with a view to structural studies and identification of interacting factors responsible for Pap1 activation. Other work currently being undertaken indicates that the Sty1/Wis1 stress signalling cascade may have a role in the Int6/Pap1 drug resistance pathway.

Multiple roles of the RAN GTPASE and the IMPORTIN-ALPHA transport receptors in fission yeast

Shelley Sazer, Makoto Umeda, Shahed Izaddoost, Gerald Lim H.W., Yoshihiro Torii and Jonathan Miller

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030 U.S.A.

The nuclear import of classical Nuclear Localization Signal-containing proteins (cNLS) depends on the Ran GTPase system and the importin-alpha family of transport receptors. Higher eukaryotes have multiple importin-alpha family members, budding yeast has just one, but fission yeast have two, making it a good model system in which to characterize the specialized roles of the importin-alpha proteins. Our investigations into the isoform-specific functions of Imp1p and Cut15p, the two importin-alpha proteins in fission yeast, show that they have both unique and common roles (Umeda et al. Genetics (2005) 171: 7-21). We are currently examining the specialized in vivo roles of Imp1p and Cut15p in fission yeast.

Ran and Importin-alpha proteins also influence nuclear envelope structure in both higher and lower eukaryotes. When the Ran GTPase is mis-regulated in fission yeast, the nuclear pore complexes are not uniformly distributed in the nuclear envelope and cells undergo unequal nuclear division followed by nuclear envelope fragmentation. Deletion of the nuclear pore complex component nup133 or overexpression of either cut15 or imp1 exacerbates these nuclear envelope defects.

During the closed mitosis of S. pombe, the nucleus undergoes dramatic changes in shape from round, to oval, to peanut, to dumbbell, before resolving itself into two discrete daughter nuclei. In order to determine the biophysical properties governing these changes in size and shape, we are developing a physical model based on current understanding of the fission yeast nuclear architecture, nuclear shapes found during normal and abnormal mitoses in fission yeast and the physical characteristics of lipid bilayers.
Nuclear transport in the fission yeast as a useful model for the nuclear import and replication of retroviruses: Functional characterization of a nucleoporin involved in retrotransposition

Srirani Sistla
and David Balasundaram

Laboratory of Nucleopore Biology,
Institute of Molecular and Cell Biology,
61 Biopolis Drive, Proteos, Singapore 138673

The propagation of TF1, a long terminal repeat (LTR)-containing retrotransposon in the fission yeast Schizosaccharomyces pombe is known to require many of the same processes used by retroviruses to complete its life cycle within the host cell and can therefore serve as a very useful model for the replication of retroviruses. Nucleoporins are proteins at the nuclear pore that play a role in trafficking proteins between the nucleus and the cytoplasm. Extensive studies have shown the organization of these proteins at the nuclear pore complex in yeast. Nup124p is a non-essential nucleoporin found in S. pombe and required for the nuclear import and activity of the retrotransposon TF1. Architecturally, it has 3 major domains, the N-terminal region encoding a putative Gag-binding region; a C-terminal large domain with 11 FXFG repeats and a C-terminal bipartite NLS-like motif. In this presentation we show that while the FXFG-repeat region of Nup124p is absolutely required for TF1 activity, the FXFG repeats themselves are not. Swapping this region with similar regions from the Nup124p orthologues, Nup153 (human) or Nup1p (S. cerevisiae) allowed complete activity of the protein to be restored. A knockdown of TF1 transposition was observed upon overexpression of the FXFG region from Nup124p as well as Nup153 and Nup1p alone and not upon expressing a FG rich region or a non-specific region of similar size from nucleoporins of S. pombe. The FXFG repeats are known to interact with β-importin transport factors. Results of our work, exploring the function of the FXFG region will be presented.

Critical residues in the C-terminal bipartite NLS-like motif that are required for TF1 function have been identified. The motif itself is well conserved among the aforementioned orthologues of Nup124p. Interacting partners to this region are being dissected to understand its specific role in the TF1 life-cycle and its phylogenetic conservation. Elucidation the mechanism of TF1 transposition would help in understanding retroviral entry into the nucleus.

nsp1 : A novel stress-responsive protein in S. pombe

Geetanjali Sundaram, Santanu Pal Chaudhuri, K.Sheelarani and Dhruabajoti Chattopadhyay
Dr. B. C. Guha

Centre for Genetic Engineering and Biotechnology, Department of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Kolkata-700019, INDIA

Our lab has been investigating the cellular responses towards Cigarette smoke using S.pombe as a model system (Chaudhuri et al., Yeast 2005; 22; 1223-1238.). Our transcript level differential display studies identified a 321 bp ORF to be highly upregulated upon exposure to Cigarette smoke Extract. Subsequent cloning and sequencing of this ORF revealed it to be a sequence orphan coding for an 11.4kDa Hypothetical protein. We have confirmed the translation of this differentially expressed transcript by demonstrating its association with polysomal fractions of fission yeast. Studies on the expression profile of this gene show that it’s expression is upregulated under most stress conditions (Oxidative stress, Nucleotide depletion, heat chock etc), osmotic shock being the only exception. We have named it nsp1 (novel stress responsive protein 1). Our studies demonstrate that the Spc1-Atf1 SAPK pathway in S.pombe regulates the expression of this ORF. Computational sequence analysis further revealed interesting features about this ORF, including a major potential for phosphorylation and glycosylation. This coupled with the observation that this small protein has an unusually high serine content (16%), indicates that phosphorylation may play a major role in regulating the function of the protein encoded by this novel ORF. We have investigated the possibility of any growth advantage or disadvantage provided to the cells during various stress conditions upon overexpression nsp1. We have also studied its sub-cellular localization by overexpressing an N-terminal EGFP tagged version of the protein in S.pombe. Flow Cytometry analysis of cells overexpressing this protein indicates that nsp1 may directly or indirectly affect the cell cycle checkpoint machinery, especially when S.pombe is subjected to heat shock (42 deg C), a condition where the transcript level of this ORF exhibit maximum induction.
Repression of ergosterol synthesis during adaptation to oxidative stress requires the new F-box protein Pof14 independently of SCF

Lionel Tafforeau¹, Sophie Bamps¹, Monique Dewez¹, Jean Vandenhauwe¹ and Damien Hermand¹ ;

¹Laboratoire de Génétique Moléculaire (GEMO), Facultés Universitaires Notre-Dame de la Paix, Rue de Bruxelles 61, 5000 Namur, BELGIUM

¹corresponding author: Damien.Hermand@fundp.ac.be

We describe a new member of the F-box family, Pof14 that forms a canonical, F-box dependent SCF ubiquitin ligase complex. The Pof14 protein has intrinsic instability that is abolished by inactivation of its F-box motif, Skp1 or the proteasome, indicating that Pof14 is degraded by an autocatalytic mechanism. Pof14 interacts with the squalene synthase Erg9, a key enzyme in ergosterol metabolism, in a membrane bound complex that does not contain the core SCF. pof14 transcription is induced by hydrogen peroxide in a Pap1-dependent manner and a pof14 deletion strain quickly loses viability in the presence of hydrogen peroxide due to its inability to repress ergosterol synthesis. A pof14 mutant lacking the F-box behaves as wild type showing that this function of Pof14 is independent of SCF. This indicates that repression of ergosterol metabolism by Pof14, and consequently modulation of membranes composition plays a key role in adaptation to oxidative stress.

SR-Protein Specific Kinase
Dsk1 Regulates the Cellular Localizations of SR Proteins by Phosphorylation in Fission Yeast Schizosaccharomyces pombe

Zhaohua Irene Tang, Amy Tsurumi, Sarah Alaee, Christopher Wilson, Cathleen Chiu, Jessica Oya, Benson Ngo

W.M. Keck Science Center, Claremont Colleges Claremont, CA 91711, USA

Dsk1p is the orthologues of human SR-specific protein kinase1 (SRPK1) in Schizosaccharomyces pombe. Dsk1p specifically phosphorylates fission yeast SR (serine/arginine-rich) proteins, Srp1p and Sp2p, and also Prp2p, the orthologue of human U2AF large subunit in vitro. However, little is known about the in vivo targets of Dsk1p and the biological consequences of the Dsk1p-mediated phosphorylation. In this study we investigated the in vivo phosphorylation of the SR proteins and the effect of Dsk1p on the cellular localizations of these SR-related proteins by ectopic expression of the corresponding GFP fusion proteins in wild-type and dsk1⁻ deletion fission yeast strains. Interestingly, Dsk1p, but not another SRPK, Kic1p, phosphorylates Sp1p in vivo. In contrast, Sp2p is phosphorylated by not only Dsk1p but also Kic1p when Dsk1p is not present in the cell. Furthermore, although the cellular distribution of the three SR-related proteins varies within the cell, their localization patterns all depend on dsk1⁻; whereas deletion in kic1⁻ exhibits little effect on their localizations. Therefore, the in vivo phosphorylation of the SR proteins by Dsk1p correlates with their dsk1⁻-dependent localization. The data prove that the SR proteins are the in vivo substrates of Dsk1p and demonstrate that Dsk1p is the major kinase responsible for the modulation of the SR protein localization in S. pombe. As Srp2p and Prp2p are known to be involved in exonic splicing enhancers (ESEs) and spliceosome assembly, respectively, our results provide strong in vivo evidence for a mechanism which regulates the cellular localization of the SR-related proteins by Dsk1p-mediated phosphorylation, thereby governing their activities in pre-mRNA splicing and/or in other cellular processes in S. pombe.
**S. pombe** importin-alpha proteins, Imp1p and Cut15, have common and unique functions.

Makoto Umeda, Shahed Izaddoost and Shelley Sazer

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030, USA.

The nuclear import of classical nuclear localization signal-containing proteins depends on importin-alpha transport receptors. In budding yeast there is a single importin-alpha gene and in higher eukaryotes there are multiple importin-alpha-like genes, but in fission yeast there are two: the previously characterized cut15 and the more recently identified imp1. Like other importin-alpha family members, Imp1p supports nuclear protein import in vitro. In contrast to cut15, imp1 is not essential for viability, but imp1 null mutant cells exhibit a telophase delay and mild temperature-sensitive lethality. Differences in the cellular functions that depend on Imp1p and Cut15p indicate that they each have unique physiological roles. They also have common roles because the imp1null and the cut15-85 temperature-sensitive mutations are synthetically lethal; overexpression of cut15 partially suppresses the temperature sensitivity, but not the mitotic delay in imp1null cells; and overexpression of imp1 partially suppresses the mitotic defect in cut15-85 cells but not the loss of viability. Both Imp1p and Cut15p are required for the efficient nuclear import of both an SV40 nuclear localization signal-containing reporter protein and the Pap1p component of the stress response MAP kinase pathway. Imp1p and Cut15p are essential for efficient nuclear protein import in **S. pombe**.

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**Gene Expression Control: From Chromatin to Proteins**

ACE2P controls the expression of several genes involved in cell separation in *Schizosaccharomyces pombe* and constitutes a part of a transcriptional cascade at the end of mitosis.

**Maria Luisa Alonso-Nuñez,**  
Ana Belén Martín-Cuadrado,  
Francisco del Rey and  
Carlos R. Vázquez de Aldana

Departamento de Microbiología y Genética, Instituto de Microbiología Bioquímica, CSIC/Universidad de Salamanca, 37007 Salamanca, Spain

*Schizosaccharomyces pombe* cells divide by medial fission through contraction of an actomyosin ring and deposition of a multilayered division septum that must be cleaved to release the two daughter cells. During the last few years, the isolation of mutants affected to different extents in cell separation has provided some insight into the mechanistic details of this process. Some of these mutants are an endo-1,3-glucanase (*agn1*), an endo-1,3-glucanase (*eng1*), an anillin homologue (*mid2*), and two transcription factors (*sep1* and *ace2*). The fact that the phenotype of *sep1Δ* or *ace2Δ* mutants is more severe than that of cells lacking *eng1*, *agn1* or *mid2* suggests that these transcription factors also control the expression of other genes involved in cell-cell separation. Here we have identified by microarray analysis a group of genes (*adg1*, *adg2*, *adg3*, *chf4*, *agn1*, *eng1*, and *mid2*) whose expression is dependent on the transcription factor Ace2p and hence they were named Ace2-dependent genes (*adg*). Northern analyses revealed that the expression of the *adg* genes is dependent on Ace2p and that ace2 transcription requires Sep1p. The expression of all of these genes varied during the cell cycle, maximum transcription being observed during septation. Mutants lacking the identified genes showed defects or delays in cell separation to different extents, but a double mutant devoid of the Eng1p endo-1,3-glucanase and the Agn1p endo-1,3-α-glucanase showed a phenotype very similar to that of ace2Δ mutants. According to these observations, these two enzymes are likely to be the major enzymatic activities involved in the dissolution of the septum during cell separation. On the other hand, it has been shown that other two proteins, Fkh2p (a forkhead transcription factor) and Mbx1p (a MADS box protein) are involved, with Sep1p, in the expression during M phase of several genes like *ace2*. Because of that, we have checked and showed that the expression of Ace2p targets is modified to different extents in these mutants.
Characterisation of Histone Posttranslational Modification in *Schizosaccharomyces pombe* using LC-MS/MS Mass Spectrometry.

Buchanan, L., Roguev, A., Shevchenko, A. and Stewart, A. F.

BIOTEC, Technische Universität Dresden, Dresden, Germany,
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.


Histone posttranslational modification (PTM) is a fundamental feature of higher order chromatin and is important in regulating chromatin structure and transcription. The combinatorial complexity of histone PTM (the “histone code”) defines another layer of information responsible for epigenetic and cell-memory phenomena. A good example of such PTM is histone H3 methylation, a modification that essentially defines different chromatin states throughout the genome and provides markers for silent chromatin (H3 K9 me) or actively transcribed genes (H3K4 and K36 me).

Bioinformatics reveals a multitude of genes in the *Schizosaccharomyces pombe* genome encoding specific protein domains implicated in histone H3 methylation. The SET domain is one of the catalytic domains responsible for histone methylation on lysine residues.

Protein domain prediction produces 12 SET domain proteins in the *S. pombe* genome. The *S. pombe* genome also contains homologues of recently identified human histone demethylases, namely Jumonji (JmJ) domain proteins (7) and SWIRM domain/Amine oxidase proteins (2).

Which of these proteins and domains are responsible for methylation and demethylation on different histone residues? To investigate this question we are using mass spectrometry (LC LTQ MS/MS) to detect and quantify global levels of histone PTM in various genetic backgrounds of *S. pombe*, in combination with traditional molecular biology. Ultimately we want to comprehensively describe the histone code in *S. pombe* and characterise the responsible proteins.

Preliminary results from this study indicate that *S. pombe* has a remarkably similar chromatin environment to higher eukaryotes and shares common features with *Saccharomyces cerevisiae*.

While retaining the fundamental features of higher eukaryotic chromatin, the complexity of the system in *S. pombe* appears to be reduced by a smaller genome size and fewer potential histone-modifying enzymes. This should increase the ease of exploring the histone PTM landscape, allowing greater resolution of the modifications and a better understanding of their inter-relatedness.

**Jmj1 is a member of Swr1 complex in *S. pombe* and has an activity at subtelomeric region**

**Authors:** Sakalar M. Cagni¹, Roguev Asseni¹, Wilhelm Brian², Shevchenko Andrej², Bähler Jürg², Stewart A. Francis

**Affiliations:** ¹BiInnovationZentrum, The University of Technology Dresden, Dresden, Germany
²The Welcome Trust Sanger Institute, Cambridge, UK
²MPI of Molecular Cell Biology and Genetics, Dresden, Germany

Covalent histone modifications such as methylation, acetylation as well as differential incorporation of histone variants are shown to co-occur with different chromatin compartments (eu- and heterochromatin) and mark active or repressed genes. Proteins having JumonjiC (JmJ) domain were shown to be involved in the maintenance of heterochromatin-euchromatin boundary and manipulation of histone modifications(1). In *S. pombe*, Jmj1 is one of the seven JmJ domain proteins. In order to identify its interaction partners, Jmj1 (Msc1) was TAP-tagged and immunoprecipitated. We found that Jmj1 is a part of a multi-protein complex that resembles *S. cerevisiae* Swr1 complex (Swr1C). In *S. cerevisiae*, Swr1C incorporates a H2A variant, H2A.Z, into nucleosomes and does not include a JmjC domain protein(2). When an immunoprecipitation experiment with Ph11 (the homolog of H2A.Z in *S. pombe*) as a bait was performed, the Swr1 complex and additionally nuclear assembly proteins were recovered. Chromatin Immunoprecipitation (ChIP) experiments showed that in swr1 and jmj1 deletion strains, Ph11 incorporation into chromatin decreased. Deletion strains of jmj1, ph11 and swr1 all showed sensitivity to TSA (a histone deacetylase inhibitor). Microarray expression analysis showed that the sets of genes affected by jmj1, ph11 and swr1 deletions overlap significantly and the upregulated genes show a localisation bias towards the chromosome ends. Hence we propose that Jmj1, in collaboration with Swr1C, functions in incorporation of Ph1 and has an activity at subtelomeric region of chromosome ends in *S. pombe*.

**References:**


RNA pol II subunit Rpb7 promotes centromeric transcription and RNAI-directed chromatin silencing


*Karolinska Institutet, Dept of Biosciences/Dept of Natural Sciences, Univ. College Sodertorn, Alfred Nobel’s Allé 7, S-141 89, Huddinge, Sweden.
**Wellcome Trust Centre for Cell Biology, Inst. of Cell and Molecular Biology, Univ. Edinburgh, Edinburgh EH9 3JR, UK.
*Karolinska Institutet, Dept of Medical Nutrition, Novum, S-141 86 Huddinge, Sweden.
**Corresponding author

In many eukaryotes RNAI mediates DNA and chromatin modifications resulting in transcriptional silencing. In fission yeast centromeric modifications are transcribed into siRNA precursors (pre-siRNAs), which are processed by Dicer to direct assembly of centromeric heterochromatin. It is not known how pre-siRNAs are structured and which RNA polymerase is responsible for their synthesis. Here we show that pre-siRNAs are synthesized by PolII. We define the centromeric pre-siRNA promoter from which initiation is exquisitely sensitive to a conditional mutation in the Rpb7 subunit of PolII. Defective Rpb7 causes reduced siRNA production and inefficient assembly of centromeric heterochromatin. Rpb7 thus provides a functional link between PolII transcription and chromatin silencing.

Role of the APC/C E3 Ubiquityl ligase in heterochromatin assembly in fission yeast

Rudra N. Dubey, Nandni Nakwai, Mitsuhiro Yanagida and Jagmohan Singh

1Institute of Microbial Technology, Sector 39A, Chandigarh-160036. India
2Department of Pharmacology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ, USA.
*Department of Gene Mechanisms, Graduate School of Biostudies, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

Gene silencing is mediated by mainly two pathways in fission yeast: post-transcriptional (PTGS) and transcriptional (TGS). Recent advances have revealed that components of PTGS initiate silencing by recruiting the machinery that sets the heterochromatin-specific histone code, i.e., methylation of H3 at Lys9. The latter is recognized by the evolutionarily conserved heterochromatin protein Swi6/HP1 (a component of TGS), which, through oligomerization, establishes the heterochromatin structure. A further stabilization of heterochromatin involves recruitment of Cohesin by Swi6. Degradation of cohesin subunit Rad21 via the pathway involving the APC/C E3 ubiquityl ligase helps to bring about sister chromatid separation.

A surprising new result indicates the involvement of the Anaphase Promoting Complex (APC) in silencing at mat, cen and rDNA loci in fission yeast. Results show that APC subunits Cut4 and Cut9 interact with Swi6/HP1 and help in stable inheritance of Swi6/HP1 (and possibly H3-Lys9-methyl) at the heterochromatin loci. Surprisingly, Swi6, in turn, is required for recruitment of APC subunits to heterochromatin, suggesting a mutually orchestrated role of Swi6 and Cut4 in facilitating the stabilization of heterochromatin and sister chromatid separation. Finally, proteolysis of the Rad21 subunit of Cohesin is required for stable binding of Swi6 to heterochromatin.

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Analysis of the SAGA transcriptional coactivator complex from *S. pombe*

Dominique Helmlinger¹, Judit Villén¹, Steven P. Gygi² and Fred Winston²

¹Department of Genetics, ²Department of Cell Biology, Harvard Medical School, Boston, MA 02115, USA

The SAGA (Spt-Ada-Gcn5 acetylase) complex is a multifunctional coactivator that regulates transcription by RNA polymerase II. SAGA was originally discovered in *Saccharomyces cerevisiae* and homologous complexes have been identified in higher eukaryotes. We have started to characterize SAGA composition and functions in *Schizosaccharomyces pombe* to characterize its roles in a yeast in which chromatin structure and modifications are similar to higher eukaryotes. Deletion of the genes encoding two SAGA core members, *spt7¹* and *ada1¹*, has shown that SAGA is critically required for growth, mating, and septation. We have also determined the biochemical composition of *S. pombe* SAGA by tandem affinity purification (TAP) on two independent core SAGA subunits (Spt7 and Ada1). Mass spectrometry analysis revealed that the 19 subunits forming SAGA in *S. cerevisiae* are conserved in *S. pombe* and co-purify with both Spt7 and Ada1. Interestingly, *S. pombe* Spt7 is present in two or more isoforms that could result from an N-terminal proteolytic processing, splicing, or post-translational modifications. Preliminary results indicated that *S. pombe* Spt7 harbors a highly acetylated and phosphorylated region, adjacent to its bromodomain. Deciphering the role of posttranslational modifications on SAGA structure and function, and their possible involvement in mating and cell division might illuminate how the specific activities of multi-protein coactivator complexes are controlled to regulate the expression of developmental genes.

Genetic analysis of fission yeast step pre-mRNA splicing factors- SpPrp18 and SpSlu7

Piyush Khandelia and Usha Vijayaraghavan

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India

Pre-mRNA splicing reactions and factors are evolutionarily conserved and predicted to function similarly in diverse metazoans. Six *S. cerevisiae* protein factors, including ScPrp18 and ScSlu7, are needed for 3' splice-site recognition and cleavage during the second-step of splicing. Direct interactions between Slu7-an essential factor, and Prp18 - a non-essential factor, underlie their spliceosome recruitment and functions therein. The intron-rich *S. pombe* genome, with multiple predominantly short introns per transcript, is an interesting model system to probe splicing mechanisms in an organism evolutionarily distant from budding yeast. We report our studies on two putative *S. pombe* splicing factors: *sppr18¹* and *spslu7¹*. In contrast to the viable but temperature-sensitive growth of ScPRP18::HIS3 we find *sppr18¹* is essential. Slu7 is essential in both yeasts. Splicing of some budding yeast pre-mRNAs, with varied spacing between the intron branch nucleotides and the 3'splice-site, differ in their dependence on ScPrp18 or ScSlu7. Genetic depletion of *sppr18¹* and *spslu7¹*, or repression of their expression, cause splicing defects for *sptH1D¹* pre-mRNAs. By examining the splicing status for several intron-containing transcripts we are investigating pre-mRNAs dependent on these fission yeast factors. Our analyses of fission yeast Prp18 and Slu7 reveal certain distinct differences. They do not interact in the two-hybrid assay; unlike the stable association between their budding yeast counterparts. Further, while ScPRP18 and ScSLU7 display functional overlap the fission yeast proteins have distinct non-overlapping functions. Comparative homology modeling of the predicted Sppr18 protein based on the crystal structure of ScPrp18 provides a structural basis for the lack of its interaction with SpSlu7. Using epitope-tagged strains for both SpPrp18 and SpSlu7 we are investigating indirect protein interactions and association with spliceosomal snRNAs. Together our current data implicate unique functions and spliceosomal interactions for SpPrp18 and SpSlu7 and suggest functional divergence of these factors with genome evolution.
Genome-wide identification targets of RNA-binding proteins

Juan Mata and Jürg Bähler

Cancer Research UK Fission Yeast Functional Genomic Group
Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus
Hinxton CB10 1SA, UK

RNA-binding proteins (RBPs) recognise specific RNA sequences and regulate every stage in the life of RNA molecules, including splicing, nuclear export, subcellular localisation, degradation and translation.

Although there are many examples of how specific RBPs regulate single transcripts, little is known about the extent of this control, and about how cells co-ordinately regulate groups of genes at the posttranscriptional level.

To get a global view of posttranscriptional regulation it is crucial to identify all the RNAs that are bound by specific RBPs. Several pioneer studies have used microarray-based approaches to achieve this goal. Their results have revealed the existence of complex networks of RBP-RNA interactions, comparable in complexity to the transcription factors networks that regulate transcription.

The first step to identify RBP targets at a genome-wide scale is to isolate an RBP together with any RNAs associated with it; the RNAs are then identified using DNA microarrays. This technique is often referred to as Rip-chip (for RBP Immunoprecipitation followed by analysis with DNA chips).

To study the global role of RBPs in fission yeast we have set up the technique of Rip-chip and we have started to apply this approach to a variety of RBPs. We will present results that validate the method and preliminary data on its application to several RBPs.

PLO1P regulates M/G1-phase specific transcription in fission yeast

Christopher J. McInerny*, Szu Shien Ng*, Kyriaki Papadopoulou* and Stephen Sedgwick†

*Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
†Division of Yeast Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Cytokinesis, the process at the end of the cell division cycle that results in separation of the two daughter cells, requires a series of precisely coordinated events. We study this process in the fission yeast Schizosaccharomyces pombe, as this organism shows significant similarities in cell separation to higher eukaryotes, including humans.

Previously, we identified a mechanism that regulates the expression of a group of genes required for cytokinesis. We characterised a protein complex that we named PBF (Pombe cell cycle box factor), composed of at least three transcription factors (Fkh2p, Sep1p and Mbx1p), that binds to a DNA promoter motif PCB (Pombe cell cycle box). This motif is found in the promoters of a number of genes required for cytokinesis, specifically expressed at the M/G1 transition.

We are interested to understand further how the PBF-PCB transcription system controls M/G1 gene expression. We previously showed that the PBF-PCB transcription system is necessary for the periodic mRNA accumulation of M/G1 transcribed genes, possibly through auto-regulation of fkh2. We also provided evidence of a stable PBF-PCB complex throughout the cell cycle, suggesting potential post-translational activation. Plo1p kinase is a prominent multi-functional mitotic regulator found in many eukaryotic systems, and we present data that components of PBF are substrates of Plo1p, thus suggesting a mechanism by which this protein kinase regulates M/G1-specific gene expression.
Comparative proteomic analysis of chromatin related complexes in *S. cerevisiae* and *S. pombe*

Assen Roguev¹, Daniel Schaf²,³, Anna Shevchenko⁴, Rein Aaslund⁴, Andrej Shevchenko⁴, A. Francis Stewart⁵

¹BIOTEC TU-Dresden, Tatzberg 47-51, 01307 Dresden, Germany
²present address: The Victor Chang Cardiac Research Institute, Level 6, 384 Victoria Street, Darlinghurst NSW 2010, Australia
³Max Planck Institute for Molecular Cell Biology and Genetics, Pfortenauerstrasse 108, 01307 Dresden, Germany
⁴Department of Molecular Biology, University of Bergen, HIB, P.O. box 7800, N-5020 Bergen, Norway

We have applied sequential epitope tagging and mass spectrometry (SEAM) approach in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* to determine and compare the proteomic environments of chromatin related protein complexes.

Initially 29 proteins in *S. cerevisiae* were selected bioinformatically on the basis of presence of chromatin related domains (SET, SANT, chromo, HDACs and Sir2-homology deacetylase proteins). They were then TAP-tagged and purified. Of the initial 29, 14 proteins were found to exist in multi-protein complexes.

Co-immunoprecipitating proteins were identified by mass-spectrometry, TAP-tagged and purified themselves.

Through this approach a number of proteins were found to be shared between two or more different protein complexes and were termed ‘proteomic hyperlinks’. Eight of these serve as links in a network of protein complexes including the major yeast histone deacetylase complex (Rpd3C), the major H4 acetyltransferase complex (Esa1/NuA4C) and two chromatin remodeling complexes, one of which (Swr1C) is responsible for incorporation of H2A.Z into chromatin.

A similar approach was then applied then in *S. pombe*. *S. pombe* was chosen mainly because of its evolutionary distance from budding yeast and vertebrates, its simplicity and ease of handling. The *S. pombe* homologs of the proteomic hyperlinks from *S. cerevisiae* were TAP-tagged, purified and protein complex members identified. While this analysis revealed remarkably conserved proteomic cores of the complexes in the two yeasts, important aspects of the network’s topology differed. The network in *S. pombe* appears to be more complex and more closely related to partial networks derived from higher organisms. To our knowledge this is the first systematic comparative proteomic attempt between different species. The nature of the proteomic hyperlinks connecting complexes of related but seemingly antagonizing functions raises many questions in regards to the targeting and regulation of such important gene expression machinery especially in the light of the recent findings on the role of Eaf3 in H3-K36Me recognition.

Genome wide patterns of histone modifications in fission yeast

Indranil Sinha, Marianna Wire’n and Karl Eklund*

Karolinska Institutet, Dept. of Biosciences/School of Life Sciences, University College Sodertorn, Alfred Nobel’s Alle’ 7, S-141 89, Huddinge, Sweden;
Tel: +46-8-6084713;
Fax: +46-8-6084510;
E-mail: karl.ekwall@sh.se

We have used oligonucleotide tiling arrays to construct genome-wide high-resolution histone acetylation maps for fission yeast. The maps are corrected for nucleosome density and reveal surprisingly uniform patterns of modifications for five different histone acetylation sites. We found that histone acetylation and methylation patterns are generally polar, i.e. they change as a function of distance from the ATG codon. A typical fission yeast gene shows a distinct peak of histone acetylation around the ATG and gradually decreased acetylation levels in the coding region. The patterns are independent of gene length but dependent on the gene expression levels. H3K9Ac shows a stronger peak near the ATG and is more reduced in the coding regions of genes with high expression compared with genes with low expression levels. H4K16Ac is strongly reduced in coding regions of highly expressed genes. A second microarray platform was used to confirm the 5’ to 3’ polarity effects observed with tiling microarrays.

By comparing coding region histone acetylation data in HDAC mutants and wild type, we found that hos2 affects primarily the 5’ regions, sir2 and clr6 affect middle regions, and clr6 affects 3’ regions. Thus, mechanisms involving different HDACs modulate histone acetylation levels to maintain a 5’ to 3’ polarity within the coding regions.
Comparative analysis the role on RNAi mechanisms of the Dictyostelium discoideum HelF gene and its putative Schizosaccharomyces pombe homologue

Huan Wang, Brendan Curran and Conrad Lichtenstein

School of Biological and Chemical Sciences, Queen Mary, University of London, London, E1 4NS, UK

RNA-induced gene silencing, first discovered in plant cells, appears to occur in the majority of eukaryotic organisms. One form of such gene regulation is post-transcriptional gene silencing (PTGS). In PTGS, dsRNAs are digested by the RNaseIII-related enzyme Dicer into 21–23bp small interfering RNAs (siRNAs), which assemble with cellular proteins to bind the target messenger RNA (mRNA) by sequence complementary. This leads to cleavage of the target mRNA thereby preventing protein synthesis.

It has already been established that the Dicer enzyme of the soil amoeba, Dictyostelium discoideum lacks a highly conserved helicase domain. Intriguingly, a genome search in Dictyostelium revealed a new gene, HelF, with high homology to the helicase domain usually found in the Dicer proteins. Moreover subsequent analysis of the S.pombe genome identified a putative homologue of the Dictyostelium discoideum HelF gene. Experimental results show that HelF is a natural nuclear suppressor of RNAi in Dictyostelium begging the questions: Do these two enzymes have overlapping functions, and does the putative S.pombe HelF homologue also negatively regulate RNAi in S.pombe?

Here using heterologous protein expression and fluorescence microscopy we have revealed that although the Dictyostelium discoideum HelF protein localizes to the nuclei of S.pombe cells, the product of the S.pombe HelF homologue does not localize to the nuclei of Dictyostelium cells. This suggests that despite their similarities these proteins have different cellular functions. Moreover, although HelF is a non-essential gene in Dictyostelium, attempts to isolate a haploid S.pombe strain carrying a HelF homologue knock-out have so far failed. We are currently attempting this knock-out in a diploid strain to test the hypothesis that the putative HelF protein is encoded by an essential gene in S.pombe. We can also use our already developed RNAi selective system to investigate the role of this HelF homologue on RNAi mechanisms in S.pombe.

huan.wang@qmul.ac.uk

Genome-wide transcript analyses in fission yeast

Brian Wilhelm, Samuel Marguerat, Chris Penkett, Stephen Watt, Jürg Bähler

Fission Yeast Functional Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, U.K.

Although the complete genome sequence for Schizosaccharomyces pombe has been available for several years, its full transcriptome remains unknown. While much of the genome content has been characterized through similarity to genes in other organisms, many other genes remain poorly described or are simply hypothetical open reading frames. Using high-densi-
Comparative analysis of mechanisms that program gene expression: role of the Gcn5 histone acetyltransferase in *S. cerevisiae* and *S. pombe*.

Yongtao Xue-Franzén**, Anna Johnsson**, Valerie Wood and Anthony Wright**

1School of Life Science, Södertörns Högskola, Sweden
2Department of Biosciences, Karolinska Institute, Sweden
3The Sanger Institute,

The budding yeast Saccharomyces cerevisiae (*S. cerevisiae*) and the fission yeast Schizosaccharomyces pombe (*S. pombe*) are thought to have diverged from one another about one billion years ago. It is therefore possible to study the conservation/divergence of mechanisms that reprogram the genome in response to changes in the external environment over a long evolutionary distance. We have shown that the Gcn5 histone acetyltransferase (HAT) is required for efficient adaptation of both organisms upon exposure to KCl and CaCl2 under identical culture conditions. Mutant strains of either yeast that lack Gcn5 grow poorly under these conditions. We have used DNA microarray analysis to compare changes in the expression of over 2000 orthologous pairs of genes during KCl adaptation in the two yeasts. While there is a group of orthologous genes that exhibit a conserved adaptation response, both yeasts also show species-specific responses to KCl-induced stress at the transcriptional level. We identified a subset of KCl adaptation genes that are Gcn5 dependent. Interestingly, these genes are almost exclusively genes that exhibit species-specific KCI adaptation responses. We conclude that the differential role of Gcn5 during KCL adaptation in the two yeasts is either (i) an example of independent evolution or (ii) divergent evolution. Both models suggest that co-regulator proteins like Gcn5 are variable in their functional role even though they are structurally conserved.

**Genome-wide Translational Control in Fission Yeast**

Daniel H. Lackner1, Traude H. Beilharz1, Samuel Marguerat1, Thomas Preiss1 and Jürg Bähler1

1Wellcome Trust Sanger Institute, Fission Yeast Functional Genomics Group, Hinxton, Cambridge, CB10 1SA, U.K.
2Molecular Genetics Program, Victor Chang Cardiac Research Institute, Darlinghurst (Sydney) NSW 2010, Australia

We are interested in global roles of translational regulation and its coordination with other levels of gene expression control. To obtain translational profiles for all mRNAs, polysome preparations are separated according to their size using a sucrose gradient and the mRNAs in each fraction (or pools of fractions) are identified and quantified with DNA microarrays. Starting with exponentially growing cells, we analyzed 12 polysome fractions using DNA microarrays containing elements for all known and predicted genes of fission yeast. This approach provided data on average numbers of associated ribosomes for most transcripts. Integration of data on mRNA steady-state levels revealed an interesting bias: the most abundant transcripts seem to be associated with many more ribosomes than less abundant transcripts, although ribosome density seems to only correlate weakly with transcript levels. We also found a strong inverse correlation between ribosome density and gene length: shorter genes tend to be much tighter packed with ribosomes than longer genes. We are now testing whether these trends depend on other mRNA features such as stability or polyA length. To obtain global data on polyA length, transcripts are fractionated using polyU columns and fractions of different tail lengths are quantified with DNA microarrays. Transcript stability is determined by measuring mRNA levels at different times after blocking transcription.

Integration of these varied data sets will provide insight into global mechanisms of post-transcriptional regulation. Moreover, combining translational and expression profiling of cells in different cell-cycle stages or subjected to various genetic and environmental perturbations will provide a genome-wide view of translational regulation in fission yeast, complementing our expression profiling data.
Mitotic and Meiotic Cell-Division Cycle

Analysis of negative regulators of the septation initiation network

*Philippe Collin and Viesturs Simanis

Swiss Institute for Experimental Cancer Research (ISREC), 155 Chemin des Boveresses, Epalinges, 1066, Switzerland.
E-mail: philippe.collin@isrec.ch.
Phone: +41-21-692-58-58

The SIN is a signal transduction network that triggers the initiation of septum formation. It is composed of four protein kinases and their regulatory subunits (pl1, cdc7 – spg1, sid1 - cdc14 and sid2 - mob1). SIN proteins are anchored to the poles of the mitotic spindle by a scaffold comprising sid4 and cdc11. Signalling is mediated by activation of the ras-superfamily GTPase spg1p. Signalling by the SIN is inhibited by mitotic kinase activity and a two-component GTPase activating protein (GAP) composed of byr4p and cdc16p. A number of other negative regulators of the SIN have been identified, including fin1, dma1, zfs1, scw1 and par1-PP2A. We will present our work studying the function and cell cycle regulation of inhibitors of the SIN.

Myo51, a fission yeast type V myosin with multiple roles in meiosis.

A. Doyle and D. P. Mulvihill.

Department of Biosciences, University of Kent, Canterbury Kent, CT2 7NJ, UK.

The fission yeast Schizosaccharomyces pombe, is a non-motile unicellular eukaryote, and has proven to be extremely useful in the study of cellular processes such as mitosis, meiosis and regulation of the cell cycle, due to its similarity to mammalian cells.

S. pombe expresses three classes of the motor protein myosin (types I, II and V) to maintain basic cellular function. Of the two type V myosins, Myo52 is involved in vacuole distribution, cytokinesis, cell growth and the organisation of the mitotic spindle, whereas the function of Myo51 has remained elusive (Cell Motil Cytoskeleton. 51:53-56).

Myo51 expression increases dramatically upon entry to meiosis (Nature Genetics 32:143-147), and we have been focusing upon identifying Myo51’s function during this cellular process.

Myo51 has a distinct localisation during meiosis and appears to have multiple functions during this event. We will describe our latest findings on this motors role in meiosis.

and the organisation of the mitotic spindle, whereas the function of Myo51 has remained elusive (Cell Motil Cytoskeleton. 51:53-56).
Neck domain independent movement of the fission yeast type V myosin, Myo52

Agnes Grallert1 Suzanne R. Edwards2 and Daniel P. Mulvihill3
1Cancer Research UK Cell Division Group, Paterson Institute for Cancer Research, Wilmslow Road, Manchester, M20 4BX. 2Department of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ.

Intra-cellular movement is a fundamental property of all cell types, as the precise sub-cellular localisation of organelles is essential for their correct function and therefore a basic requirement for cell viability. Many organelles and molecules are actively transported throughout the cytoplasm by molecular motors. Myosins Vs are one such motor protein. They are dimeric actin based motor proteins, which transport organelle cargoes throughout a cell. They possess a long neck region that has previously been hypothesised to allow the protein to move rapidly by taking large steps along actin cables. As yet very little is known about their regulation or precise cellular function.

The genome of the fission yeast, Schizosaccharomyces pombe, encodes only 5 myosins, thus providing the perfect model system in which to study the regulation and function of individual myosins. Using fluorescent labelling, the S. pombe type V myosin, Myo52, can be visualised as vesicle like dots, which move rapidly along actin cables throughout the fission yeast cytoplasm. We will present data from our mutant analysis of the Myo52 protein. Removing the long IQ motif containing neck region had little affect on Myo52 velocity, demonstrating that a property other than step size determines this motor protein’s cellular velocity. In contrast mutations within the motor domain affecting the motor protein’s affinity for nucleotide and actin fail to complement the growth defects associated with the myo52Δ allele. Models will be presented which attempt to explain these findings.

A role for the septation initiation network in the formation of the cytokinetic ring in fission yeast

Olivier Hachet and Viesturs Simanis

Swiss Institute for Experimental Cancer Research (ISREC)
Chemin des Boveresses 155
CH-1066 Epalinges Switzerland

In most eukaryotes, cytokinesis is mediated by a contractile actomyosin ring (CAR). The formation of the CAR depends on the reorganization of the actin cytoskeleton at the onset of mitosis. In Schizosaccharomyces pombe, the onset of septation is temporally coordinated with the end of mitosis through the combined action of the septation initiation network (SIN) and the polo kinase Plo1p. The function of the SIN is to trigger the formation of the division septum and the contraction of the CAR after completion of chromosome partitioning. CAR assembly occurs at the onset of mitosis, independently of SIN signaling. However, ectopic activation of spg1 is sufficient to trigger CAR assembly in interphase, though how this is achieved remains unclear. We are making use of spg1 overexpression to evaluate the involvement of the SIN in CAR assembly.

We characterized the properties of rings induced by spg1 overexpression in G2 arrested cells. Using ring markers such as cdc15-GFP, rlc1-GFP and myp2-YFP, we found that interphasic spg1 induced rings are functional in that they can contract, however most fail to be correctly positioned. This observation show that interphasic spg1 induced CAR lack some spatial information suggesting a bypass of the CAR positioning mechanism or a failure to maintain CAR positioning. Using different genetic backgrounds, we addressed the requirements for the formation of interphasic spg1 induced CAR. Our results suggest a requirement of the SIN pathway for the formation of such rings. These observations suggest that, although the SIN appears not required for ring assembly, the SIN pathway may participate in this process.
Lsk1p and Lsc1p form a CDK-cyclin complex that positively regulates the Septation Initiation Network and is required for Ser-2 phosphorylation of the regulatory heptad repeat found in the carboxy terminal domain of RNA polymerase II

Karagiannis J, and Balasubramanian MK.

Laboratory of Cell Division, Temasek Life Sciences Laboratory, Singapore 117604

In *Schizosaccharomyces pombe* cytokinesis is monitored by a checkpoint system capable of delaying cell cycle progression and promoting actomyosin ring stability. Key components of the checkpoint are the phosphatase, Clp1p, and the Septation Initiation Network (SIN), which function in a positive feedback loop to lengthen the duration in which cells are competent for cytokinesis. To identify novel regulators of this system we screened a genome-wide bank of kinase deletion mutants and identified one knockout strain, *lsk1Δ*, that displayed a striking fragmentation of the actomyosin ring upon perturbation of the cell division machinery. We demonstrate that Lsk1p – a member of the cyclin dependent kinase (CDK) family – forms a physical complex with the previously uncharacterized cyclin, Lsc1p, and that both deletion mutants display indistinguishable phenotypes. In addition, genetic and physiological analysis demonstrate that Lsk1p-Lsc1p acts in parallel to Clp1p to promote actomyosin ring stability upon checkpoint activation. Remarkably, deletion of either gene is capable of rescuing the lethal, multi-septate phenotype conferred by constitutive hyper-activation of the SIN, strongly suggesting that the Lsk1p-Lsc1p complex acts to stabilize the actomyosin ring through its action as a positive regulator of this module. Intriguingly, Lsk1p is most closely related to the family of CDKs that function, at least in part, by modulating the phosphorylation status of a repeated regulatory heptad found in the carboxy terminal domain of RNA polymerase II. Consistent with such a role we are able to demonstrate that Ser-2 phosphorylation of the heptad is abolished in both *lsk1Δ* and *lsc1Δ* mutants.

Fission yeast MES1 is an APC/C substrate and is directly recognized by WD40 activator proteins

Yuu Kimata and Hiro Yamano

Marie Curie Research Institute, Oxted, Surrey, RH8 0TLL United Kingdom

In meiosis, to ensure successive chromosome segregation without intervening DNA replication, the prevention of complete inactivation of the Cdc2 activity is required between meiosis I and meiosis II. We have recently shown that the fission yeast Mes1 protein has a role in maintaining a certain amount of Cdc13 in this period by inhibiting the APC/C activity. Here we investigate the molecular mechanism of APC/C inhibition by Mes1. Mes1 has putative D-box and KEN-box in its sequence. Over-expression of wildtype Mes1 in fission yeast arrests cells at metaphase coincident with accumulation of Cdc13 and Cut2, whereas expression of D-box and KEN-box double mutant does not. To ensure the idea that Mes1 is an APC/C substrate, we show that Mes1 is destroyed in an APC/C- and Fizzy-dependent manner in Xenopus egg extracts, which requires intact D-box and KEN-box. Furthermore Mes1 is efficiently polyubiquitinated by APC/C in vitro. These data strongly suggest that Mes1 inhibits APC/C-dependent destruction of Cdc13 as a competitive substrate. We also show by using site-specific photo-crosslinking techniques that Mes1 directly binds to the Fizzy family of proteins from various species, suggesting that APC/C activator proteins are able to recognize D-box and KEN-box independently of APC/C and this ability is conserved in WD40 domains of activators.
Role of the Septation Initiation Network during meiosis

A. Krapp, P. Collin, A. Cokoja, S. Dischinger, E. Cano and V. Simanis
ISREC, Epalinges, Switzerland

When nutrients are abundant, cells of the fission yeast *Schizosaccharomyces pombe* grow as rods, dividing by medial fission after formation of a medially placed cell wall or division septum. Septum formation is triggered by a group of proteins called the septation initiation network, or SIN. Ectopic activation of the SIN can uncouple septum formation from other cell cycle events, while failure of SIN signalling gives rise to multinucleated cells due to a failure of cytokinesis. When starved, *S. pombe* cells of opposite mating type will fuse to form a diploid zygote that undergoes meiosis producing four spores. No septa or contractile rings are formed during this process, but SIN proteins are expressed and localise to the SPB. Moreover, the localisation pattern of SIN activators and inhibitors suggests that the SIN is activated during the second meiotic division. In agreement, SIN mutants cannot form spores, while all the other steps of meiosis appear normal. This defect in spore formation is due to a failure in the proper development of the forespore membrane.

Functional characterization of Nap1 protein in cell cycle progression of *Schizosaccharomyces pombe*

Eva Lambea, Maribel Grande, Sandra López-Avilés and Rosa Aligué.

Department of Cell Biology, University of Barcelona. C/ Casanova 143. 08036-Barcelona. Spain.

Nap1, a member of the Nap/Set family, has been described in human cells as a histone chaperone protein that promotes nucleosome assembly *in vitro*. In budding yeas, Nap1 has also been described as a member of mitotic signalling network that controls cell growth during mitosis and appears to require shuttling of Nap1 between nucleus and cytoplasm. In order to establish this mitotic signalling network we have studied the role of the homologue Nap1 protein in *Schizosaccharomyces pombe*. We have found that Nap1 is required for the B-type cyclin Cdc13 activity. Deletion of nap1 leads to a delay in the onset of mitosis and we show that this delay is rescued with the *cds1* deletion, which is a kinase involved in the activation of the replication checkpoint. In contrast, the mitotic delay due to *nap1* deletion is not rescued with *chk1* deletion, which responds to the DNA damage checkpoint. Therefore, it indicates that the deletion of *nap1* gene activates specifically the replication checkpoint. These results suggest a model in which the role for Nap1 during DNA synthesis is linked with the onset of mitosis.

In order to further characterize the role of Nap1 during cell cycle progression we have purified Nap1 binding proteins by affinity chromatography and we have identified Sda1 as a protein that binds directly Nap1. In Saccharomyces cerevisiae it has been described that Sda1 is involved in the synthesis of cyclins during G1 progression.

Altogether, these results suggest a model in which the role of Nap1 during DNA synthesis is linked with the onset of mitosis.
The dual role of the mitotic kinase Srk1 in fission yeast

Sandra Lopez-Aviles, Maribel Grande, Eva Lambea and Rosa Aligue

Department of Cell Biology, University of Barcelona. C/ Casanova 143. 08036-Barcelona. Spain.

In fission yeast, the Chk1 and Cds1(Chk2) checkpoint kinases block mitotic entry by inhibiting the Cdc25 phosphatase in response to replication arrest or DNA damage. In this work we show that the stress-activated kinase, Srk1, also inhibits mitotic entry by phosphorylating Cdc25. We find that overexpression of Srk1 kinase causes cell cycle arrest in late G2 phase, an effect which is dependent on the catalytic activity of Srk1. Conversely, cells lacking srk1 enter mitosis prematurely. The G2/M arrest caused by overexpression of Srk1 is due to hyperphosphorylation of Cdc2 on Tyr15 and can be attributed, primarily, to inhibition of Cdc25 activity. Consistent with this we find that Srk1 interacts with Cdc25 in vivo. Most importantly, we find that Srk1 phosphorylates the N-terminal non-catalytic region of Cdc25 by Srk1 in vitro, at the same sites phosphorylated by the Chk1 and Cds1(Chk2) kinases, and that phosphorylation of Cdc25 on these sites is necessary for Srk1 to impose a G2 arrest. Furthermore we show that overexpression of Srk1 causes Cdc25 to accumulate in the cytoplasm, an effect which is dependent on Rad24, a member of the14-3-3 protein family. Given these observations, we propose a model in which Srk1 inhibits Cdc25, avoiding a premature onset of mitosis.

In addition to this role during a normal cell cycle, we show that Srk1 regulates Cdc25 in response to environmental stress. Treatment of wild type cells with osmotic stress leads to the accumulation of Cdc25 in the cytoplasm and to an increase of the Cdc25-Rad24 binding, whereas this behaviour is not observed in srk1-deleted cells. This effect is mirrored in vivo by the stabilization of Cdc25 in the wild type strain compared to the strain lacking Srk1. Moreover, the activity of Srk1 increases dramatically following the exposure of cells to osmotic stress. Taken together, these results suggest that, in a similar manner to Chk1 and Cds1 in response to genotoxic stress, Srk1 is responsible for the cell cycle arrest and Cdc25 stabilization following a non-genotoxic environmental insult.

Characterization of the cellular function of the ubiquitin ligase complex SCF<sub>pof</sub>

Yasmine Mammun, Satoshi Katayama and Takashi Toda

Laboratory of Cell Regulation, London Research Institute, Cancer Research UK
44 Lincoln’s Inn Fields, London WC2A 3PX, UK

Ubiquitination and subsequent degradation of proteins regulate many important cellular processes. Key regulators of cell cycle and division are targeted for degradation by a family of E3 ubiquitin ligases, termed Skp1-Cdc53-F-box (SCF) complexes. In this enzyme complex the F-box proteins act as receptors to bind and recruit substrates thereby conferring the specificity of the ubiquitin transfer. We have characterized the <i>S. pombe</i> F-box protein Pof3, which contains in addition to the F-box two independent protein interaction domains, an N-terminal TPR and a C-terminal LRR motif. Pof3 is part of the SCF complex, as it interacts with Pcu1 (the fission yeast Cullin-1) and Skp1.

In the absence of Pof3 cells exhibit a number of phenotypes including appearance of lagging chromosomes and a high rate of chromosome loss. pof3 cells are highly sensitive to UV and show G2 delay due to constitutive activation of the DNA damage checkpoint, which is required to prevent lethal mitosis. Loss of Pof3 additionally causes substantially shortened telomeres and desilencing of heterochromatin regions. Taken together these phenotypes hint a central role of SCF<sub>pof</sub> in protection of genome integrity.

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The fission yeast Chs2p protein interacts with the type-II myosin Myo3p and is required for the integrity of the actomyosin ring.

Rebeca Martín-García* and M.-Henar Valdivieso.

Ph: +34 923 121589. Fax: +34 923 224876.

In Schizosaccharomyces pombe cytokinesis requires the function of a contractile actomyosin ring. Fission yeast Chs2p is a transmembrane protein structurally similar to chitin synthases that lacks such enzymatic activity. Chs2p localisation and assembly into a ring that contracts during division requires the general system for polarised secretion and some components of the actomyosin ring. Chs2p interacts physically with the type-II myosin Myo3p showing a physical link between the plasma membrane and the ring. In chs2Δ mutants the actomyosin ring integrity is compromised during the last stages of contraction and it remains longer in the midzone.

Additionally, chs2Δ cells are hypersensitive to a minor perturbation of the actomyosin ring. In a synchronous culture, chs2Δ cells exhibit a delay in septation with respect to the control strain. All these results show that Chs2p participates in the correct function of the medial ring.

Putative β-1,3-glucanosyltransferases in S. pombe

María de Medina-Redondo, Carlos R. Vázquez de Aldana and Francisco del Rey.

Instituto de Microbiología Bioquímica, Departamento de Microbiología y Genética, CSIC/Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain.

The cell wall of Schizosaccharomyces pombe is a dense network of polysaccharides, composed of α-galactomannans, α-1,3-glucan and β-glucan (linear β-1,3-glucan, β-1,6-glucan and β-1,6-glucan branched β-1,3-glucan). β-1,3-glucan chains are synthesized by a glucan synthase complex, and remain unorganized and alkali-soluble until covalent linkages occur between β-1,3-glucans and other cell wall components. In Saccharomyces cerevisiae and in Candida albicans, proteins encoded by the GAS and PHR genes have β-1,3-glucanosyltransferase activity, belonging to the GHF72 family. All of them are attached to the membrane through a glycosylophosphatidylinositol (GPI).

A search of the S. pombe database for proteins with sequence similarity to the S. cerevisiae Gas1p resulted in the identification of the gas1p, gas2p, gas4p and gas5p proteins (the products of the SPAC19B12.02c, SPBC29A10.08, SPBC342.3 and C11E3.13c ORF, respectively). Three of these genes (Sp gas1, Sp gas2 and Sp gas5) are expressed periodically during vegetative growth. Sp gas4 has a basal expression during vegetative growth, but its mRNA levels increase during sporulation. gas2Δ and gas4Δ cells showed no apparent growth defect in either rich or minimal medium, neither at 32°C nor at 37°C. However, gas5Δ cells showed a slower division rate at 37°C and thicker cell walls at this restrictive temperature. gas4Δ diploids cells divide normally in rich medium and undergo meiosis correctly in media lacking nutrients. In contrast, this mutant cannot complete sporulation, and forms aberrant spores which are not totally individualized. To determine the subcellular localization of these proteins, fusions with the yellow fluorescent protein (YFP) were constructed, under the control of the native promoter of each gene. So far, we have been able to determine the localization of gas1p protein in the cell wall all around the cells, and also in the septa. gas4p protein is located in the spore wall.
Biochemical analysis of the fission yeast tropomyosin, Cdc8.

Kalomoira Skoumpia, Sheran Attanapola, Arthur C. Coulton, Michael A. Geeses, and Daniel P. Mulvihill

Department of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ.

Cytokinesis, the division of a cell brought about by the contraction of an actin-based contractile ring, is an essential process, conserved in all eukaryotes. Much of what we understand today about the process of cytokinesis and its regulation comes from studies using the fission yeast, Schizosaccharomyces pombe. Tropomyosin, an essential actin-binding protein is required for the maintenance and the stabilization of actin cables throughout the cell cycle, including those incorporated into the cytokinetic actomyosin ring (CAR).

We present data from a cross-discipline study of the fission yeast tropomyosin, Cdc8, which has previously been shown to be essential for CAR formation and therefore for cytokinesis to occur in S. pombe (Nature 360: 84-7). Facilitated by the use of a novel antibody (raised against the full-length Cdc8 protein), we have characterised a number of cell biological and biochemical properties of Cdc8. Both immunofluorescence and GFP tagging methodologies confirm that Cdc8 associates with the actin ring during mitosis, as reported previously. In addition these reagents reveal that the fission yeast tropomyosin associates with actin filaments during interphase. Consistent with this, western blot analysis demonstrate that Cdc8 protein levels do not vary through the cell cycle, and suggests that the protein is subjected to constant post-translational modifications. Biochemical and kinetic analysis of purified endogenous Cdc8 revealed that the protein is acetylated in vivo, and this modification modulates the protein’s ability to associate with actin, thus regulating Cdc8 function in vivo.

Rgf3p is a specific Rho1-GEF that regulates septum synthesis during cytokinesis in fission yeast.

Virginia Tjadura, Patricia Garcia and Yolanda Sánchez

Instituto de Microbiología Bioquímica, CSIC/Universidad de Salamanca and Departamento de Microbiología y Genética, Universidad de Salamanca. Campus Miguel de Unamuno. 37007, Salamanca, Spain. E-mail: ysm@usal.es

Schizosaccharomyces pombe cells divide by binary fission through the formation of a septum that divides the mother cell into two equal daughter cells. At the onset of mitosis a contractile actomyosin ring (CAR), essential for cell division, is assembled in the medial region of the cell but it only contracts at the end of mitosis when the two nuclei have separated and the mitotic spindle has disassembled. At the same time of ring contraction, the synthesis of the primary septum, mainly composed of linear 1,3-β-glucan, begins and it follows as the CAR contraction proceeds to form a plate at the division site. The secondary septa, with a composition similar to the cell wall, are then deposited to each side of the primary septum. The cleavage of the primary septum at the end of cytokinesis liberates the two daughter cells. Thus proper formation and cleavage of the division septum is essential for cell division and to maintain cell integrity during cell separation.

The synthesis of the 1,3-β-glucan, the main component of the primary and secondary septa, in the medial region is carried out by at least two different 1,3-β-glucan synthases whose catalytic subunits are coded by the cps1'/bgs1' and bgs4' genes. The regulatory subunit of the 1,3-β-glucan synthase is the small GTPase Rho1p which acts as a molecular switch varying from an inactive state bound to GDP to an active state bound to GTP.

We identified rgef3' as the gene affected in the ehs2-1 (quinocandin hyper-sensitive) mutant. ehs2-1 is defective in 1,3-β-glucan synthesis and is thermosensitive, at the restrictive temperature cells lyse as doublets. Mutant cells lacking rgef3' are unviavible.

Rgf3p is a specific Rho1p GEF (Guanine nucleotide Exchange Factor). Rgf3p activates Rho1p promoting the change of the GDP bound to the GTPase by GTP and hence increasing the 1,3-β-glucan synthase activity. rgef3' expression is cyclic with a peak in M-phase of the cell cycle and it exclusively localizes at the medial region of the cell where the contractile ring is located and the division septum is assembled. Early in mitosis, Rgf3p forms a ring-like structure that contracts to a dot during the latest stages of mitosis and disappears when the septum is completely formed. rgef3Δ spoires arrest with two nuclei and an actomyosin ring but they are unable to form any septum and thus are unable to divide and propagate.

Flanking the RhoGEF catalytic domain, Rgf3p contain two regulatory domains, a Serine-Threonine rich region at the amino terminus and a CNH (crotephin kinase Homology) domain at the carboxy terminus; this one is essential for Rgf3p activity. The presence of these domains suggests that Rgf3p and thus Rho1p functions could be regulated by interaction with other proteins to switch on 1,3-β-glucan synthase activity.
Opportune retirement of Hrs1p, the organiser of astral microtubule arrays for the horsetail nuclear movement, ensures smooth spindle formation at the onset of Meiosis I

Kayoko Tanaka, Shinya Okamoto, Ayano Kagami and Masayuki Yamamoto

Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan, e-mail: ktanaka@biochem.s.u-tokyo.ac.jp

Upon commitment to sexual differentiation, cells exchange the mating factor signal which induces dramatic reprogramming of microtubule architecture to form an astral microtubule array emanating from the spindle pole body (SPB)1. During meiotic prophase, an oscillatory nuclear movement termed horsetail nuclear movement (HNM) takes place2. HNM is led by the astral microtubule array aided by the dynein-dynactin complex and is proposed to facilitate the alignment of homologous chromosomes necessary for efficient meiotic recombination3,4.

We have shown that a meiosis-specific SPB component Hrs1p (also known as Mcp6) is a key molecule to remodel microtubules into the horsetail-astral array (HAA)5. Deletion of Hrs1p impaired HAA formation, leading to compromised HNM. Ectopic expression of Hrs1p during the mitotic cell cycle resulted in the formation of a HAA-like astral microtubule array, which drove an oscillatory nuclear movement in interphase cells. Hrs1p interacted with components of the γ-tubulin complex (γ-TuC) as well as with a meiotic SPB component, suggesting that Hrs1p facilitates formation of the HAA, responsible for the vigorous HNM, by stabilising connection between the SPB and minus ends of microtubules.

Expression of Hrs1p is restricted to meiotic prophase. Upon the onset of meiosis I, Hrs1p abruptly disappeared from the SPB even when it was forcibly expressed under the regulation of a strong nmt1 promoter. To see the effect of ectopic Hrs1p expression during meiosis, we isolated a few hrs1 mutants in which mutated Hrs1 proteins were stabilised at the SPB during meiosis I and II. In one of the mutants (hrs1-1), relative timing of the spindle formation after the cessation of HNM was delayed and malformed spindles were observed. These results indicate a possible mechanism to down-regulate Hrs1p at the onset of meiosis I to ensure a smooth spindle formation.

References

Timely destruction of S. pombe Rad54 by the Anaphase Promoting Complex

Michelle Trickey and Hiro Yamano

Marie Curie Research Institute, Oxted, Surrey, RH8 0TL, United Kingdom

The Anaphase Promoting Complex (APC)/cyclosome is a large multi subunit E3 ligase which is known to orchestrate the cell cycle transition from mitosis to G1. Originally identified as the factor required for cyclin B degradation, the APC/C is now known to be required for the timely degradation of numerous proteins, which include securin, Cyclin A, Cdc6, Xid, UbcH10, geminin and Plk1. The APC/C functions with the aid of co-activator proteins known as Fizzy/Cdc20/Sip1 and Fizzy-related/Cdh1/Ste9, these interact with APC/C from metaphase to anaphase and during G1 respectively. It has been shown that both Fizzy and Fizzy-related are destroyed in an APC/C dependent manner. The APC/C degrades proteins, which carry destruction boxes (D-box), a nine-residue motif RXLXXXXN/D/E and/or KEN boxes. Using an in vitro assay utilising Xenopus laevis egg extracts we have identified Rhp54, the S. pombe homolog of the human and S. cerevisiae homologous recombination protein Rad54, as a target of the APC/C. However, no other members of the Rad52 recombination family in S. pombe, or human and S. cerevisiae homologs of Rhp54 are targets. In vitro Rhp54 destruction is dependent upon a KEN box within its far N-terminus and Fizzy-related activated APC/C with which it interacts in an APC/C independent manner. Studies in S. pombe have found that Rhp54 destruction is restricted to the G1 phase of the cell cycle and is dependent upon the S. pombe Fizzy-related homolog Ste9. However, mutation of the KEN box to AAA is not sufficient in S. pombe to stabilise Rhp54, and an addition factor resides within its N-terminus.
Cellular roles of the Cdc37 molecular chaperone in fission yeast

Emma Turnbull, Jun Liang, Ina Martin and Peter Fantes

Institute of Cell Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, UK

Cdc37 is a molecular chaperone found in fungi and animals. In mammalian cells it interacts strongly with the heat-shock protein Hsp90, another chaperone, but direct interaction appears to be much weaker in fungal systems (1,2).

Most client (substrate) proteins of Cdc37 are protein kinases. We have shown that the cell cycle regulatory kinase Cdc2 is a major client of Cdc37, by a combination of genetics, cell physiology and biochemistry (3). When Cdc37 function is impaired in cdc37ts mutants, cells arrest in G2 with a phenotype very similar to that of cdc37ts mutants. In arrested cdc37ts cells, Cdc2 kinase activity is greatly reduced, as is the level of Cdc2 associated with its mitotic cyclin partner Cdc13, indicating that Cdc37 is required for assembly or maintenance of the active complex. On the other hand, the tyrosine 15 phosphorylation status of Cdc2 is not affected.

In a separate study, we have been carrying out a genetic screen with the aim of identifying novel clients of Cdc37, and have identified candidates. Several of these are the products of genes with known roles in cell cycle progress and cell morphogenesis: we are testing whether they interact directly with Cdc37.


Cytoskeleton and Cell Morphogenesis

Non-core components of the fission yeast γ-tubulin complex

Andreas Anders and Kenneth E. Sawin

Wellcome Trust Centre for Cell Biology, Edinburgh University, Swann Building, Mayfield Road, Edinburgh, EH9 3JR, United Kingdom

Relatively little is known about the in vivo function of individual components of the eukaryotic γ-tubulin complex (γ-TuC). We identified three genes, gfh1+, mod21+ and mod22+, in a screen for fission yeast mutants affecting microtubule organization. gfh1+ is a previously characterized γ-TuC protein weakly similar to human γ-TuC subunit GCP4, while mod21+ is novel and shows limited similarity to human γ-TuC subunit GCP5. We show that mod21p is a bona fide γ-TuC protein and mod21Δ mutants are viable. We find that gfh1Δ and mod21Δ mutants have qualitatively normal but quantitatively reduced numbers of active interphase microtubule organizing centers in vivo, and this is exacerbated by mutations in mod22+. Simultaneous deletion of gfh1p, mod21p and alp16p, a third non-essential γ-TuC protein, does not lead to additive defects, suggesting that all three proteins contribute to a single function. Co-immunoprecipitation experiments indicate that gfh1p and alp16p are codependent for association with a small “core” γ-TuC, while mod21p is more peripherally associated, and that gfh1p and mod21p can form a sub-complex independent of the small γ-TuC. Interestingly, sucrose gradient analysis suggests that the major form of the γ-TuC in fission yeast may be the small complex. We propose that gfh1p, mod21p and alp16 act as facultative “non-core” components of the fission yeast γ-TuC and enhance its microtubulenucleating ability.
Self-organization of cytoplasmic microtubule arrays in fission yeast

Rafael E. Carazo-Salas¹ and Paul Nurse¹,²

¹Cell Cycle Laboratory, 44 Lincoln’s Inn Fields, Cancer Research UK, London Research Institute, WC2A 1PX, United Kingdom
²Rockefeller University, 1230 York Avenue, NY10021, USA

Animal cells can acquire highly ordered microtubule patterns via a combination of structurally enforced assembly and dynamical (motor protein-mediated) self-organization. We show that interphase microtubule arrays in the fission yeast also self-organize. This organizational process requires the microtubule-interacting genes mod20, tip1, ase1 and klp2, but not the spindle pole body. Moreover, this process allows the dynamical generation of a central position in the cell independently of pre-defined spatial cues. We suggest that microtubule self-organization may be essential for the robust generation of intracellular coordinates throughout eukaryotes.

btn1, the orthologue of the Batten disease gene CLN3, is involved in the maintenance of cylindrical rod shape morphology and polarised cell growth

S Codlin¹ and S E Mole¹,²

¹MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London WC1E 6BT, United Kingdom.
²Department of Paediatrics & Child Health, and Biology, UCL.

We have previously reported btn1, the Schizosaccharomyces pombe homologue of the human Batten disease gene, CLN3, as being involved in vacuolar homeostasis. Btn1 is a transmembrane protein that traffics slowly to the vacuolar membrane via the endomembrane system. Here we show that btn1Δ is temperature sensitive for growth at 37°C. Cell death is due to cell lysis, which occurs within two to three cell cycles and 18 h following heat stress. Early temperature sensitive growth defects include swelling and bundling of interphase microtubules leading to curved shaped cells within 15 mins of the temperature shift, and by 7h, all cells were curved. Also, cells exhibited defects in NETO transition after completion of the first round of cytokinesis at 37°C, resulting in monopolar growth. The loss of rod-shaped morphology was accompanied by a progressive failure in F-actin patch movement. First, F-actin re-polarisation following activation of the heat stress response pathway was severely delayed, and following subsequent rounds of mitosis, F-actin patches were either loosely polarised and monopolar or exhibited random cortical localisation. Subsequent cell cycles resulted in the formation of pear-shaped cells, with daughter cells showing total rounded, depolarised cell growth. Cell swelling occurred first at the non-growing end, suggesting a defect in the correct positioning of the polarisome machinery in an attempt to pass through NETO. A genetic interaction was established with for3, supporting a role for Btn1 in F-actin polarisation. Cell lysis was found to be due to defective cell wall composition - btn1Δ cells were highly sensitive to zymolase and swelling and lysis, but not cell curving and monopolarity, were rescued by the addition of 1 M sorbitol to osmotically stabilise cells. The localisation of Btn1 became more endosomal and bipolar upon a temperature shift to 37°C, and this localisation was actin dependent and essential for the rescue of the polarity defects in btn1Δ.

Btr1p therefore, links the endomembrane system with both the actin and microtubule cytoskeleton, in a heat stress response pathway. Since Batten disease is a lysosomal storage disorder with progressive loss of neurons, specialised and highly polarised cell types, fission yeast is proving to be a good model system to understand the molecular basis of this disorder.
Role of Phosphorylation in regulating CLIP-170 and EB1 Fission Yeast Homologues, TIP1 and MAL3

Andréia Feijão and Damian Brunner

European Molecular Biology Laboratory, EMBL, Heidelberg

CLIP-170 and EB1 protein family members are plus end-tracking proteins that accumulate to growing microtubule tips being implicated in the local control of microtubule dynamics and attachment of microtubules to the cell membrane or kinetochores. Tip1p, the fission yeast CLIP-170 homologue is required for targeting of microtubules to cell ends. It spatially regulates microtubule dynamics suppressing catastrophe in the middle regions of the cell, but not at the cell ends. On the other hand the EB1 homologue, mal3p, is a general promoter of microtubule growth. It localizes all along the microtubules and accumulates on their tips, from where it disappears preceding catastrophes. The mechanisms that allow tip1p and mal3p to influence and regulate microtubule dynamics are not yet understood and its also not known what regulates their activities. This may involve phosphorylation because both tip1p and mal3p are phosphorylated. In this context, we are investigating the role of tip1p and mal3p phosphorylation. We show that both mal3p and tip1p are phosphoproteins and that the phosphorylation state of mal3p clearly changes along the cell cycle. We are currently investigating which sites are phosphorylated in these proteins and which kinases could be responsible for their phosphorylation.

Force Provides a Local Cue for the Organization of Microtubules in Fission Yeast

Dietrich Foethke,1 Damian Brunner,1 Francois Nédélec1

1Cell Biology and Biophysics
European Molecular Biology Laboratory
Meyerhofstr. 1, 69117 Heidelberg, Germany

To understand how the complex layout of the microtubule network in interphase arises from the properties of single dynamic microtubules, we created a computer simulation of an interphase cell and compared it with experimental observations. Using overdamped Langevin equations we built a 3-dimensional model of the cell containing the nucleus together with its associated microtubules within a fixed shape. Microtubule properties such as mechanic elasticity and production of force by polymerization are considered together with different models of how microtubule dynamics might be regulated. With the help of the simulation we compared the influence of these models on the geometry of the microtubule network and on the positioning of the nucleus. We find that force dependent microtubule dynamics can explain most of the features observed in wild type cells.
Mapping functional regions of btm1, the Schizosaccharomyces pombe orthologue of the human Batten disease gene CLN3

R. Haines1, S. Codlin1, and S. E. Mole1,2

1MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London, WC1E 6BT, United Kingdom
2Departments of Paediatrics & Child Health and Biology, University College London

btm1 has been reported by our laboratory to be involved in vacuole homeostasis, and to be a functional homologue of the human gene CLN3. Mutations in this gene cause juvenile neuronal ceroid lipofuscinosis (JNCL) or Batten disease, a neurodegenerative lysosomal storage disease. Since S. pombe cells have over 50 vacuoles (the equivalent of the mammalian lysosome) and exhibit striking polarised growth, as do neurons, we have concluded that the fission yeast system provides an excellent tool for the study of JNCL. We have shown that btm1Δ cells have larger vacuoles that are also less acidic, and that this can be rescued by overexpression of GFP:Btm1p. btm1Δ cells also lose rod-shaped morphology upon a temperature shift to 37°C, with defects including cell curving, inability to repolarise actin patches and failure to undergo NETO. In addition we have shown that the ability of GFP:Btm1p to rescue these defects is dependent on its location, implying that Btm1p has more than one function and that controlling the location of the protein may be functionally important.

The most common disease-causing mutation in CLN3 is a 1 kb internal deletion that occurs on 81% of disease chromosomes and results in the loss of a large region of homology between the two proteins. When overexpressed in btm1Δ cells, GFP:Btm1p containing an internal deletion that models this mutation (GFP:Btm1Δ), is localised to the endoplasmic reticulum and the golgi complex, but not to early endocytic compartments unlike full length GFP:Btm1p. Furthermore it was unable to rescue the cell curving defect observed in btm1Δ cells after 4 hours at 37°C. However this protein was able to partially rescue the vacuole size defect in btm1Δ cells, suggesting that it retains partial function. We have also designed a set of deletion and truncation constructs to map regions of the protein required for controlling its intracellular location and its varied functions (curving, polarised growth and vacuole homeostasis). In addition we have used site directed mutagenesis to model all disease causing missense mutations of CLN3 in btm1 and expressed these in our btm1Δ strain. Since all of these mutations occur at residues that are conserved in the S. pombe protein they are likely to be essential for function. These studies have revealed residues that are required for correct trafficking of the protein, as well as those that have impacts, both positive and negative, on vacuole size and pH. The results from this study will help to define the complex mechanism of Btm1p action and its apparent role in cytoskeletal-related functions and vacuole homeostasis, and will add considerably to our understanding of the molecular basis of JNCL.

Network from spindle pole body for cell morphogenesis

Dai Hirata

Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi Hiroshima 739-8530, JAPAN

Cell morphogenesis and the cell cycle are coordinately regulated. In fission yeast, the growth polarity dynamically changes during 3 stages of the cell cycle, initiation of growth upon cell division, NETO (new end take off), and septum formation. However, the coordinated mechanism remains elusive. Recently, we have found that the conserved proteins, MO25-like Pmo25, GC kinase Nak1, Drosophila Furry-like Mor2, NDR kinase Orb6, and Mob2 constitute a morphogenesis network that is important for polarity control and cell separation. These proteins are essential for cell viability and mutations result in failure to establish rod shape upon septum formation. Pmo25 is localized at mitotic SPBs and septum, and act upstream of Orb6. We have found that localization patterns of Pmo25 to the SPB are asymmetrical, reminiscent of components of the septation initiation network (SIN). Further analysis has indicated that the SIN is required for Pmo25 localization, in which Nak1-Orb6 kinase activities are under the control of the SIN. We propose that Pmo25 plays a connecting role between the SIN and the morphogenesis network. In this presentation, we will present a new molecule involved in the network.
Tomography shows the way of interphase microtubules in fission yeast

J.L. Höög*, C. Schwartz, A.T. Noon, E.T. O’Toole, D.N. Mastronarde, J.R. McIntosh, C. Antony*

*EMBL, Heidelberg, Germany; The Boulder Laboratory of 3D EM of Cells, Boulder Colorado, USA

Cellular organisation and the spatial positioning of protein complexes within a cell are critical for the maintenance of cellular architecture and directed cell growth. Fission yeast (Schizosaccharomyces pombe) has proven to be an excellent model for studying cell division and cell morphogenesis. Maintaining the polarity of these rod shaped cells as well as targeting polarity factors to the right location requires an intact microtubule network. Interphase microtubules are arranged into 4-5 cytoplasmic bundles, oriented along the long cell axis. Based on fluorescence microscopy data, the individual microtubules within a bundle are believed to grow and shrink independently of each other.

Electron tomography is ideally suited for a detailed study of microtubule organisation, as it enables the investigation and reconstitution of a volume with optimal resolution. Using this method, we have investigated the exact range of the number of microtubules in a single bundle and the details of microtubule organization at both the cell tips and in the nuclear area, where microtubule nucleation occurs. Solving the microtubule organization within these cells requires the 3-D reconstruction of volumes larger than those represented by a single tomogram. Thus, we have used montage to cover a greater volume of cells, and in some cases we gained an entire cell volume. This is, to our knowledge, the first time a whole eukaryotic cell has been reconstructed to this resolution. Large volumes provide further information about the structure of microtubule bundles as well as their respective orientation and interactions with other organelles i.e. mitochondria.

Cell Polarity Determinants Tea1p, Tea4p and Pom1p Inhibit Cell Division at Cell Ends in Fission Yeast

Yinyi Huang, Ting Gang Chew, Wanzhong Ge, Hongyan Wang, Naweed I. Naqvi and Mohan K. Balasubramanian

Cell Division Laboratory, Temasek Life Sciences Laboratory and the Department of Biological Sciences, National University of Singapore, Singapore e-mail: mohan@ttl.org.sg

The mechanisms positioning the plane of cell division are crucial to all cells. While bacteria appear to choose their division plane by negative regulatory mechanisms that prevent cell division at inappropriate sites, most eukaryotic cells do so by signals that positively influence this process. Cells of the fission yeast Schizosaccharomyces pombe are cylindrically shaped with hemispherical ends and divide through the use of an actomyosin based contractile ring. The metazoan anillin-related Mid1p is required for selection of the division site in fission yeast. Intriguingly, we find that, although mid1 mutants misplace the division septa, the misplaced septa are occluded from the cell ends. This process, referred to as tip-occlusion, is essential for viability of Mid1p-defective cells and requires tip-localized kelch repeat protein Tea1p and associated factors, Tea4p / Wsh3p, and Pom1p (tip-complex). In a genetic screen aimed at identifying molecular mechanism(s) of tip occlusion we identified Cyk3p, an SH3 domain containing protein that localizes to the actomyosin ring and cell ends in a tip-complex independent manner. The tip complex appears to negatively regulate cell end localized actomyosin ring proteins such as Cyk3p in order to achieve tip occlusion. Our studies reveal interesting analogies between the tip-complex and the bacterial Min-proteins, both of which prevent cell division at inappropriate sites.
Microtubules, MAPs, and motors: How to organize the microtubule cytoskeleton.

Marcel Janson, Isabelle Loiocrine, and Phong Tran

University of Pennsylvania, Philadelphia, PA, USA

The microtubule cytoskeleton is essential for cellular processes such as mitosis, organelle transport, and cell polarity. The key organizer of microtubules is the microtubule organizing center (MTOC). All MTOCs are composed of multi-protein complexes and share three general properties: a) They nucleate microtubules. b) They arrange the microtubules into functional patterns. And c) They attach the microtubules to their proper organelle targets. In recent years, the molecules that are localized to the centrosome, the primary MTOC in animal cells, have been catalogued and much progress has been made in understanding the mechanism by which the γ-tubulin ring complex (γ-TuRC) located at the centrosome nucleates microtubules. However, most cytoplasmic γ-TuRCs are not located at the centrosome, and their cellular functions are unknown. Furthermore, while the canonical centrosome arranges radial arrays of microtubules which are attached to the nucleus, many highly differentiated cell types – neurons, myotubes, and polarized epithelial cells – have linear arrays of microtubules which are not attached to the nucleus. The mechanisms which generate linear arrays of microtubules are unknown.

The fission yeast Schizosaccharomyces pombe is an ideal organism to study linear arrays of microtubules and MTOCs because they are genetically tractable, optically convenient for high spatial-temporal resolution imaging and analysis, and have MTOCs which organize four linear arrays of microtubules. There are three classes of MTOCs in the fission yeast: the centrosome-equivalent spindle pole body (SPB), the equatorial MTOC (eMTOC) which forms during cytokinesis, and multiple interphase MTOCs (iMTOCs). Works from ours and several other labs have identified mto1p/mto2p, ase1p, and klp2p as playing key roles in the organization of the interphase microtubule arrays. Using a combination of in vivo live cell imaging and in vitro motility assays with purified proteins, we are investigating the molecular mechanism of how antiparallel linear arrays of microtubules are formed. We discovered that: 1) mto2p recruits γ-TuRCs to pre-existing microtubules and activates de novo nucleation of a new microtubule on the pre-existing microtubule, 2) ase1p preferentially bundles the new and old microtubules into an antiparallel array, and 3) this new antiparallel microtubule array is then pulled to the site of the iMTOC by the minus end kinesin klp2p. Our results suggest a model where microtubules, MAPs, and motors interact in a coordinated manner to organize linear and dynamic microtubule structures.

Control of growth polarity upon perturbed DNA replication

Muneyoshi Kanai¹, Takashi Toda² and Dai Hirata³

¹Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi Hiroshima 739-8530, JAPAN
²Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, PO Box 123, 44 Lincoln’s Inn Fields, London WC2A 3PX, UK

In fission yeast, at a specific point in the G2 phase, growth polarity switches drastically from monopolar to bipolar. This phenomenon is called NETO (New End Take Off). For NETO to take place, two requirements have to be fulfilled, a critical cell size and completion of DNA replication. However, the mechanism underlying NETO remains to be understood. Here we show that S-phase checkpoint pathway is required for the maintenance of the monopolar growth in the pol1/mon7 mutant. Indeed, the over-expression of either Rad3 or Cds1 kinase was able to inhibit the switch to bipolar under the normal growth condition. Further, the activity of the Dyrk family Pom1, essential for the switch to bipolar, in the pol1 mutant was higher than that in the G1-arrested cdc10 mutant with monopolar growing manner. These results suggest that the Rad3-Cds1 kinases coordinate NETO with completion of DNA replication by regulating a molecule(s) that acts downstream of Pom1.
Screening for the mutants that have the defect in cell size control

Shunsuke Kubota, Muneyoshi Kanai, Kazunori Kume, Daisuke Urata, Tetsuya Goshima, and Dai Hirata

Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi Hiroshima 739-8530, JAPAN

In fission yeast, *Drosophila Furry-like* Mor2, in concert with the NDR/Tricornered kinase Orb6, becomes localized at the polarized growing regions and plays an important role in the restriction of growth zone(s) to which CLIP170-like protein Tip1 is targeted. Further the *mor2* mutation induces a Wee1-dependent G2-delay, indicating that the mutation activates the mechanism coordinating growth polarity with cell cycle progression. However, the mechanism inducing the Wee1-dependent G2-delay remains elusive.

To identify a molecule(s) involved in the regulation of *Wee1*, we tried to isolate the mutants that have the defect in cell size control like *wee1* mutant (*weel: WEE1-Like mutant*). Wild-type cells were mutagenized with NTG and cultured at 36°C for 5 h. Using this culture, we isolated the small cells by elutriation. From this collection, we screened for the temperature-sensitive and small cell size mutants. In this screening, we isolated 33 *wee1* mutants and classified into at least 3 loci including the *wee1* mutation.

Fission yeast MO25 protein is essential for cell morphogenesis

Kazunori Kume¹, Muneyoshi Kanai¹, Takashi Toda¹ and Dai Hirata¹

¹Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi Hiroshima 739-8530, JAPAN

²Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, PO Box 123, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

In fission yeast, *Drosophila Furry-like* Mor2 plays an essential role in cell morphogenesis in concert with the NDR/Tricornered kinase Orb6. Mutations of these genes result in the loss of cell polarity. Here we show that the conserved proteins, MO25-like Pmo25, GC kinase *Nak1*, Mor2, and Orb6, constitute a morphogenesis network that is important for polarity control (establishment and maintenance) and cell separation. Pmo25 was localized at mitotic SPBs and then underwent translocation to the dividing medial region upon cytokinesis. Our results indicated that Pmo25 interacts with Nak1 transiently at SPBs and is required for both the localization and kinase activity of Nak1. Pmo25 and Nak1 were essential for Orb6 kinase activity. Further, the Pmo25 localization at the SPBs and the Nak1-Orb6 kinase activities during interphase were under the control of the Cdc7 and Sid1 kinases in septation initiation network (SIN), suggesting a functional linkage between SIN on SPBs and the network for cell morphogenesis following cytokinesis.
Characterisation of Tip1p-interacting proteins in *Schizosaccharomyces pombe*

Lindsay Murrells, Mika Toya, and Damian Brunner

1European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany.
2Present Address: Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, Lincoln’s Inn Fields Laboratories, London WC2A 3PX, United Kingdom

In fission yeast, growth is restricted to the cell ends and, as a consequence, cells are rod-shaped cylinders. Correct positioning of the growth zones and the maintenance of polarity requires the microtubule cytoskeleton, which in turn is regulated by microtubule-associated proteins (MAPs). One such protein is Tip1p, a microtubule plus-end tracking protein, homologous to human CLIP170. Tip1p is transported to the plus-ends of microtubules by the kinesin Te2p, where it is important for the spatial regulation of microtubule dynamics, suppressing catastrophe at the cell cortex in the central regions of the cell, but not at the cell ends. The presence of Tip1p at the plus-ends of microtubules therefore ensures that microtubules efficiently target the cell ends.

Although we now have a good understanding of how Tip1p regulates microtubule dynamics, the mechanisms and proteins that modulate the activity of Tip1p are not yet understood: Tip1p could be a component of many different protein complexes and such additional roles for Tip1p, involving interactions with different cellular structures, are expected.

To identify additional roles of Tip1p, screens have been carried out in the lab to identify proteins that interact with Tip1p *in vitro*. Initially, four Tip one-interacting (Toi) proteins were identified from a pulldown using bac terially-expressed GST-Tip1p, chemically cross-linked to Proteina beads using an anti-GST antibody, as bait. Proteins isolated from an SDS gel were identified using mass spectrometry, however only those proteins giving a clear signal above background could be detected using this method. In order to more sensitively detect Tip1p interactors, an isotope-coded affinity tag (ICAT) method was employed and six additional candidate Tip1p-interactors were identified (collaboration with E Brunner, University of Zurich), which are currently under investigation.

Pob1 functions in the secretory pathway, interacting with Rho3 and the exocyst complex in fission yeast

Kentaro Nakano, Mika Toya, Naomi Kamasawa, Aki Yoneda, Masako Osumi, and Masayuki Yamamoto

1Department of Structural Biosciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8577, 2Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Hongo, Tokyo 113-0033, 3Department of Chemical and Biological Sciences, Faculty of Science, Japan Women’s University, Tokyo 112-0015, Japan

Cell morphogenesis is coordinated with several steps including establishment of cell polarity, organization of polarized cytoskeleton, and trafficking of membrane and other cell materials. In fission yeast, round, bent or T shape mutants have been studied as morphological defective mutants. We have previously shown that pob1-664 mutant cells show a lemon-like shape at the restrictive temperature (Toya et al., 1999).

Here we study how Pob1p is involved in the maintenance of cell morphology. Electron microscopy using the high-pressure freezing and freeze-substitution method revealed that, in the pob1-664 cells, presumed secretory vesicles (~90 nm in diameter) were accumulated along F-actin cables at the restrictive temperature. This indicates that Pob1 may play an important role in vesicle transport associated with actin cables. Rho3, a small-GTPase, was obtained as a multi-copy suppressor of pob1-664 mutation. Rho3 has been shown to have functions at least in two pathways: controlling localization of For3 (Nakano et al., 2002) and modulating exocyst complex including Sec8 (Wang et al. 2003). Given that the pob1-664 cells showed a vesicle accumulation phenotype similar to sec8-1 mutant cells, we studied the functional interaction of these genes. pob1-664sec8-1 double mutant cells showed a severe growth defect at the permissive temperature. At the restrictive temperature, Sec8p was delocalized in pob1-664 cells, while Pob1 localized properly at the cell tips or division site in sec8-1 background. This indicates that the localization of exocyst complex depends on the localization of Pob1.

These results suggest that Pob1 plays a role in maintenance of cell morphol ogy determining appropriate sites for the secretory vesicle targeting by the localization of the exocyst complex.
**Schizosaccharomyces pombe**

Rho5, a protein highly homologous to Rho1, is involved in stationary phase cell survival and spore morphogenesis.

Sergio A. Rincón, Beatriz Santos and Pilar Pérez

Instituto Microbiología-Bioquímica. Departamento de Microbiología-Genética. CSIC/Universidad de Salamanca. 37007 Salamanca. Spain

Rho GTPases are key molecules that regulate several cellular functions, such as cell polarity, secretion or cell wall biogenesis. *Schizosaccharomyces pombe* has six Rho GTPases, Cdc42 and Rho1-Rho5. Rho1 is an essential protein involved in the regulation of the cell wall biosynthesis since it is the regulatory subunit of the β-(1,3)glucan synthase. Rho1 is also important for the maintenance of actin patches. Rho5 is a highly homologous protein to Rho1 (86% identity). We hypothesized that Rho5 might be acting in a similar way than Rho1. However, rho5Δ mutant cells show no morphological or cell wall defects. In order to make sure that rho5Δ is not a pseudogene, we checked the expression of the gene.

* rho5Δ expression was hardly detected during log phase growth but was induced under nutritional starvation conditions. Rho5 overproduction caused a similar, but less penetrant, phenotype than Rho1 overproduction. Importantly, overexpression of rho5Δ but no other rho genes was able to rescue the lethality of rho1Δ cells. Rho5 has a role in stationary phase because the rho1Δ rho5Δ strain had reduced viability during stationary phase compared to that of rho1Δ.

To get information about the possible function of Rho5 in sporation, we made a dominant-negative (rho5T20N) and a hyperactive (rho5G15V) allele of rho5Δ in a homothallic background and both strains showed severe ascospore formation defects. The rho5T20N strain produced four ascospores less refringent and very sensitive to glusulase. These spores showed a very faint signal of Mde10-GFP, a protein that localizes to the ascospore wall, suggesting weak spores walls. On the other hand, the rho5G15V mutant produced four aberrant spores with some wall thickenings, and slightly sensitive to glusulase. These results suggest that Rho5 functions in a similar, but less efficient way than Rho1, plays a non-essential role during stationary phase, and participates in spore wall formation.

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**Multi-mode and multi-step cortical anchoring of tea1p**

Hilary Snaith and Ken Sawin

Wellcome Trust Centre for Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, UK

Tea1p is the key cell polarity regulator in fission yeast. It is an 1147 amino-acid protein which contains six N-terminal kelch repeats and a largely alpha-helical coiled-coil C-terminal domain. Cells lacking tea1p are unable to establish proper bipolar growth and as a result grow in a monopolar manner, often forming additional growth axes. tea1Δ mutants are also defective in the cortical localization of other polarity factors, including the membrane protein mod5p and the tea1p-related protein tea3p. Tea1p itself is predominantly localised at cell tips, following targeting by association with growing microtubule ends. It is subsequently anchored at cell tips via an unknown mechanism that requires both the tea1p C-terminus and mod5p. Once at the cell tips tea1p organised a zone of polarised filamentous actin which drives growth.

Currently there are three major outstanding issues in relation to how tea1p functions in microtubule-mediated cell polarity in fission yeast. Firstly, how tea1p is associated with, and transported on, microtubule plus ends? Secondly, how does tea1p become anchored at the cortex at cell tips? Thirdly, how does tea1p interact with the actin cytoskeleton? In this work we address the second of these questions: tea1p cortical anchoring. We show that the tea1p-related protein tea3p, binds independently to both mod5p and tea1p, and that tea1p and mod5p can also interact directly, independent of tea3p. Despite their related structures, different regions of tea1p and tea3p are required for their respective interactions with an essential central region of mod5p. We demonstrate that tea3p is required for proper cortical localization of tea1p, specifically at non-growing cell tips, and that tea1p and mod5p are independently required for tea3p localization. Further, we find that tea3p is co-transported with tea1p by microtubules to cell tips, but this occurs only in the absence of mod5p. Together these results suggest that independent protein-protein interactions among tea1p, tea3p and mod5p collectively contribute to tea1p cortical anchoring at cell tips, and allow us to propose a multi-step and multi-mode model for this process. According to the model, tea1p is initially in a transport complex on microtubule plus ends, in association with other plus-end binding proteins. (1) Upon reaching cell tips tea1p interacts with mod5p. This interaction is important for localisation of tea1p at the cortex but is by itself insufficient for the correct steady-state localization of tea1p and mod5p. (2) Tea1p then further interacts with cortically-localized tea3p. This may occur at both cell tips but is functionally important primarily at non-growing tips. (3) We also suggest that at growing cell tips there may be a parallel, tea3p-independent pathway of tea1p retention, involving both a tea1p-mod5p interaction and an interaction of tea1p with other cortical proteins. (4) Finally, we propose that in addition to the mod5p-dependent pathway of cortical tea1p anchoring, a complementary mechanism exists whereby tea1p and tea3p functionally interact both in the cytoplasm and at the cortex to “bypass” mod5p. This bypass operates only in the absence of mod5p and therefore may represent a homeostatic mechanism that can maintain polarity fidelity irrespective of the precise levels of mod5p.
Modeling of the regulation of fission yeast growth modes

Saša Svetina

Institute of Biophysics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Fission yeast growth machinery acts within localized growth zones which are during interphase at the rounded ends and during the division process in the middle of this rod-like cell. A wild type cell first grows in a monopolar growth mode at the preexisting cell end. Later on in the cell cycle a bipolar growth mode is established, by the growth being induced also at the new cell end. Several gene products and their mutual interactions have already been revealed that are involved in the positioning of the growth machinery and thus in the regulation of fission yeast growth modes. Therefore the attempts to integrate the existing information in terms of a model seem to be justified. In a switch-like model introduced recently it was assumed that different growth modes correspond to different stable steady states of an underlying regulatory circuit which was described in terms of a logical circuit based on three binary variables. The signaling pathways through which the system variables act on each other were represented by circuit linkages expressed as logical operations. Different types of mutants were predicted by abolishing a single or more circuit linkages. The bipolar growth mutants obtained in this manner could be essentially grouped into those that arise due to the loss of cell ability to inhibit the activators of the monopolar growth mode, and those that arise due to the loss of cell ability to activate the bipolar growth mode. In order to relate the predictions of the proposed regulatory circuit to underlying molecular mechanisms, different known gene products that act as polarity factors have been assigned to different circuit linkages. In the present work we include into the assignment analysis the literature data on growth initiation sites of different bipolar growth mutants, and also take into consideration recently described polarity factors Slg2p and Tea4p.


DYRK-family protein kinase, Pom1, interacts with Rho GAP

Hisashi Tatebe1, Kentaro Nakano2, Attila Glatz1 and Kaz Shiozaki1

1Section of Microbiology, University of California, Davis, California, USA
2Department of Structural Biosciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, JAPAN

The DYRK (dual-specificity tyrosine regulated kinase) family members have a Tyr-X-Tyr motif in the activation loop and are structurally related to MAP kinases that have a Thr-X-Thr motif instead. DYRKs autophosphorylate the Tyr-X-Tyr motif, resulting in full activation to phosphorylate Ser/Thr residues in substrate proteins. DYRK genes have been identified ubiquitously among eukaryotes and implicated in the control of neuronal cell growth. A mutation in a DYRK gene in Drosophila, mini-brain, brings about marked reduction in the adult brain size. Human DYRK1A is among the genes mapped to the Down syndrome critical region.

S. pombe Pom1 DYRK localizes at cell tips and septation sites in a manner dependent on the cell-end marker Tea1 and Tea4/Wsh3. The $\Delta$ pom1 mutant is defective in NETO and shows monopolar growth with cytoskeletal actin concentrated to the growing end. We have found that, like other DYRKs, Pom1 is indeed Tyr-phosphorylated. A yeast 2-hybrid screen for proteins that interact with Pom1 has identified Rga4, one of the nine Rho GTPase-activating proteins (GAPs) in S. pombe. Rga4-GFP is localized to cortical regions except cell tips, showing "corset"-like staining around the cell. Interestingly, $\Delta$ pom1 cells cannot maintain this characteristic localization pattern of Rga4. The arga4 mutant is viable but actin patches are less polarized and ~50% of the mutant cells fail NETO. We will discuss the role of Rga4 and Rho-family GTPase in cell polarity, together with our latest results.
Mitotic Spindle

Cdc2 phosphorylation of fission yeast Dis1 similar to XMAP215/TOG improves segregation accuracy via metaphase kinetochore localization

Keita Aoki, Yukinobu Nakaseko, Kazuhisa Kinoshita, Gohta Goshima and Mitsuhiro Yanagida*

Department of Gene Mechanisms, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
*Present address: Max Planck Institute of Molecular Cell Biology and Genetics, D-01307 Dresden, Germany.
1Present address: Department of Cellular and Molecular Pharmacology, University of California-San Francisco, San Francisco, CA 94107

Spindle formation and microtubule dynamics are vital for chromosome segregation. Kinetochore microtubules are shortened in anaphase to bring separated chromatids toward the opposite spindle poles. Fission yeast Dis1, similar to frog XMAP215, human Top, fly mini spindles, nematode Zyg-9 and budding yeast Stu2 that are required for proper microtubule dynamics, is immuno-coprecipitated with the central centromere DNAs in mitosis. We here report that Dis1 is hyper phosphorylated by Cdc2 in mitotic metaphase and rapidly dephosphorylated in anaphase. Whilst Dis1-6A mutant cells that substitute all of the six Cdc2 sites can produce colonies at 22-36°C, they lose minichromosome at a high frequency, become hypersensitive to a tubulin poison and synthetic lethal with mis12-537, a centromere defective mutant. Cdc2 phosphorylation of Dis1 improves the fidelity of chromosome segregation through conferring on Dis1 the ability to locate at metaphase kinetochores. The signals of GFP-tagged unphosphorylatable Dis1-6A are greatly diminished in the metaphase kinetochores, but instead present along the short spindle. The wild type Dis1 is enriched at the plus and minus ends of microtubules, respectively, in metaphase and anaphase. The transition is regulated by Cdc2 phosphorylation and dephosphorylation. Dis1-6A resembles and partly substitutes Aip14/Mtc1, another XMAP215-like protein in fission yeast.

Characterisation of the fission yeast DASH-kinetochore complex

Karen Crawley, Takashi Toda

Cell Regulation Laboratory, London Research Institute, Cancer Research UK

Bipolar attachment of the kinetochore to the mitotic spindle is a crucial step for sister chromatid separation during mitosis. The kinetochore is a specialised proteinaceous structure formed on centromeres that is required for tethering the spindle. It consists of many sub complexes including inner core platform proteins and outer proteins. In the budding yeast Saccharomyces cerevisiae, the DASH complex is an essential complex consisting of 10 components and plays a vital role in chromosome segregation. All 10 homologues of DASH have been recently identified in the fission yeast, Schizosaccharomyces pombe, an organism that contains a larger and more complex centromere structure. In this organism all the DASH genes are not essential but nonetheless play an important role in chromosome segregation. Deletion mutants of the DASH genes display chromosome instability and are synthetically lethal with deletions of the kinesin-like molecules Klp5/6 (kinesin-8). Klp5 and Klp6 are known to play a role in promoting bipolar attachment of the spindle to the kinetochore. Thus DASH may regulate microtubule attachment at the kinetochore in concert with Klp5/6, thereby generating tension. We have screened for DASH mutants in a klp5 null background by selecting survivors that are functional at the permissive temperature but lose DASH function when shifted to the restrictive temperature. We are characterising the mitotic phenotypes of these mutants to gain insight into DASH function during mitosis.

This work is partly performed under collaboration with Dr. Jonathan Millar’s group (Sanchez-Perez et al., 2005). Sanchez-Perez, I., Renwick, S.J., Crawley, K., Karig, I., Buck, V., Meadows, J.C., Franco-Sanchez, A., Fleig, U., Toda, T., and Millar, J.B. (2005). The DASH complex and Klp5/Klp6 kinesin coordinate bipolar chromosome attachment in fission yeast. EMBO J. 24, 2931-2943.
localised coiled-coil protein that is recently identified to be the orthologue of budding yeast Spc110p and human kendrin, has been shown to play an essential role in bipolar spindle formation and chromosome segregation. Also it is known that overproduction of Pcp1 results in SPB abnormalities. With the aim to unravel the role for Pcp1 in spindle nucleation and its regulation during the cell cycle, we are currently examining mitotic phenotypes of conditional mutants of pcp1 that were isolated in the lab. Preliminary observations of the mutants indicated multiple mitotic defects including chromosome misregulation, deformed mitotic spindles and delayed mitotic progression.

The DASH complex and Klp5/Klp6 kinesin co-ordinately control chromosome bi-orientation.

Franco-Sanchez A., Sanchez-Perez 

In order for each set of sister chromatids to be separated to opposite spindle poles, each kinetochore must be bound to microtubules from one pole and its sister kinetochore to microtubules from opposite pole, a phenomenon known as spindle bi-orientation. The mechanism governing the establishment of spindle bi-polarity has been examined in some detail in budding yeast, in which each kinetochore is bound by only a single microtubule (2-4 in fission yeast). In this mechanism are involved a number of microtubule binding proteins and kinetochore associated proteins. These proteins are phosphorylated by the Aurora like Ipl1 kinase, which triggers microtubule detachment. One of these complexes is DASH, which is essential in budding yeast and forms both rings and spiral structures on microtubules in vitro. However in fission yeast posses at least two pathways to establish a correct bi-polar mitotic spindle (DASH is not essential), one that requires DASH and one that does not. We carry out a genetic analysis to establish any link between DASH complex and kinetochore associated proteins as Mal2p, Mts6p or Mis12p and Kinesins as Klp5p or Klp6p. We were unable to construct a DASH complex deleted strain an either Klp5 or Klp6 deleted, indicating that DASH complex and kinesins Klp5/Klp6 perform an essential over-lapping function. On the other hand double mutants between deleted DASH complex and either others kinesins (Klp1, Klp2, Klp3 or Kip4), are viable indicating that the interaction between the DASH and Klp5/Klp6 is specific.

To examine the nature of the essential role shared by DASH and Klp5, spores lacking both genes could germinate. The cells had an elongated mitotic spindle and separated spindle poles indicating that the cells had entered but failed to exit mitosis. In conclusion, we find that mitotic centromere associated Klp5/Klp6 kinesin complex is essential in cells lacking components of the DASH complex. Cells lacking both complexes undergo a first cell cycle arrest in mitosis due to a failure to establish chromosome bi-orientation.
Characterisation of a new allele of CUT12: A Spindle Pole Body component implicated in the control of commitment to Mitosis

Daphné Garcin, Agnes Grallert, Anna Poziemba-Niedbala, Iain Hagan

Paterson Institute for Cancer Research (CRUK), Christie Hospital, Wilmslow Road, Manchester M20 4BX, United Kingdom. dgarcin@picr.man.ac.uk

In eukaryotic cells, entry into mitosis is tightly occurs once a trigger level of Cdc2-CyclinB activity is reached. This trigger is then amplified by a positive feedback loop to promote complete entry into mitosis. Dissecting the mechanisms that control the activation of this complex remains a challenge. Cut12/Stt1 is a spindle pole body (SPB) component that has been involved in the commitment to mitosis as it genetically interacts with cdc25 and the Polo kinase, plo1. In this study, a novel cut12 allele, cut12.2, is being characterised. It is a thermo-sensitive loss-of-function allele of cut12: cut12.2 cells enter mitosis but nucleate microtubules from only one of their two SPBs. The lower penetrance of cut12.2 compared to the one of cut12.1 has enabled us to clone multi-copy suppressors of cut12, such as CyclinB. New genetic interactions between Cut12 and proteins involved in cell division have also been uncovered: for example the dominant activating mutation in cdc22, cdc2-3w, suppresses the conditional lethality of cut12.2.

Mad3 and its regulation of the APC/C

Kevin G. Hardwick, Matylda Szczanieka, Emma King, Julie Blyth and Sjaak van der Sar

The Wellcome Trust Centre for Cell Biology, University of Edinburgh King's Buildings, Mayfield Road, Edinburgh, EH9 3JR, UK

The spindle checkpoint prevents cells with spindle or kinetochore defects from initiating chromosome segregation. Mutations in the Mad (mitotic arrest defective) or Bub (budding uninhibited by benomyl) genes inactivate the checkpoint and allow cells with defective spindles to proceed through mitosis. Such cell divisions lead to chromosome loss, aneuploidy and death.

Mad3, Mad2 and Slp1(Cdc20) co-immunoprecipitate in the mitotic checkpoint complex (MCC), and it is thought that formation of this complex inhibits the activity of Slp1-APC/C and thereby the metaphase to anaphase transition. However, there may be other mechanisms whereby spindle checkpoint proteins inhibit Slp1-APC/C.

Here we describe multiple mechanisms whereby Mad3 regulates the activity of Slp1-APC/C. Mad3 (and BubR1) contain two conserved KEN boxes. Mutation of either of these KEN boxes abrogates the fission yeast spindle checkpoint. We will provide evidence that these signals are crucial for MCC formation, and for the interaction between the Mad2/Mad3 checkpoint proteins and mitotic APC/C.

In addition, we find that phosphorylation of Mad3 by aurora kinase is a key requirement for the spindle checkpoint response to the lack of tension at yeast kinetochores.
Identification of the Alp14-interacting protein using yeast two-hybrid screening

Chiho Ikebe and Takashi Toda

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

Bipolar spindle formation during prometaphase is vital for accurate chromosome segregation in anaphase. The Dis1/TOG microtubule-associated protein (MAP) family is evolutionarily conserved in all eukaryotes from yeasts to humans. All this family proteins localize to microtubule-organizing centers (MTOCs) that include the spindle pole bodies (SPBs) in yeast and the centrosome in animals. This family plays a pivotal role in microtubule functions, including mitotic spindle formation, kinetochore function and cell polarity and morphogenesis.

In the fission yeast, the two Dis1/TOG MAPs homologs, Alp14 and Dis1 have been identified. Alp14 was originally identified in our laboratory as one of morphological mutants (alp stand for altered polarity). Alp14 consists of two separable domains. The N-terminal domain contains 2 TOG domains that by themselves consist of 5 HEAT (Huntingtin-Elongation A-TOR) repeats, which are believed to be involved in protein-protein interaction. On the other hand, the C-terminal region possesses a microtubule-binding activity that is required for targeting of this protein to the SPB via binding to Alp7, the TACC (transforming acidic coiled-coil-containing) homolg. We previously showed that Alp14 is required for bipolar spindle formation. In its absence, spindle-kinetochore interaction is not established, resulting in chromosome mis-segregation. That means Alp14 acts as a structural bridge between the kinetochore and the spindle, thereby facilitating microtubule-kinetochore attachment. In order to identify more Alp14-interacting proteins, we undertook yeast two-hybrid screening, using Alp14 as a bait. We isolated several proteins that interact with Alp14. We have started to characterize these integrators and the results of the functional analysis will be presented.

Dynamics of Spindle Checkpoint Proteins

K. May and K. Hardwick

The Wellcome Trust Centre for Cell Biology, University of Edinburgh, Kings Buildings, Mayfield Rd., Edinburgh EH9 3JR, Scotland.

The mitotic spindle checkpoint prevents progression of mitosis unless all chromosomes are correctly attached to the spindle and tension is generated. Bub1 and Bub3, Mad1-3 and Mph1 are essential components of the spindle checkpoint. On activation of the checkpoint they localise to the site of chromosome/spindle microtubule attachment, the kinetochore. Although these proteins have been well characterised it is still unclear how they function to generate the signal which ultimately leads to APC inhibition and, once generated, how this signal is turned off. To gain insight into the roles of these proteins we have used time-lapse microscopy and FRAP analysis to study the dynamic localisation of these proteins during the cell cycle and on activation of the spindle checkpoint.

Bub1 and 3, and Mad1, 2 and 3 localise to the kinetochore early in an unperturbed mitosis. As mitosis progresses we observe differences in localisation between these proteins. Mad1 and 2, which interact with each other constitutively, become localised to the spindle and spindle pole body (SPB) while the Bub1, Bub3 and Mad3 signals are lost as anaphase progresses. Unlike the other checkpoint proteins Mad1 and 2 localise to the nuclear periphery during interphase. We show that this interaction is mediated by the N-terminal coiled coil domain of Mad1 and the S.pombe Mlp homologue Alm1. We are investigating the significance of these interactions with respect to the spindle checkpoint.
Dissociation of mitotic spindle position from the timing of anaphase onset in fission yeast

John C. Meadows and Jonathan B.A. Millar

Division of Yeast Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

It has previously been proposed that in fission yeast mitotic spindle position is monitored by a checkpoint that controls the timing of anaphase onset. This checkpoint is activated by treatment of cells with Latrunculin A, an inhibitor of actin polymerisation, and requires a subset of spindle assembly checkpoint proteins including Bub1, Bub3, Mad3 and Mph1. We find that cells lacking Mto1, a centrosomin-like protein, have mis-positioned spindles but are not delayed in the timing of sister chromatid separation. Instead we find that Latrunculin A delays the onset of anaphase and causes mitotic spindles to collapse. This effect is exacerbated in cells lacking Ase1, a microtubule associated protein (MAP), which stabilises anti-parallel spindle midzone microtubules. These results suggest that Latrunculin A delays the onset of anaphase by destabilising formation of a bipolar mitotic spindle rather than by causing spindle mis-orientation.

Characterisation of the spindle checkpoint kinase Mph1 in fission yeast

Laura Milne and Kevin Hardwick

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

The spindle checkpoint monitors the kinetochore-microtubule interactions to ensure accurate segregation of sister chromatids during mitosis to prevent aneuploidy. It arrests cells at the metaphase to anaphase transition in response to lack of tension and/or kinetochore-microtubule attachment until the problem can be corrected and anaphase can progress.

Mph1, the fission yeast homologue of Mps1, is one of the kinases involved in the spindle checkpoint. Deletion of Mph1 results in cells with a compromised spindle checkpoint. They do not arrest in response to spindle damage by drugs such as Benomyl or mutations like the cold sensitive tubulin mutant nda3, but attempt to continue through anaphase with the result being cells showing cut phenotypes and ultimately cell death due to chromosome loss.

Mph1 is phosphorylated throughout the cell cycle and the levels of this do not change except during checkpoint activation, for example in response to spindle damage, when it becomes hyperphosphorylated. This modification is Bub1-independent and we are currently testing other kinases for their involvement in Mph1 phosphorylation.

In addition to a role in the spindle checkpoint Mph1, as well as Bub1 (another spindle checkpoint kinase) also has a role in chromosome segregation. These are the only spindle checkpoint components in fission yeast involved in chromosome segregation. In a deletion mutant a high percentage of lagging chromosomes and chromosome loss is observed, much the same as in a Bub1 deletion. Bub1 is also known to have a role in chromosome segregation. When a double deletion of Mph1 and Bub1 is made an additive effect is seen with respect to chromosome segregation. The percentage of lagging chromosomes and chromosome loss almost doubles with respect to the single mutants. We are currently using live cell imaging to study these chromosome segregation defects in more detail.
Regulation of exchanging the daughter nuclei and co-symmetric localization of Cdc7 on the SPBs in the dikaryotic cell division of S. pombe.

Koei Okazaki and Osami Niwa
(Kazusa DNA Research Institute, Japan)

We found that, if a temperature-sensitive SIN mutant is returned to permissive temperature after binucleation at the non-permissive temperature, cells restart propagation with the two nuclei. The two nuclei that had been associated each other during interphase with a Kip2-dependent mechanism began synchronized back-to-back mitoses. Each spindle appeared to only extend outward during early anaphase B. However, at a certain point in anaphase B, the spindles began to extend inward to facilitate the exchange of the inner daughter nuclei. The onset of inner nuclei exchange coincided with the completion of bundling the split cytokinetic actomyosin fibers into a fine ring. It also coincided with the time point when the localization of Cdc7-GFP on the SPB becomes asymmetric, suggesting mechanisms coupling these three events. In accordance with this notion, inhibition of actomyosin ring formation with latrunculin B or heat-inactivation of the temperature-sensitive cdc7-24 gene product induced the precocious onset of nuclear exchange. The Cdc7-GFP signal persisted preferentially on both of the outer SPBs but disappeared from both of the inner SPBs. Like haploid cell division, the Cdc7-GFP positive SPBs were in most cases “new” SPBs in dikaryotic cell division. The head-to-head spindle orientation was not compromised in the presence of latrunculin B. This orientation appeared to be established at an early phase of SPB separation and maintained throughout the most part of mitosis. Transient disruption of cytoplasmic microtubules by a low temperature treatment in G2 phase compromised partially the head-to-head spindle orientation in subsequent dikaryotic cell division. We propose a model that the new SPBs are built opposite to the nuclear partner, and the new-and-old SPB polarity is maintained by the SPB-associated cytoplasmic microtubules during G2 phase.

Optical tweezers in fission yeast: Mechanism of nuclear and septum positioning

Isabel Raabe¹, Leonardo Sacconi², Francesco Pavone¹, Iva Tolic-Nørrelykke²

¹Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany
²European Laboratory for Non-linear Spectroscopy, Via Nello Carrara 1, 50019 Sesto Fiorentino (Florence), Italy

Cells of Schizosaccharomyces pombe have a centrally placed nucleus and divide by fission at the cell center. Microtubules are required for the central position of the nucleus. Genetic studies suggested that the position of the nucleus may determine the position of the septum. Alternatively, the septum may be positioned by the spindle, or by morphogen gradients or reaction diffusion mechanisms. We investigated the role of microtubules in nuclear positioning, as well as the role of the nucleus in septum positioning, by displacing the nucleus with optical tweezers. A displaced nucleus returned to the cell center by the pushing force exerted by microtubules against the cell tips. Nuclear displacement during interphase or early prophase resulted in asymmetric cell division, whereas displacement during prometaphase resulted in symmetric division as in unmanipulated cells. These results suggest that the division plane is specified by the pre-dividing nucleus. Since the yeast nucleus is centered by microtubules during interphase but not in mitosis, we propose that the establishment of the division plane at the beginning of mitosis is an optimal mechanism for accurate symmetric division in these cells.
Precise spindle cutting using a picosecond laser

Isabel Raabe, Sven K. Vogel, Iva Tolic-Norrelykke

MPI of Molecular Cell Biology and Genetics Dresden, Germany

Laser cutting experiments have led to important discoveries in cell biology (Berns et al., Int. Rev. Cytol. 1991). With this technique it is possible to ablate specific structures inside a cell locally and with rapid time resolution. Here we use a low-cost and easy to use picosecond pulsed 405 nm laser diode to dissect GFP-labeled spindle microtubules in fission yeast. Laser irradiation at short exposure times induced spindle bleaching, whereas longer exposures resulted in partial or complete cutting of the spindle. The bleached and partially cut spindles typically continued elongating. The completely cut spindles broke into two segments, which crossed each other or moved away from each other. The segments later reconnected to form a functional elongating spindle in ~50% cases. Mitosis and cytokinesis proceeded normally in most irradiated cells, though the separation of the daughter cells was delayed in comparison to non-irradiated cells. We conclude that a picosecond pulsed laser diode can be used for precise ablation of fluorescently labeled structures in the smallest and most genetically tractable model system for eukaryotic cell division.

Dynamic Studies of Checkpoint Protein Interactions in Fission Yeast S. pombe

Patricia E. Rischitor and Kevin Hardwick

Institute of Cell Biology, University of Edinburgh, UK

Cells must accurately replicate their chromosomes during S phase and then segregate the genetic material equally during mitosis. Failure to correctly replicate and segregate chromosomes results in the gain or loss of genetic information, which may cause lethality or promote the genetic changes that predispose higher eukaryotic cells to cancer. The spindle assembly checkpoint is an elegant regulatory system that delays the onset of anaphase until each and every chromosome has established a bipolar orientation. The components of this system, Mad and Bub proteins are known to form dynamic protein complexes, which partially are activated through their interaction at and with the kinetochores. This poster focuses on two strategies of analyzing such interactions in S. pombe, one strategy being based on FRET technique (Fluorescence Resonance Energy Transfer), which allows direct testing whether two proteins (one donor, eg. Mad2-CFP and one acceptor, eg. Mad1-YFP) come in very close proximity and can form complexes. New tags, with pair fluorescent fluorophores (CFP/YFP; Cerulean/Citrine) for donor/acceptor checkpoint proteins were constructed, and consequently several methods for measuring FRET – acceptor photobleaching and sensitized fluorescence – are being used. Our other strategy is to study the “molecular output” of the interactions between kinetochores and spindle checkpoint proteins. Bub1 protein is thought to act as a “kinetochore scaffold”, as its presence at kinetochores is required for targeting other checkpoint proteins (eg. Bub3 and Mad3). Using an ectopically targeted Bub1 scaffold to telomeres (Bub1-GFP-Taz1Myb), one can test whether Bub1 is sufficient to recruit other proteins to these sites. This will give more insights into deciphering the actual role of kinetochores in checkpoint signalling.
Regulation of chromosome bi-orientation in fission yeast

Isabel Sanchez-Perez, Steven J. Renwick, Karen Crawley, Inga Karig, Vicky Buck, John C. Meadows, Alejandro Franco-Sanchez, Ursula Fleig, Takashi Toda and Jonathan B.A. Millar

1 Division of Yeast Genetics, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

2 Laboratory of Cell Regulation, Cancer Research UK London Research Institute, Lincoln’s Inn Fields Laboratories 44 Lincoln’s Inn Fields, London WC2A 3PX, UK

3 Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany.

In order for each set of sister chromatids to be separated to opposite spindle poles, each kinetochore must be bound to microtubule(s) from one pole and its sister kinetochore to microtubule(s) from the opposite pole, a phenomenon known as chromosome bi-orientation or bi-polar chromosome attachment. Fission yeast is an excellent model system in which to study this process for multiple reasons.

Firstly, fission yeast centromeres closely resemble those in animal cells in that they are large (~35-110 kb) and contain multiple inverted repeat units which are important for establishing centromeric cohesion. Secondly, unlike budding yeast, spindle microtubules are only nucleated during mitosis and each kinetochore is bound to multiple (2-4) microtubules. Finally, many of the proteins of fission yeast and animal cell kinetochores are structurally and functionally conserved.

We have sought factors that are important for the establishment of chromosome bi-orientation in fission yeast. We have identified a truncated allele of dam1 as a multicopy suppressor of the sensitivity of cdc13-117 (cyclin B) and ma3-1 (EB-1) cells to thiabendazole, a microtubule poison. Dam1 binds to the plus end of spindle microtubules and kinetochores as cells enter mitosis. Dam1 is a component of a heterotetrameric complex, variously called the Dam1/DASH/DDD complex that forms rings around the microtubule in vitro. In fission yeast cells lacking Dam1, or other components of the DASH complex, anaphase is delayed due to activation of the spindle assembly checkpoint and lagging sister chromatids are frequently observed and occasionally sister chromatid pairs segregate to the same spindle pole. We find that one of the DASH proteins, Dad1, is also a component of the Mis6 kinetochore sub-complex, suggesting it might tether microtubule associated DASH complex to the kinetochore. Finally, we show that the DASH complex is essential in cells lacking the mitotic centromere associated Klp5/Klp6 kinesin complex. Cells lacking both Dam1 and Klp5 undergo a first cell cycle arrest in mitosis due to a failure to establish bipolar chromosome attachment. We will discuss the roles of DASH and Klp5/Klp6 in establishing chromosome bi-orientation.

Microtubule organisation via regulation of microtubule-associated proteins TACC/Alp7 and TOG/Alp14 throughout the cell cycle

Masamitsu Sato and Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, 44 Lincoln’s Inn Fields, London, WC2A 3PX, United Kingdom

Centrosomal protein TACC (transforming acidic coiled-coil protein) and TOG (tumor overexpressed gene) are conserved MAPs that regulate microtubule dynamics in many aspects of cellular phenomena. TACC and TOG form a complex and the localisation of TOG to the centrosome is dependent upon TACC. In fission yeast, the orthologs of TACC (Alp7) and TOG (Alp14) function throughout the cell cycle. During interphase, Alp7 and Alp14 are required to organise cytoplasmic microtubule structure, and in mitosis, they play key roles in formation of bipolar spindle and establishment of amphitelic microtubule-kinetochore attachment in the nucleus. Therefore, localisation of Alp7–Alp14 complex must be regulated in order to execute multiple functions at each location. Since yeast cells undergo closed mitosis in which the nuclear envelope does not break down, Alp7–Alp14 complex needs to translocate from the cytoplasm to the nucleus upon mitotic entry and vice versa upon mitotic exit.

Here we show that Alp7/TACC possesses an ability to actively transport Alp7-Alp14 complex into the nucleus. Surprisingly, nuclear import of Alp7-Alp14 occurs not only during mitosis but also even during interphase, in which Alp7-Alp14 complex is exported to the cytoplasm. During mitosis, cells are utilising molecular schemes which accumulate Alp7-Alp14 in the nucleus effectively. Nucleocytoplasmic shuttling of microtubule-associated proteins TACC-TOG via the Ran GTPase system is an efficient way to regulate both interphase and mitotic microtubules in a spatial and temporal manner. Regulatory circuit of TACC-TOG as a possible cargo of Ran might be conserved as a mechanism underlying microtubule dynamics and spindle formation.
Msd1p, a novel protein which localises to mitotic SPBs and spindle MTs, is implicated in anchoring of spindle MTs to SPB and proper chromosome segregation

Mika Toya and Takashi Toda

Laboratory of Cell Regulation, Cancer Research UK, London Research Institute, 44 Lincoln’s Inn Fields, London, WC2A 3PX, UK

Accurate transmission of chromosomes during mitosis is critical for maintaining genome integrity. In fission yeast, the spindle pole body (SPB) plays pivotal roles in formation of proper bipolar spindle, thereby ensuring accurate chromosome segregation. We cloned a gene which encodes a novel coiled-coil protein during the screening to find multi-copy suppressors for the alpha-tubulin mutant, atb2-983. The encoded protein localised to mitotic SPBs and spindle MTs, and no specific localisation is evident during interphase, hence we named it Msd1p (Mitotic Specific Dot). The msd1 deletion was viable but resulted in minichromosome loss phenotype. In addition, msd1 mutants showed abnormal spindle MTs that slipped through SPB(s) in ~50% of mitotic cells. The ends of slipped-through spindle MTs were less dynamic and Alp4p, a component of gamma-tubulin complex, was observed at these sites, suggesting that they are minus ends of MTs. DAPI staining showed that DNA was unequally segregated in a part of these cells. A potential role of Msd1p in anchoring spindle MTs to SPBs and its implication in proper chromosome segregation will be discussed.

Identification and characterisation of Soc2, a novel regulator of microtubule stability

Nadeem Shaikh, Steven Renwick, Vicky Buck, and Jonathan B.A. Millar

Division of Yeast Genetics, National Institute for Yeast Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

To identify novel regulators of spindle microtubule stability we isolated multicopy suppressors of the sensitivity of cdc13-117 (cyclin B) cells to thia bendazole, a microtubule poison. We identified five distinct genes including cdc13 (this clone), a truncated allele of dis2 type 1 phosphatase (8 clones), a small heat stable inhibitor of type 1 phosphatase (1 clone) and a truncated allele of dam1, which encodes a component of the heterodecameric DASH complex that links the +TIPS of spindle microtubules to kinetochores. In addition we identified soc2 (7 clones), which encodes an essential protein that contains a UBA (Ubiquitin associated) and an arfGAP (GTPase activating protein) domain. Both the UBA and arfGAP domains of Soc2 are necessary to suppress thia bendazole sensitivity of cdc13-117 cells and viability of Δsoc2 cells. Notably, the soc2 gene was previously identified as a multicopy suppressor of the cold sensitivity of cdc2-r4 cells (GeneDB, Sanger Centre), although we have been unable to repeat this observation. Instead we find that soc2 additionally suppresses the sensitivity of Δmal3 cells to thia bendazole, suggesting that Soc2 is a general regulator of microtubule stability. However live analysis of soc2-gfp cells revealed that Soc2 does not bind microtubules but appears as multiple rapidly moving dots underneath the plasmamembrane which concentrate at the cell tips and division septa. We are currently exploring the possibility that Soc2 may act as a GAP for multiple ARFs (ADP-ribosylation factor) including Arf1 and Arf2, which are involved in vesicle trafficking, and Alp41, which is required for α-tubulin biogenesis.
Genetic analysis of the cut12 gene highlights roles for RNA metabolism in fission yeast cell cycle control

* Victor A. Tallada, Alan J. Bridge, Patrick Emery and Iain Hagan

Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, UK, vatalada@pirc.man.ac.uk (Fax +44 161 466 3109)

Commitment to mitosis is regulated by MPF. MPF is inhibited by phosphorylation of p34<sup>cdc2</sup> by protein kinases related to Wee1. Removal of this phosphate by Cdc25 promotes entry into mitosis. Full-scale commitment to mitosis involves the promotion of Cdc25 activity and down regulation of Wee1 in a positive feedback loop that is triggered by active MPF and involves the protein kinase polo. Fission yeast cdc25.22 mutants can divide if cdc2, wee1 or cut12 are also mutated. Cut12 is an essential spindle pole body (SPB) component that associates with the fission yeast polo kinase Plo1. Recessive cut12 mutants block spindle formation while the dominant cut12.s11 (stf1.1) mutant permits division of cells which lack Cdc25 and promotes the premature association of Plo1 with the SPB and activation of its kinase activity. The recruitment of Plo1 to the interphase SPB, and the suppression of cdc25.22 by cut12.s11 is dependent upon the function of Plo1 itself. Activating Plo1 activity independently of the status of Cut12 enables cdc25.22 cells to divide. The most attractive hypothesis to explain these observations is that the association between Plo1 and Cut12 on the SPB plays a critical role in the feedback loop that regulates commitment to mitosis. We have used a genetic approach to study Cut12 function in greater detail. We have isolated mutations at five loci which can simultaneously suppress the cut12.1 mutation and confer a cold sensitive lethal growth defect. All of the identified genes are involved in RNA production, processing or translation. Interestingly, most of the mutants show cell cycle defects, including monopolar spindle phenotypes that are strikingly reminiscent of the cut12.1 defect, defective spindle pole structure, altered interphase microtubules arrays and nuclear envelope defects. These findings suggest that enhanced production of cell cycle regulators may underlie the suppression of cut12.1 by these mutations. Consistently we have found that elevating the levels of Cdc25 with a mutant that lacks sequences in the 5’UTR, that link Cdc25 production to translation competence of the cell, suppresses cut12.1.

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The Mitotic Role and Regulation of Kinesin-8 Klp5 and Klp6 in Fission Yeast

Amy Unsworth and Takashi Toda

London Research Institute, Cancer Research UK, 44 Lincoln’s Inn Fields, London, WC2A 3PX, UK

It is vital for cells to segregate their DNA equally during mitosis. To do this, chromosomes must be captured by microtubules and bipolar attachment established. Chromosomes then congress at the cell equator before being segregated to opposite poles. These chromosome motions are powered by microtubule dynamics and also by motor proteins such as kinesin and dynein. There are, however, intriguing motor proteins belonging to the Kinesin-8, -13 or -14 families that influence microtubule dynamics by depolymerising microtubules. Most of those characterised play roles in spindle assembly and chromosome segregation.

Klp5 and Klp6 are two such mitotic microtubule depolymerising kinesins (kinesin-8) in fission yeast, which are thought to function together as a heterodimer. They localise to cytoplasmic microtubules during interphase and upon mitotic entry, translocate to mitotic spindles and kinetochores, and then to the spindle midzone during anaphase. We aim to explore the role and regulation of these proteins during mitosis. We have done live microscopy analysis in deletion mutants and found that chromosome congression prior to segregation does not occur. We are also studying the significance of heterodimerisation of Klp5 and Klp6 and are analysing the behaviour of ATPase “rigror” mutants.
Regulation of the Chromosomal Passenger proteins by Shugoshin2 in fission yeast mitosis

Vincent Vanoosthuyse, Sergey Prykhozhij and Kevin G. Hardwick

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

Fission yeast has two members of the Shugosin family, Sgo1 and Sgo2. Although Sgo1 has clearly been established as a protector of centromere cohesion in meiosis I, the roles of Sgo2 remain elusive. Here we show that Sgo2 contributes to proper chromosome segregation in mitosis by regulating the Chromosomal Passenger Complex (CPC). Interestingly, whilst Sgo2 has a pronounced effect on the localisation of Bir1/Survivin on chromosomes in early mitosis, it has only a milder effect on the localisation of the two other known CPC components Pic1/INCENP and Ark1/AuroraB. Consistent with this, co-immunoprecipitation experiments reveal that the Bir1/Pic1 complex, but not the Ark1/Pic1 complex, is destabilised in the absence of Sgo2. This suggests that there are multiple chromosomal passenger complexes, subject to distinct regulation. Interestingly, the absence of Sgo2 has only a minor effect on chromosome segregation in a normal unperturbed mitosis but very significantly increases the rate of chromosome loss and chromosome attachment defects (syntely, merotely) following the release from a spindle checkpoint arrest. This suggests that Sgo2 is an important factor for the successful recovery from a spindle checkpoint arrest. We also show that Sgo2 and the CPC localise to telomeres. Interestingly their telomere localisation is dependent on the spindle checkpoint kinase Bub1. We discuss the possible roles of Sgo2 and the CPC on telomeres at the metaphase to anaphase transition.

Pre-anaphase Spindle Positioning

Sven K. Vogel, Isabel Raabe and Iva Tolic-Norrevykke

Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Pfortenauerstrasse 108, 01307 Dresden, Germany.

Correct positioning of the mitotic spindle in eukaryotic cells is necessary for an adequate separation of sister chromatids before cytokinesis. It also directs symmetric versus asymmetric cell division and thereby influences cell fate choice in many developing cells. It is therefore of fundamental interest to understand the mechanisms underlying the positioning of the mitotic spindle. Schizosaccharomyces pombe is an excellent model system to study spindle positioning because of the small number of microtubules and the reproducible and highly symmetrical cylindrical cell shape. The cell geometry itself most likely contributes to the spindle alignment during anaphase B when the spindle length exceeds the width of the cell. In order to examine positioning of the spindle that is not confined by the cell geometry, we focused on the spindle alignment during pro-metaphase (phase II).

We observed mitosis in wild type cells expressing GFP-α2-tubulin and measured the spindle length and angle in three dimensions. The mitotic phases were determined by examine the spindle elongation rates. We show that the spindle gets aligned already in pro-anaphase. The angle at the beginning of phase II was 37.6 ± 2.2 (mean ± SEM, n=46) which represents a mis-aligned spindle (by definition >30 degrees), whereas by the end of phase II the angle decreased to 27.4 ± 1.7 (mean ± SEM, n=46) which represents an aligned spindle.

Previous studies have shown that during pre-anaphase intra-nuclear astral microtubules nucleate from the spindle pole bodies. We hypothesize that those astral microtubules may contribute to the spindle alignment. Alternatively, asymmetric properties of the nuclear envelope might promote directed movement of the spindle pole bodies and hence align the spindle. We are currently examining these hypotheses.
DNA Metabolism

Characterization of the S. pombe homologues of the human proteins Translin and TRAX

Ron Ben Yosef, Orly Laufman and Haim Manor

Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel

Translin is a single-stranded DNA and RNA binding protein. It is highly conserved in evolution and homologues are found in various mammals, aves, insects, plants and in S. pombe, but not in S. cerevisiae. A Translin homologue designated TRAX, which specifically interacts with Translin but does not bind DNA or RNA, is also found in all these organisms. The human and the mouse Translins selectively bind single stranded G rich DNA and RNA sequences, including (GT)n microsatellites, (TTAGGG)n telomeric repeats and certain types of mRNAs. Based primarily on their nucleic acid binding characteristics, various functions have been suggested for Translin and TRAX. However, a definition of their functions in precise molecular terms is lacking. We reasoned that S. pombe would be most suitable for such functional analysis and therefore initiated a study of the S. pombe Translin (spTranslin) and TRAX (spTRAX). First, we cloned and expressed the two yeast proteins in E. coli. We have shown that, like the previously characterized human Translin, the spTranslin is an octamer of identical subunits that specifically binds (GT)n repeats, though at a lower affinity. However, unlike the human Translin, spTranslin binds the corresponding RNA transcripts, (GU)n, with a much higher affinity. By using the yeast two-hybrid technique, we showed that the spTranslin and the spTRAX specifically interact. We also deleted the Translin and/or TRAX ORFs in S. pombe and found that these genes are non-essential. Currently, we are searching for proteins that may form specific complexes with spTranslin and/or spTRAX in the yeast cells. For this purpose, we constructed S. pombe strains in which the spTranslin ORF was fused to the Tandem Affinity Purification (TAP) tag either through its N terminus, or through its C terminus. We are purifying native complexes including the tagged spTranslin from these cells by affinity chromatography. SDS-PAGE of these complexes has already revealed the presence of other proteins in these complexes. The identity of these proteins will be determined by mass spectrometry. We are also constructing similar strains expressing TAP-tagged spTRAX and will use affinity purification for identification of proteins that are specifically associated with the spTRAX.

Analysis of GINS function in the fission yeast cell cycle

Chen Chun Pai¹, Ignacio Garcia Llorente², Xiaowen Yang¹, Stuart MacNeil² and Stephen Kearsey²

¹Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK
²Institute of Molecular Biology and Physiology, University of Copenhagen, Selvgade 83H, 1307 Copenhagen K, Denmark

Studies in Xenopus and Saccharomyces cerevisiae have shown that the GINS complex plays an essential role in the initiation and elongation stages of DNA replication. To analyze this Sld5-Psf1-Psf2-Psf3 tetramer in Schizosaccharomyces pombe, we have constructed conditional Psf1 and Psf2 mutants where the proteins are fused to an estradiol-binding domain. On estradiol depletion, both of these strains show a DNA replication arrest as judged by flow cytometry. Inactivation of Psf1 and Psf2 in G2 allows normal execution of mitosis and cytokinesis, followed by arrest in the subsequent S phase. In addition, we also found that inactivation of Psf1 in meiosis causes arrest in pre-meiosis S phase, suggesting that GINS is also required for the meiotic cell cycle. Levels of the Psf2 subunit of GINS are constant during the cell cycle and during G1 arrest by nitrogen starvation. Using an in situ chromatin binding assay, we have shown that the binding of Psf2 to chromatin is periodic in fission yeast and occurs during S phase. To investigate the relevance of GINS function to the chromatin association of other replication proteins, we are examining how the chromatin binding of DNA polymerase alpha and Cdc45 is affected by Psf1 inactivation.
Dual roles for Homologous recombination in break-induced loss of Heterozygosity in fission yeast

Jason Cullen, Sharon Hsueh, Carol Walker, John Prudden, Boon-Yu Wee, Anoushka Davé and Tim Humphrey

MRC Radiation and Genome Stability Unit, Harwell, Didcot Oxfordshire, OX11 0RD, UK.

Loss of heterozygosity (LOH) is the most common form of genome instability in cancer cells. Despite its potential initiating role in tumorigenesis, the mechanisms by which such genetic alterations arise are poorly understood. We have investigated whether DNA double-strand breaks (DSBs) can cause LOH by screening for auxotrophic marker loss ~30kb away from an HO-endonuclease break-site within a non-essential minichromosome in Schizosaccharomyces pombe. Extensive break-induced LOH was observed in ~2% of cells and was associated with large non-reciprocal translocations involving a homologous chromosome which resulted from both allelic crossovers and break-induced replication. Non-reciprocal translocations were found to require the homologous recombination (HR) genes rhp51’ (RAD51’), rad22’ (RAD52’), rhp55’ (RAD55’) rhp54’ (RAD54’), and mut81’ , in addition to the MRN component nbs1’. Surprisingly, break-induced LOH resulted predominantly from de novo telomere addition at the break-site in these HR-defective strains, although at reduced levels when HR was disrupted at later stages. As levels of de novo telomere addition were reduced in rad50Δ, rad50S or exo1Δ backgrounds, in which end processing is compromised, these results together suggest that de novo telomeres are added preferentially to resected ends. HR therefore performs dual roles in both facilitating LOH through the generation of non-reciprocal translocations and in preventing LOH, possibly through competition with telomerase recruitment factors for resected DSB.

Cellular response to UVA radiation in fission yeast

Delphine Dardalhon, Anne Reynaud, Eveline Sage and Stefania Francesconi

(CNRS/ Institut Curie, UMR 2027, Centre Universitaire d’Orsay, 91405 Orsay, France)

Ultraviolet (UV) radiation comprises approximately 5.4 % of total solar spectrum and, within the UV spectrum, only UVA (320-400 nm) and UVB wavelengths (280-320 nm) reach Earth’ surface. UVA itself comprises more than 90 % of total solar UV radiation. While UVB radiation directly damage DNA by inducing formation of dipyrimidine photoproducts, UVA radiations mainly generate reactive oxygen species (ROS) that may damage DNA as well as lipids and proteins. Indeed, UVA radiation are known to induce oxidative stress response pathways and formation of low levels of oxidized bases (mainly 8-oxoguanine) and dipyrimidine photoproducts. While an effort has been done to understand the role of UVA radiation in DNA damage and cancerogenesis, very little is known on the effects of UVA-induced stress response and on the impact of UVA radiation on cell cycle progression. Thus, we decided to study the cellular response to UVA radiation in S.pombe and we will present data on the effects of UVA on cell cycle progression.
Multiple distinct mechanisms regulate de novo telomere addition at DNA double-strand breaks in fission yeast

Anoushka Davé, Boon-Yu Wee, Jason Cullen, Carol Walker, John Prudden and Tim Humphrey

MRC Radiation and Genome Stability Unit, Harwell, Didcot, Oxfordshire, OX11 0RD, UK.

Loss of heterozygosity (LOH) is a common form of genetic instability in cancer cells that is thought to promote tumorigenesis through functional inactivation of tumour suppressor genes. However, the mechanisms leading to LOH and how they are suppressed in normal cells are poorly understood. We have investigated mechanisms of LOH distal to an HO-endonuclease-induced DNA double-strand break (DSB) within a non-essential minichromosome, Ch16, in Schizosaccharomyces pombe. We find that break-induced LOH in a wild-type background is infrequent (~2%) and results from homologous recombination (HR)-dependent allelic crossovers and break-induced replication (BIR). In contrast, DSB induction in HR mutants results in similar LOH levels but mainly through de novo telomere addition at the break-site, indicating a key role for HR in preventing de novo telomere addition at DSBs. We further find that disrupting Rqh1, the S. pombe homologue of the human tumour suppressor, BLM, in an HR-deficient background leads to a dramatic increase in LOH levels compared to the corresponding single mutants, predominantly through de novo telomere addition. Moreover, truncating the minichromosome arm by adding a telomere directly distal to the break-site in HR-deficient strains results in a striking increase in de novo telomere addition at the break-site. A further increase in de novo telomere addition was observed when Rqh1 was also deleted in this context. We have thus identified three distinct factors – disruption of HR, deletion of Rqh1 and location of the break-site proximal to a telomere, which when combined, result in remarkably high levels of break-induced LOH through de novo telomere addition. These data identify multiple distinct mechanisms which function to regulate levels of de novo telomere addition at a break-site in fission yeast and together are likely to play an important role in maintaining genome stability in eukaryotes.

Capture of extranuclear DNA at fission yeast double-strand breaks

Anabelle Decottignies

Catholic University of Louvain, Faculty of Medicine, Cellular Genetics. 74+3, Avenue Hippocrate, 1200 Brussels, BELGIUM.
anabelle.decottignies@gece.ucl.ac.be

Proper repair of DNA double-strand breaks (DSBs) is necessary for the maintenance of genomic integrity. A new simple assay was used to study extrachromosomal DSB repair in Schizosaccharomyces pombe. Strikingly, DSB repair was associated with the capture of endogenous fission yeast mitochondrial DNA (mtDNA) at high frequency. Capture of mtDNA fragments required the Lig4p/Pku70p non-homologous end-joining (NHEJ) machinery. The Mre11 complex Rad32p/Rad50p/Nbs1p was also required for efficient capture of mtDNA, supporting a role for the complex in promoting intermolecular ligation. MtDNA capture frequency was highly increased in fission yeast cells grown to stationary phase, a phenomenon requiring vacuolar carboxypeptidase activity. Further genetic requirements for mtDNA fragment production and transfer to the nucleus are currently under investigation. Several pieces of evidence suggest that DSB repair-driven chromosomal integration of mtDNA may not exclusively occur under experimental conditions. Firstly, recent studies reported the association of human genetic diseases with de novo insertions of mtDNA in the nuclear genome including a patient exposed to Chernobyl radiations. Secondly, systematic sequencing of nuclear genomes from budding yeast, human and various plant species revealed that integration of mtDNA fragments occurred during evolution and is probably an ongoing process. Accordingly, this study unraveled the presence of mtDNA sequences in the fission yeast nuclear genome. In the second part of the work, co-transformation experiments revealed that microsatellite DNA from higher eukaryotes competed with fission yeast mtDNA for insertion at DSBs. Finally, this study revealed that, in NHEJ-deficient cells, capture of extranuclear DNA at DSBs was observed if homologies -as short as 8 bp- were present between DNA substrate and DSB ends. Hence, whether driven by NHEJ, microhomology-mediated end-joining or homologous recombination, DNA capture associated with DSB repair is a mutagenic process threatening genomic stability.
Regulation of DSC1 by The Replication Checkpoint

Chaitali Dutta, Prasanta Patel, Anna Oliva*, Janet Leatherwood* and Nicholas Rhind

University of Massachusetts Medical School, USA
*State University of New York, Stony Brook, USA

Transcriptional regulation in response to replication arrest is important for cell survival and genomic stability. However, the mechanism by which checkpoint regulates transcription is not well understood. The exception is in Saccharomyces cerevisiae, where a checkpoint specific transcription factor up-regulates the enzymes that are needed to produce more deoxynucleotides. This transcriptional strategy does not appear to be conserved. We are using Schizosaccharomyces pombe to study checkpoint dependent transcriptional regulation.

In fission yeast the checkpoint not only up-regulates the genes that are needed to produce more deoxynucleotides but also up-regulates many genes normally expressed during S phase with no obvious checkpoint function. This observation suggests the checkpoint regulates the entire S-phase transcription program. We hypothesize that in response to HU, the checkpoint kinase Cds1 maintains high level of S phase transcripts by regulating DSC1 (DNA synthesis control), the transcriptional factor complex responsible for normal S-phase transcription.

In order to confirm our suspicion that the checkpoint controls the entire S-phase transcription machinery we analyzed the global transcriptional induction in response to HU by gene array in collaboration with Dr. Janet Leatherwood (Stony Brook NY). We find that the activated checkpoint affects all S-phase transcripts and this response is Cds1 dependent. Furthermore, our data suggests that checkpoint has no effect of S-phase transcription when DSC1 activity is compromised, corroborating the hypothesis that DSC1 is the checkpoint target.

Currently, our study is focused on regulation of DSC1 activity by the checkpoint. Studies have shown that C-terminal 61 amino acids of Cdc10, a major component of the DSC1, are crucial for its regulation. We have shown that bacterially expressed GST-tagged Cdc10 is phosphorylated in vitro by Cds1 and have mapped the phosphorylation of Cdc10 to C-terminal serines. We are currently investigating the checkpoint dependent in vivo phosphorylation of Cdc10 by western blotting and isoelectric focusing. In addition we are examining the requirement of in vivo Cdc10 phosphorylation in checkpoint response.

The fate of dysfunctional telomeres through the cell cycle

Miguel Godinho Ferreira* and Julia Promisel Cooper

Telomere Biology Laboratory, 44 Lincoln’s Inn Fields, Cancer Research UK, London WC2A 3PX, United Kingdom.
*Present address: Telomere and Genomic Stability Laboratory, Instituto Gulbenkian de Ciência, Lisboa, Portugal.

Telomeres, the natural chromosome ends of eukaryotes, have unique properties that distinguish them from damage-induced DNA ends. Most human somatic cells lack telomerase, the enzyme responsible for generating telomeres. Proliferation of cells lacking telomerase leads to telomere depletion and ultimately to telomere loss. When telomere function is lost, chromosome ends are treated as DNA breaks that, in most cases, leads to cell death. If the cell survives, chromosomes will be joined by their ends yielding dicentric entities that can break upon mitosis. This, in turn, causes unequal distribution of genetic information to daughter cells as well as to the formation of new unprotected ends. The ensuing genomic instability is thought to be involved in the development of cancer.

Our studies have shown that Taz1 (orthologue of the human telomere binding proteins TRF1 and TRF2) is central to telomere protection in fission yeast. Surprisingly, cells lacking Taz1 are as viable as wild type cells in optimal growth conditions. However, in G1-arrested taz1Δ cells, chromosomes undergo lethal end-to-end fusions via non-homologous end-joining (NHEJ). In dividing taz1Δ cells, which spend the majority of the cell cycle in S and G2 phases, chromosome ends undergo homologous recombination (HR) that protects dysfunctional telomeres from end-fusions. The profile of DNA repair at taz1Δ telomeres reflects the reciprocal regulation of NHEJ and HR through the cell cycle; so that NHEJ is higher in G1 than in other stages of the cell cycle and, conversely, HR repair is higher in G2 than in G1.

In conclusion, Taz1 protects chromosome ends from being recognized as deleterious breaks and used as substrates of DNA repair. The outcome of these processes at telomeres varies greatly through the cell cycle, leading to chromosome-end fusions and lethality in G1 and chromosomal rearrangements in G2. We propose to investigate the mechanisms underlying chromosome-end protection and to understand the outcomes of its failure.
Stalled replication forks process in absence of Cds1

B. Froget, S. Lambert, P. Meister, and G. Baldacci

UMR2027, Institut Curie, Orsay University, France.

During replication, cells have to coordinate DNA synthesis with cell cycle progression, checkpoints and recombination. A defect in these processes may result in genetic instability. Hydroxyurea (HU) treatment slows down replication forks progression by deregulating the nucleotide pool, and results in DNA replication checkpoint activation, mediated by the Cds1 kinase, which maintains replication forks integrity. In absence of this checkpoint (cds1-d strain), we have previously shown that in response to HU treatment: 1) cells are unable to complete DNA replication, 2) aberrant DNA structures accumulate at collapsed replication forks and 3) the formation of aberrant DNA structures depends on homologous recombination. These results suggested that Cds1 prevents unscheduled recombination events at HU-induced stalled replication forks. In this context, we used Chromatin Immunoprecipitation (ChIP) to determine whether recombination proteins are recruited at stalled forks in absence of DNA replication checkpoint. We observed that the recombination protein Rad22 is recruited on early and late replication origins in response to HU treatment in cds1 deleted strain. Moreover, replication factories containing PCNA foci and Polε foci are less abundant after HU treatment in cds1 deleted cells compared to wild type strain. These results suggest that in absence of DNA replication checkpoint, a replication complex destabilization occurs at stalled forks, which could be targeted by nucleases. In line with this, using PFGE analysis, we observed that cds1 deleted cells experience DNA fragmentation in response to HU treatment. Moreover, this DNA fragmentation depends on the endonuclease Mus81 and is independent of Rhp51. Altogether, our data are consistent with a role for Cds1 in replication forks integrity protection by preventing aberrant stalled forks processing by DNA repair enzymes leading to unscheduled recombination events.


The role of Rad9 phosphorylation in recovery from stressed replication fork in fission yeast

Kanji Furuya, Francesca Paderi, Antony Carr

DNA checkpoint is one of the mechanism to prevent the genomic instability, and the defect causes cell death, senescence, neural degeneration or tumorigenesis. DNA replication is one of the event which causes genomic instability when things go wrong and DNA checkpoint pathway is shown to monitor the process mainly by stabilising the replication fork structure.

Here we focus on the Rad9 protein and show its phosphorylation contribute to the replication fork stability possibly by modulating post-replicative repair pathway. Rad9, which forms PCNA-like structure together with Hus1 and Rad1, is phosphorylated on Thr225 (inside PCNA domain) by Rad3 kinase.

We focused on this Thr. 225 because the phosphorylation become dependent on Rad9-Cut5/Rad4 complex only at replication fork. The mutant which Thr225 is no longer phosphorylated rescues post replicative repair (PRR) pathway mutant rhp18-d. Although the mutant showed a weak Camptothecin sensitivity, it showed hyper recombination phenotype and hyper-mutator phenotype, and showed fragmented chromosomes after camptothecin treatment. Surprisingly, the threonine 225 mutant failed to activate chk1 upon replicative stress, but manage to survive by activating Mad2 checkpoint which probably is activated by abnormal chromosome structure. These phenotype is seen only under replicative DNA damage but not on G2 DNA damage. We hypothesize Rad9 phosphorylation somehow modulate the way the replication fork is repaired thus ensures proper activation of PRR and replication checkpoint pathway and thus, we are currently trying to see the difference in stalled replication fork between mutant and wild type.
Rad4/Cut5 interact with Cdc2/Cdc13 in response to replication stress and DNA damage

Valerie Garcia and Antony Carr

Fission yeast Cut5/Rad4 and its budding Yeast, Xenopus and Human homologues are required for both the DNA replication process and for S phase checkpoints. Cut5/Rad4 is an essential protein containing 4 BRCT domains which are common to many DNA repair/checkpoint proteins. Although Rad4 interacts with several proteins implicated in DNA replication and checkpoint functions (Drc1, Rad9, Crb2 and Chk1), its molecular function is still not understood. We show that Rad4 is phosphorylated after treatment of cells with HU or DNA damaging agents, and these phosphorylation events are dependent upon the Rad3 kinase. We observed that the phosphorylation of Rad4 is also partially dependent upon the Cdc2 kinase. Cdc2 is the sole cyclin-dependant kinase in S. pombe and has been found to activate recombination pathways (Caspers et al, 2002). We have shown that Rad4 is phosphorylated in vitro by Cdc2, and identified phosphorylation sites by mass-spectrometry. The function of these sites in vivo is under investigation and will be presented. Furthermore, Cdc13 was identified as a Rad4 partner in TAP purification of Rad4 complex from extracts prepared from HU treated cells. This HU induced interaction was confirmed by co-immunoprecipitation. Altogether, these results position the Rad4-Cdc13/Cdc2 complex as a key intermediate in the stabilisation or processing of stalled replication forks.

A novel Double Strand Break system in Schizosaccharomyces pombe, specific for Single Strand Annealing

Stephen Gill, Petra Werler, Anthony M Carr and Johanne M Murray

Genome Damage & Stability Centre, University of Sussex, BN1 9RQ, UK

The repair of double strand breaks (DSBs) is essential for genomic stability and cell survival. Experimentally, DSBs can be introduced non-specifically through exposure to irradiation and genotoxic chemicals, whereas utilisation of an endonucleases allows introduction of DSBs at specific sites. In Saccharomyces cerevisiae a number of assays have been developed which utilise HO endonuclease recognition sites to induce a single DSB at a specific location within the genome, allowing the kinetics of DSB repair to be studied. However, this technique is not widely used in Schizosaccharomyces pombe. We have designed an HO system specifically to the study the single strand annealing (SSA) pathway, about which little is known about in S. pombe.

The system comprises two halves of the LEU2 marker gene, integrated in chromosome II, separated by non-essential DNA containing the ura4 gene with an HO recognition site and the hygromycin resistance gene. The two halves of the LEU2 gene contain 500 bp of homology between them which can utilised for SSA. The HO endonuclease is expressed from a plasmid under the control of the inducible nmt promoter. Once expressed, the HO endonuclease generates a single DSB within the ura4 gene. Following break formation and strand resection, SSA is used to repair the break, leading to the formation of a fully functional LEU2 gene. Thus cells switch from ura4+/LEU2- to ura4-/LEU2+.

The requirement for proteins known to be involved in SSA in S. cerevisiae was investigated using this system. While broadly in agreement with data from S. cerevisiae (Rad22 (RAD52) is required but Rhp51 (RAD51) is not) there were a number of differences. S. pombe Rad16/Swi10, homologues of the RAD1/RAD10 flap endonuclease, which is essential for SSA in S. cerevisiae, were shown to be important, but not essential for SSA in S. pombe. Interestingly, the checkpoint protein Rad17, like S. cerevisiae homologue RAD24, was found to be essential for SSA. Furthermore, we found that, unlike its S. cerevisiae homologue SRS2, Srs2 was not required for checkpoint recovery following DSB induction.
Bypass of replication arresting DNA lesions: Involvement of S. pombe mhp1\(^1\) and mhp2\(^2\)

Ralph Gruber\(^1\), Jonathan Framton\(^1\), Wilfried Kramer\(^1\) and K. Anke Schürer\(^1\)

\(^1\)Leibniz Institute for Age Research-Fritz Lipmann Institute, Beutenbergstr. 11, D-07745 Jena, Germany, Email: aschuer@flf-leibniz.de
\(^2\)University of Göttingen, Institute for Microbiology and Genetics, Dept. of Molecular Genetics and Preparative Biology, Grisebachstr. 8, D-37077 Göttingen, Germany
\(^3\)Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton BN1 9RQ, Great Britain

In S. pombe two sequence homologues of S. cerevisiae MPH1, mhp1\(^1\) and mhp2\(^2\), can be identified.

S. cerevisiae MPH1 (mutator phenotype) constitutes a new pathway for error-free bypass of DNA lesions during replication that requires homologous recombination but is distinct from post-replicative repair. Mph1 is conserved in archaea and eukaryotes with FancM, defective in the rare disorder Fanconi Anaemia, as the human homologue.

We constructed both mhp1\(^1\) and mhp2\(^2\) mutants (mhp1 homologue pombe) in S. pombe and have started to characterise the phenotypes of both single and double mutants. We found similar phenotypes as shown for mhp1 from S. cerevisiae supporting the hypothesis of mhp1\(^1\) and mhp2\(^2\) being orthologues of bakers yeast Mph1. We got evidence for an apparent separation of function diversification during evolution. However, none of the S. pombe genes was able to complement the S. cerevisiae mhp1 phenotypes (sensitivity to DNA damaging agents and spontaneous mutator phenotype). Further characterisations including genetic interaction studies with genes from post-replicative repair, from homologous recombination and other genes involved in reinitiation of stalled replication forks are presently in progress. These analysis may also open new perspectives on the function of FancM, the human MPH1/mhp1\(^1\)/mhp2\(^2\) homologue.

Smc5/6 complex is required for recombination repair at collapsed replication forks

Anja Irmisch, Eleni Ampatzidou and Jo Murray

Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, BN1 9RQ, UK

Structural Maintenance of Chromosome (SMC) proteins are highly conserved proteins involved in higher-order chromosome organization. In eukaryotes they exist in three conserved multi-subunit protein complexes. The Smc1/3 complex (cohesin) mediates sister chromatid cohesion, while the Smc2/4 complex (condensin) facilitates chromosome condensation at mitosis (Hirano, 2002). The function of the third complex, which comprises Smc5, Smc6, and four stoichiometric non-Smc core complex components (Nse1-4), is less clear. All components are essential and loss of Smc5/6 complex function results in chromosomal fragmentation during S phase. Hypomorphic mutants are defective in HR repair and segregation of the rDNA (Torres-Rosell et al., 2005; Lehmann et al., 1995).

In S. pombe Smc6 is nuclear and predominantly localises to the nucleolus. However, Chromatin immunoprecipitation (ChIP) experiments show that it associates with DNA throughout the genome. Epistasis analysis, ChIP and visualisation of replication intermediates by 2-D gels suggest that Smc6 is required at a late stage in homologous recombination at collapsed replication forks. The role of the Smc6 complex in regulation of recombination at stalled forks will be discussed.

The role of *rrp1* and *rrp2* genes in the repair of DNA damage in *Schizosaccharomyces pombe*

Pawel Karpinski*, Agnieszka Dyjakiewicz and Dorota Dziadkowiec

Institute of Genetics and Microbiology, Wrocław University, Przybyszewskeiego 63/77, 51148 Wrocław, Poland.

* present address: Department of Genetics, Wrocław Medical University, Marcinkowskiego 1, 50368 Wrocław, Poland

Homologous recombination is an important process assisting in the repair of DNA damage in all studied organisms. In *Schizosaccharomyces pombe* a Rhp51 recombinase (functionally homologous to RecA protein in E.coli and Rad51 in *S.cerevisiae*) and mediators, Rhp55 and Rhp57 (Rad55 and Rad57 in *S.cerevisiae*), take part in homologous pairing and strand exchange, essential to the homologous recombination repair process. It was previously shown that Sfr1 and Swi5 form a protein complex that is involved in Rhp51-dependent but Rhp55/57 independent recombination repair and it is suggested that this complex is a second mediator for Rhp51 recombinase. Here, we report an analysis of a novel DNA repair protein, Rrp1 that has SNF2N, zf-C3HC4 and helicases C domains. The strain devoid of the *rrp1* gene is slightly sensitive to high doses of UV, but not to MMS. Δ*rrp1Δrhp51* double mutant shows the same UV sensitivity as a Δ*rhp51* single mutant, indicating that Rrp1 functions in the Rhp51-dependent repair process for the UV-induced DNA damage.

While a Δ*rrp1* mutant is less sensitive to UV than a Δ*sfr1* mutant, the Δ*rrp1Δsfr1* double mutant shows the same sensitivity as Δ*sfr1* single mutant. On the other hand, *rrp1* mutation has an additive effect on DNA repair phenotype of a Δ*rhp57* mutant. These results indicate that Rrp1 functions independently of Rhp55/57 and possibly takes part in a sub-pathway of Sfr1-dependent recombination repair. The phenotypes of a deletion mutant of a *rrp1* parologue, *rrp2*, are also investigated in order to determine if this gene is involved in DNA replication/repair.

**Premeiotic G1 to S transition is affected by recombination proteins, and mutual inhibition is observed for two recombination hotspots**

Jürg Kohli, Eveline Doll, Monika Molnar, Benjamin Sakem, Emil Parvanov

Institute of Cell Biology, University of Berne, Baltzer-Strasse 4, CH-3012 Berne, Switzerland e-mail: juerg.kohli@izb.unibe.ch

Three types of fission yeast meiosis are used for experimental analysis. The shortest and most synchronous meiosis is induced by temperature shift of strains carrying the pat1-114 mutation. A longer prophase and poorer synchrony are displayed by the azigotic *h+/h* meiosis of diploids induced by nitrogen starvation. The natural azigotic meiosis used for recombination analysis in crosses of haploid strains is not suitable for the study of the timing of prophase events. A systematic analysis of strains carrying mutations in genes that are coding for proteins involved in meiotic sister chromatid cohesion, chromosome pairing, double-strand break (DSB) formation, and repair of DSBs, showed that the start of premeiotic S phase (and of subsequent events) is advanced for up to two hours in several mutants (eg rec11, rec12, rec14), like wild-type in a few mutants (*mde2, dmc1*), and delayed in one case (*rec8*). A preliminary model on the regulation of the premeiotic G1 to S transition will be presented.

The meiotic recombination hotspot at *ura4A* was correlated with a double-strand break (DSB) 500 bp upstream of the gene. The dependence of *ura4A* hotspot activity and DSB formation on proteins modifying chromatin structure and enhancing the M26 hotspot was investigated. In particular, it was found that *ura4A* hotspot and DSB was not dependent on intact Pcr1, which is required for hotspot activity at ade6-M26. Nevertheless, recombination analysis indicated that the two hotspots (distance 15 kb) mutually inhibit each other to a significant extent for inter- and intragenic recombination. In addition the hotspots showed competition (deficit of double events at both hotspots) which was a minor part of the overall reduction.
Streptomyces phage φC31 and φBT1 integrases in fission yeast

Nicholas CO Lee and William RA Brown

Institute of Genetics, Queens Medical Centre, Nottingham University, NG7 2UH, UK

Site specific recombinases belong to two main families; the tyrosine and serine recombinases. The tyrosine recombinase family include the enzymes Cre and Flp which are widely used in genome manipulation in metazoans. These enzymes promote reversible reactions between identical sites and are thus unsuited to promoting insertions, translocations or inversions. We are trying to develop techniques for manipulating the centromeric DNA of fission yeast in vivo. The serine recombinases family includes members that promote unidirectional reactions between two different attachment sites of about 40bp in the absence of additional proteins and are thus potentially ideal for exactly the type of genome manipulation to which Cre and Flp are unsuited. The Streptomyces phage φC31 and φBT1 integrases are examples of such proteins. We have shown that these integrases promote both excision and insertion reactions in fission yeast but the reactions do not go to completion. Incomplete reaction due to attachment site damage is a feature of the reactions promoted by the φC31 integrase in vertebrate cells and probably arises because the reaction intermediate is recognized as DNA damage. We are investigating whether this is also true in fission yeast and the nature of the nucleases that are required for the inappropriate repair of the reaction intermediate.

A bioinformatics approach to predict origin efficiencies as a function of the genomic context

Legouras Ioannis1, Kouretas Panagiotis2, Nurse Paul3, Lygeros John3, Lygerou Zoi3

1Département of Medicine, University of Patras, Greece
2Département of Electrical Engineering, University of Patras, Greece
3Rockefeller University, New York, NY, USA

Following the publication of the fission yeast genome (Wood V. et al, 2002), high throughput approaches to investigate origin function became feasible. Recent analyses of DNA replication across the fission yeast genome have mapped origin positions and estimated their firing efficiencies. Such analyses have shown that in order to function, origins must satisfy certain criteria; they must have an AT content above average (>69%) for a sufficiently large segment of DNA (~1kb). However, not all potential origins will fire in every cell cycle: origin efficiencies vary from below 10% (the experimental detection limit) to around 80% and origin selection in each individual cell is stochastic. The stochastic nature of origin selection in fission yeast has long been speculated and was recently proved with single molecule analysis (Patel et al, 2006). Due to this stochasticity as well as the limitations of the experimental techniques used, it is strongly speculated that more origins exist. Observed efficiencies are a function of the intrinsic probability of a given region of the genome to act as an origin and by the rate at which it is converted to the post-replicative state by passive replication from nearby origins. To detect a possible greater number of origins than the ones currently mapped and to define intrinsic firing efficiencies of known origins, we are implementing a bioinformatics approach that correlates the following in a genome-wide level: known origin data (positions and efficiencies), AT richness and the locations of transcribed regions. This approach will be insightful in terms of the possible effects of AT content and nearby transcribed DNA on origin efficiencies. AT richness is crucial for origin establishment, but it remains elusive if it can also determine efficiency. Furthermore disturbance of the replication machinery due to the nearby transcription machineries has long been considered detrimental to origin establishment. Current progress in this area will be presented with emphasis on the various aspects of genomic context that allegedly affect intrinsic origin efficiencies, namely AT content, genes and chromatin structure.
Control of RNR by the Signalosome (CSN) and checkpoints

Cong Liu, Thomas Caspari and Antony M. Carr

Genome Damage and Stability Centre, University of Sussex, Falmer Brighton BN1 9RQ.

The COP9/signalosome complex (CSN) is highly conserved multi component protein complex with homology to the 19S regulatory lid complex of the proteosome. The signalosome is required for the removal of covalently attached Ned8 from cells. In S. pombe CSN co-purifies with the Cullin 4 homolog (Pcu4), the Ddb1 and Cdt2 (a WD-repeat protein). Null mutations in csn1, csn2, pcu4, ddb1 and cdt2 genes in S. pombe are DNA damage sensitive and exhibit slow DNA replication because of misregulation of RNR. In wild type cells in G1, G2 and mitosis the small subunit of RNR (Suc22) is sequestered in the nucleus, while the large subunit (Cdc22) is distributed throughout the cell. In S phase, Suc22 relocalises to the cytoplasm.

Re-localisation of Suc22 does not occur in these null mutants. Upon irradiation of G2 cells, Suc22 is also exported from the nucleus to the cytoplasm in wildtype cells. Again, this does not occur in the null mutants. The inability to relocalse Suc22 explains the DNA damage sensitive phenotypes.

Deletion of spd1, that encodes a small protein with similarity to S. cerevisiae Sml1, results in constitutive cytoplasmic localisation of Suc22 and suppresses the slow S phase and radiation sensitivity phenotypes of csn1, csn2, pcu4, ddb1 and cdt2 mutants. Spd1 degradation is ubiquitin-dependent and CSN-Pcu4-Ddb1-Cdt2 dependent in S phase, but checkpoint pathway-independent. In contrast, in response to irradiation of G2 cells, Spd1 degradation is ubiquitin-dependent, CSN-Pcu4-Ddb1-Cdt2-dependent and checkpoint pathway-dependent. We have shown that Cdt2 acts as an adaptor protein, bringing Spd1 to the CSNPcu4-Ddb1 complex, and that the key regulatory step in Spd1 degradation is the presence of the Cdt2 adaptor.

Interactions of SpMcm10 with DNA replication factors

Karen Moore, Simone Contini and Stephen J. Aves

School of Biosciences, University of Exeter, Exeter EX4 4QD, UK
K.A.Moore@exeter.ac.uk

S. pombe mcm10 (cdc23) encodes an essential protein of 593 amino acids that binds to chromatin. Mcm10 homologues are found in all eukaryotes where they function in DNA replication but their precise roles remains elusive. Temperature sensitive mcm10 mutants display a typical cell division cycle arrest phenotype at 36°C with elongated cell morphology indicative of an intact checkpoint. SpMcm10 is required for both the initiation and elongation phases of DNA replication, but it is not necessary for pre-replicative complex formation; it is, however, essential for activation of the replication complex. Consistent with a role at initiation SpMcm10 interacts physically with four subunits of the origin recognition complex (Orc1, Orc2, Orc5 and Orc6). We have used yeast 2-hybrid analysis to demonstrate a physical interaction with three subunits of the MCM2-7 replicative helicase (Mcm4, Mcm5 and Mcm6), two GINS subunits (Psf2 and Sld5) and Rad4/Cut5. In addition to the physical interactions with Mcm4-6 genetic interactions have also been demonstrated with mcm2 as well as mcm4, mcm5 and mcm6. Recombinant Mcm10 has single stranded DNA binding activity in vitro and activates DNA polymerase α via the p180 catalytic subunit. In vivo we find no genetic or yeast 2-hybrid interactions between Mcm10 and DNA polymerase α catalytic subunit, or either of the primase subunits. Chromatin binding assays have shown that SpMcm10 is required to load Cdc45 onto chromatin after pre-replicative complex formation. In vitro Mcm10 is required to stimulate phosphorylation of Mcm4 by Dfp1/Hsk1 kinase. In Saccharomyces cerevisiae interactions with Dna2, DNA polymerase ε and PCNA indicate that it is involved in the elongation phase. Intriguingly, interactions with a variety of other proteins have been reported including Sir2 and Sir3 suggesting a role in gene silencing. The S. pombe Mcm10 amino acid sequence contains motifs associated with some bacteriophage primases and this has led to the hypothesis that Mcm10 is a primase. However, both mcm10 and spp1 are essential genes and over-expression of mcm10 is insufficient to rescue spp1-4 mutants in which the primase catalytic subunit is defective.
Aberrant chromosome rearrangement induced by replication fork stalling at a palindrome

Ken‘ichi Mizuno, Johanne Murray and Antony Carr

Genome Damage and Stability Centre, University of Sussex, Brighton, BN1 9RQ, UK.

Genomic rearrangements linked to aberrant recombination are associated with cancer and human genetic diseases. Such recombination has indirectly been linked to replication fork stalling. We have previously established a system, designated RuraR, to induce replication fork stalling (RFS) at a specific site in a controllable manner, using a defined replication fork barrier sequence RTS1 and trans-acting replication fork block protein Rtf1 (Lambert et al, 2005). In the RuraR system, RFS is overcome by homologous recombination between RTS1 sites leading to inversion of the intervening ura4 marker and rarely to site-specific gross chromosome rearrangements. We have constructed a derivative palindrome system, Rura-arur, by inserting another copy of the ura4 gene in reverse orientation. In contrast to the RuraR system, the viability of the palindrome system is dramatically reduced when replication fork stalling is induced. Southern blot analysis and PFGE show that RFS at the palindrome leads to rapid chromosomal rearrangement and the formation of dicentric and acentric sister chromatids. The cells progress through G2 and enter mitosis with normal kinetics suggesting that the rearrangements occur during S phase and the products do not activate the G2/M DNA damage checkpoint. The dicentrics align correctly at metaphase with bipolar attachment to the spindle but are then torn apart at anaphase leading to a catastrophic mitosis. The generation of the non-equivalent sister chromatids is dependent on the RTS1-Rtf1 system and a subset of recombination and checkpoint proteins. These results lead us to conclude: 1) RFS at the palindrome triggers the chromosomal rearrangements through homologous recombination; 2) the generation of non-equivalent sister chromatids is dependent upon the palindromic sequences, which are able to self-pair, forming intrastrand hairpin structures; and 3) the palindrome system provides an inducible system with which to analyse the behaviour of dicentric chromosomes.

Sap1 associates with replication origins and is required for the replication stress response pathway

Chiaki Noguchi and Eishi Noguchi

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102

To achieve faithful DNA replication, eukaryotic cells are equipped with a dedicated sensor-response system, termed the replication checkpoint. This checkpoint is activated by stalled replication forks and required for accurate transmission of genetic information. Stalled forks are inherently unstable structures that are prone to collapse, regression and recombination, posing serious threats to genomic integrity. In humans, defects in this checkpoint cause genetic instability, leading developmental and neurological defects and a predisposition to cancer. Recently, our investigations revealed that two proteins, Swi1 and Swi3, form a replication fork protection complex (FPC) that plays a central role in the activation of the replication checkpoint and the stabilization of stalled forks. To further understand the mechanisms of the replication checkpoint, we have carried out screens to identify genes that genetically interact with FPC. Here we describe one of FPC interacting factors, Sap1, a protein bound to DNA sequences required for switching of sexual identity in the fission yeast Schizosaccharomyces pombe. We show that Sap1 is required for replication checkpoint signaling. Inactivation of Sap1 causes DNA damage represented by Rad22 DNA repair foci during S-phase, indicative of fork damage. A pulsed-field gel electrophoresis analysis shows that the sap1 mutants have a defect in resumption of DNA replication after fork arrest. We also provide evidence by chromatin immunoprecipitation that Sap1 associates with a replication origin. Our blast search reveals that Sap1 is conserved throughout evolution. We propose that Sap1 is required for the maintenance of replication forks in eukaryotic cells.
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Checkpoint pathway regulates Post Replicative Repair thought Damage Dependent Rad9t225 phosphorylation

Francesca Paderi

Genome Damage and Stability Centre, Science Park Road, University of Sussex Falmer, Brighton East Sussex BN1 9QJ, UK

Checkpoint pathways respond to changes in DNA structure. Its activation ensures the integrity of DNA by delaying cell cycle upon DNA damage. We focus on Thr 225 phosphorylation site on checkpoint protein rad9. Thr 225 phosphorylation does not involve checkpoint activation after general DNA damage, but it is suggested that this phosphorylation is required for the repair at the damaged replication fork. We here show that rad9 Thr 225 interacts with rhp18 post replication repair pathway (PRR) protein. After CPT (camptothecin) treatment, which causes replicative DNA damage, we observe that rad9 T225 mutation rescues rhp18-d. Synchronous culture experiment shows cell cycle delay induced by CPT in rhp18-d mutant is compromised by rad9 T225 mutation. Consistently Chk1 phosphorylation is reduced in rhp18-d - rad9 t225 background.

Our data suggest that Thr225 residue specifically responds to S phase damage to generate checkpoint signal. The function of Thr 225 may involves DNA processing on damaged replication fork; the phosphorylation at Thr 225 site may acts as a coordinator for polimerase network at damaged replication fork.
Msn complex and the spindle checkpoint

Hayatu Raji, Edgar Harstuiker

UK

Cells have functional checkpoints that ensure critical steps are not initiated until prior steps have been accurately completed. DNA damage and spindle defects would go undetected in the absence of these checkpoints, which can lead to cancer and aneuploidy, both of which can be deleterious to the survival of the cell. The Msn complex is involved in double strand break repair and the complex is thought to be involved in the early stages of DNA damage repair. In this study, S. pombe msn mutants are shown to be sensitive to spindle depolymerisation drugs. When observed under the microscope the mutants show defective septum formation and sister chromatid mis-segregation after treatment with thiabendazole. This indicates that the cells are unable to arrest at mitosis following thiabendazole treatment. The results of this study suggest that DNA repair proteins are involved in the spindle checkpoint in S. pombe.

Involvement of Cid1 poly(A) polymerase and the sub-telomeric rqh2 DNA helicase gene in a common pathway following inhibition of DNA replication

Olivia S Rissland, Abigail Stevenson, Li Ping Liew, Shao-Win Wang, Daniel Lackner, Juan Mata, Jürg Bähler and Chris J Norbury

1Sir William Dunn School of Pathology and 2Department of Zoology, University of Oxford and 3Wellcome Trust Sanger Institute, Hinxton, UK.

S. pombe cid1 was identified through a screen for S-phase cell cycle checkpoint genes. Surprisingly, the Cid1 protein is a cytoplasmic non-canonical poly(A) polymerase. We hypothesized that, upon S phase arrest, Cid1 might regulate the poly(A) tail length of checkpoint gene mRNAs. Using microarray hybridisation, we identified putative targets as those RNAs whose levels changed upon Cid1 deletion. One of the most striking changes occurred in the levels of rqh2/SPAC212.11: these transcripts were dependent on Cid1 cds1Δ mutants arrested in S phase. Rqh2 is the second member of the RecQ DNA helicase family to be identified in S. pombe. RecQ helicases are associated with several human disorders. For instance, mutations of the BLM helicase, which is the closest relative of Rqh2, cause the cancer predisposition disorder Bloom's syndrome. The rqh2 gene is thought to be present in four sub-telomeric copies, at each arm of chromosomes I and II; at least one of these copies is expressed after telomere erosion or replication stress. The open reading frame itself contains sequences encoding the RecQ helicase domain and sequences similar to centromeric dh repeats. Unlike rqh1, the other RecQ helicase family member in S. pombe, rqh2 is normally silenced in sub-telomeric heterochromatin. This silencing occurs by both the RNAi/RTS pathway, which is dependent on transcription of rqh2-associated dh sequences; and an RNAi-independent pathway, which itself is dependent on the telomere-associated protein Taz1. However, upon expression, rqh2 transcripts undergo a distinctive pattern of the 3' end processing, which suggests that this may be a direct Cid1 target. We propose that after replication stress Cid1-mediated cytoplasmic polyadenylation of rqh2 mRNA occurs and allows Rqh2 translation. Rqh2 itself may then stabilise and/or resolve stalled DNA replication forks, particularly those that are telomere proximal, and may also maintain the S-M checkpoint signal.

The telomere protein Taz1 is required for semi-conservative DNA replication through telomeres

Ofer Rog, Kyle Miller and Julia Promisel Cooper

Telomere Biology Laboratory, Cancer Research UK, 44 Lincoln’s Inn Fields, London WC2 3PX, UK

Telomeres are bound by a complex of proteins that regulates both chromosome end-protection and the activity of telomerase, which synthesizes the terminal repeats. Here we show that a telomeric DNA binding protein is also required for efficient semi-conservative replication of the bulk of the telomeres, and explore possible causes and consequences of fork-stalling at telomeres.

Fission yeast Taz1, an ortholog of the human telomere binding proteins hTRF1 and hTRF2, binds double-stranded telomeric repeats and regulates diverse telomere functions. Using two-dimensional gel electrophoresis, we find that loss of Taz1 results in stalled replication forks both within and directly adjacent to the telomere. Taz1 is also required for efficient replication through internally positioned telomeric sequences, regardless of their orientation in relation to the direction of replication. Therefore, telomere sequences lacking Taz1 constitute an obstacle to the replication machinery. Despite the impairment of telomere replication in the absence of Taz1, telomeres in taz1Δ cells are very long, suggesting that in taz1Δ cells, the very long telomeres are re-synthesized in every S-phase by telomerase. To test this idea, we monitored telomeres following loss of telomerase activity. Strikingly, taz1Δ cells lose their telomeres immediately upon telomerase loss, in contrast to wild type cells, which exhibit gradual attrition of telomeres. This observation supports the idea that taz1Δ telomeres break during S-phase and erode abruptly in the absence of telomerase. We will present our progress in understanding why Taz1 is required to orchestrate passage of replication forks through telomere sequences, and whether specialized helicases are required for this process. We will also address possible genetic interactions linking stalled forks to the chromosome mis-segregation exhibited by taz1Δ cells at cold temperatures.

High-resolution analysis of cohesin association along fission yeast chromosomes II and III

Christine Schmidt¹, Yuki Katou¹, Katsu Shirahige² and Frank Uhmann²

¹Chromosome Segregation Laboratory, Cancer Research UK, London Research Institute, Lincoln’s Inn Fields Laboratory, 44 Lincoln’s Inn Fields, London WC2A 3PX, United Kingdom
²Center for Biological Resources and Informatics, Division of Gene Research, and Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 226-8501, Japan

After replication, sister chromatids are held together by the cohesin complex which persists on chromosomes until mitosis when the dissolution of sister chromatid cohesion at anaphase-onset allows the spindle to pull sister chromatids apart. We have performed analysis of cohesin association along fission yeast chromosomes II and III using different cohesin subunit chromatin immunoprecipitates for hybridisation to high-density oligonucleotide microarrays (ChIP on chip).

In budding yeast, the transcription machinery has been shown to locate cohesin to sites between convergently transcribed genes. Likewise, in fission yeast, cohesin peaks are preferentially but less exclusively localised to these intergenic regions forming cluster-like arrangements. Less than half the convergence sites are occupied by cohesin whereas in budding yeast almost all of these sites are used. Interestingly, although cohesin peaks appear less confined to places between convergently transcribed genes they seem to be highly restricted to intergenic regions in general.

We have monitored cohesin dynamics during mitotic chromosome segregation in a highly synchronous culture released from G2. Surprisingly, the number of cohesin association sites increases towards anaphase, possibly due to the fragmentation of single cohesin peaks observed in G2. It will be interesting to determine the sites at which cohesin is loaded onto chromosomes. This might help to explain the cluster-like arrangement of cohesin association sites and could also be a crucial step for identification of possible cohesin removal sites during mitosis.
Fission yeast Cid12 has dual functions in chromosome segregation and checkpoint control
Abigail Stevenson, Thein Z. Win, and Shao-Win Wang
Department of Zoology, University of Oxford, South Parks Road, Oxford, United Kingdom

Fission yeast Cid12 is a member of the Cid1 family of specialised poly(A) polymerases. Like cells lacking cid1, cid12Δ mutants were shown to have checkpoint defects when DNA replication was inhibited. Here, we show that Cid12 is also required for faithful chromosome segregation and that mutation of amino acid residues predicted to be essential for poly(A) polymerase activity resulted in loss of Cid12 function in vivo. Cells lacking Cid12 had an increased chromosome segregation failure rate due to precocious loss of sister chromatid cohesion at the centromere but not along the chromosome arms. In keeping with a recently described function for Cid12 in RNAi-mediated heterochromatin assembly, this was accompanied by an accumulation of polyadenylated transcripts corresponding to naturally silenced repeat elements within heterochromatic domains, with consequent defects in centromeric gene silencing. These cells also suffered increased meiotic defects and their viability was dependent on the spindle checkpoint protein Bub1. To account for the effects of Cid12 on various aspects of DNA metabolism, including chromosome segregation and the checkpoint control, we suggest that Cid12 has dual functions in RNAi silencing and regulating mRNA stability.

What is the functional significance of the bouquet structure?

Kazunori Tomita, and Julia Promisel Cooper
Telomere Biology Laboratory, Cancer Research UK, London, UK
E-mail: kazunori.tomita@cancer.org.uk

Telomeres are nucleoprotein structures that form and protect chromosome ends and play a critical but poorly understood role in meiosis. Telomere function changes profoundly as cells proceed from the mitotic to meiotic cell cycle. In early meiosis, telomeres gather at the centromere to form the so-called ‘bouquet’ structure observed in diverse eukaryotes. This is particularly striking in fission yeast, as the telomeres localize to the spindle pole body (SPB) during meiotic prophase, in contrast to the mitotic interphase configuration in which the centromeres localize to the SPB. The S. pombe telomere binding protein Taz1 is required for telomere clustering at the SPB. Taz1 also protects telomeres from being recognized as DNA breaks and subjected to inappropriate DNA repair reactions. During the G1 arrest that precedes meiosis, end-to-end fusions form between taz1Δ telomeres; these fusions are mediated by Lig4-dependent nonhomologous end-joining. Thus, taz1Δ mutants have not only impaired telomere clustering but also chromosome fusions through meiotic cell-cycle. To assess the importance of telomere clustering without worrying about chromosomal fusions, we characterized meiotic phenotypes in lig4Δ taz1Δ cells. 50% of lig4Δ taz1Δ asci are abnormal. This defect is partially suppressed by the concomitant deletion of mad2, suggesting that defects in telomere clustering trigger a checkpoint-mediated block to meiotic progression. We monitored the SPB and microtubules during meiosis to investigate the basis for the spore formation problem in lig4Δ taz1Δ cells. We found defective SPB separation and segregation, as well as severe spindle defects in some lig4Δ taz1Δ zygotes. Because Taz1 is a component of the meiotic SPB, we checked the timing of telomere dissociation from SPB. Telomeres dissociate from the SPB almost simultaneously with SPB division. We propose that telomere dissociation from the SPB is an initiating event of meiosis I and controls the timing of SPB division through Mad2 inactivation.
Role of intra-S phase checkpoint proteins at stalled replication forks

Ellen Tsang

Genome Damage and Stability Centre
University of Sussex

The intra-S phase checkpoint coordinates a variety of cellular responses to replicative stress. Lambert et al. have recently shown that several recombinogenic pathways occur to bypass stalled replication forks in the site-specific fork stalling system “RuraR”. Here the S. pombe ura4 gene was flanked by two copies of RTS1, a Replication Termination Sequence from the mat locus (Lambert et al., Cell 2005). The role of the present study is to examine the interplay between checkpoint proteins and recombination at stalled forks in RuraR. We present data showing an elevated level of Rad22 (Rad52 homologue) recruitment to stalled forks in the absence of the Rad3 checkpoint protein, but not in the absence of Rad9, Rad17, Cds1 or Chk1. Rad22 recruitment also spreads further along the chromosome away from the site of fork stalling in the absence of Rad3. This suggests an involvement of Rad3 in fork processing that is independent of the Rad9/Rad17/Cds1/Chk1 pathways.

Characterisation of the role of sumoylation in the DNA damage response and genome stability

Watts FZ, Andrews A, Ho JCY, Taylor DL and Zhou L

Genome Damage and Stability Centre,
School of Life Sciences, University of Sussex, Falmer, Brighton, E Sussex

SUMO (small ubiquitin-like modifier) is covalently attached to proteins. Unlike ubiquitin, SUMO does not target proteins for degradation. In contrast, it affects protein localisation, protein–protein interactions and protein activity. S. pombe mutants defective in sumoylation are sensitive to DNA damaging agents (DDA) and the microtubule inhibitor, thabendazole (TB2). This implies that sumoylation has roles in the DNA damage response and genome stability. In order to more precisely identify the roles of sumoylation we have been characterising the S. pombe SUMO ligases (Nse2 and Pl1) and the SUMO proteases (Ulp1 and Ulp2).

Nse2 is essential for viability and is a member of the Smc5-6 complex (1). It is required for sumoylation of Smc6, and a SUMO ligase null mutant of Nse2 is sensitive to DDA. In contrast, deletion of pl1 is not lethal: cells are not sensitive to DDS, but are sensitive to TB2 and display loss of silencing at the centromere (3). Null mutants of ulp1 (required for the majority of the processing of SUMO to the mature form) display aberrant cell and nuclear morphologies, suggesting that multiple processes are affected in this strain (2). ulp2 null mutants resemble pl1, d null mutants, but with subtle differences. The various roles of these proteins will be discussed.

Analysis of Cohesin Function in Fission Yeast

Saeeda Bhatti, Tracy Riddell and Christopher J. McInerny

Division of Biochemistry and Molecular Biology, Institute of Biological and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

Survival of an organism is dependant on the accurate transmission of genetic information from one cell to its daughters. Such faithful transmission requires accuracy in the replication of DNA, precision in chromosome distribution and the ability to survive spontaneous and induced DNA damage.

Cohesin is a chromosomal protein complex holding replicated sister DNA strands together after synthesis. It plays a critical role in the successful separation of sister chromatids in both mitosis and meiosis for which there are specific cohesin proteins. The fission yeast Schizosaccharomyces pombe (S.pombe), has two cohesin genes rec8+ and rad21+ that are specific to meiosis and mitosis respectively. Expression of both genes is regulated during their respective cell cycles, with maximum transcript and protein being present during DNA replication.

Long before the essential role of cohesin in chromosome segregation was recognized investigators found that one of the sub-units was involved in repair in S.pombe. Increase in cell survival during DNA replication has been attributed to the formation of two identical sister chromatids, the physical linkage of sister chromatids by the cohesin complex is vital for double strand break (dsb) repair. This central role of cohesin in DNA dsb repair became clear when mutants of rad21+ had decreased survival after exposure to ionizing radiation.

Here we analyse the effects of inducing double strand. The alkylating agent methyl methane sulfonate (MMS) was used to mimic the effects of ionizing radiation. Increasing concentrations of MMS were administered to three S. pombe strains, wild-type with functional Rad21p, a rad21+ mutant strain and a strain in which the meiotic cohesin rec8- was expressed in the mitotic cycle, replacing the Rad21p. Expression of rec8- in this manner serves as a useful tool for investigating the meiotic specific role of Rec8p and allows us to determine if there is an overlap in cohesin function.

These experiments revealed mitotic and meiotic specific roles for the two cohesins. When Rec8p was expressed in the mitotic cycle replacing the Rad21p cell survival decreased, thus indicating that Rec8p does not have a role in DNA repair.

A Mathematical Model of the Initiation of DNA Replication

Orsolya Kapuy*, Attila Csikász-Nagy and Béla Novák

Molecular Network Dynamics Research Group of the Hungarian Academy of Sciences and Budapest University of Technology and Economics, Hungary

*email: okapuy@mail.bme.hu
phone: + 36-1-463-2910

DNA replicates once, and only once per cell cycle. After exit from mitosis prereplicative complexes are formed on the origins of replication. During S phase these prereplicative complexes are transformed to postreplicative complexes. Proper initiation of S phase in fission yeast requires Cdc10 mediated transcription. Cdc10 transcribes Cig2 cyclin (the regulatory subunit of the S phase Cdk/cyclin complex), Cdc18 (the licensing factor of the replication), and Cdc18 (the inhibitor of Cdk). We incorporated the major regulations of this pathway into a simple mathematical model of the fission yeast cell cycle. The model includes the effect of the unreplicated DNA checkpoint (Rad3 system), and can explain not only wild type behavior, but many other situations as well (e.g. HU block caused S phase block, cdc18+ and cdc18A- mutants).

Our model reveals two “oscillators” centered around the two cell cycle regulating Cdk/cyclin complexes: Cdc2/Cig2 and Cdc2/Cdc13. The Cdc2/Cig2 “oscillator” is not essential for mitotic cycle, because the Cdc2/Cdc13 “oscillator” can initiate both S and M phases. The Cdc2/Cig2 “oscillator” can work in the absence of Cdc2/Cdc13, but it drives only periodic S phases. Both “oscillators” can work in the absence of Cdc18, but their interaction is sensitive to Cdc18 level. We propose that Cdc18 plays a role of coordinating their action: up-regulation of Cdc18 level might block Cdc2/Cdc13 “oscillator” and let the Cdc2/Cig2 “oscillator” periodically initiate S phase.

1 D. Herm, N. Feraha and P. Nurse: Cdc18/Cdc6 plays a central checkpoint role in restraining mitosis during DNA replication (submitted)
Gross chromosomal rearrangements and elevated recombination at an inducible site-specific replication fork barrier.

S. Lambert¹, A. Carr², and G. Balldacci1.

¹UMR2027, Institut Curie, Orsay University, France.
²Genome Damage and Stability Centre, University of Sussex, Brighton, UK.

Genomic rearrangements linked to aberrant recombination are associated with cancer and human genetic diseases. Such recombination has indirectly been linked to replication fork stall. Using fission yeast, we have developed a genetic system to induce a site-specific fork barrier at a nonhistone/DNA complex. We used the RTS1-dependent fork stalling, previously identified at the mat locus, which is able to stall replication fork in a polar way. To create a region of DNA difficult to replicate, we integrated RTS1 sequences on either side of the ura4 gene on chromosome III, to create RuraR. By controlling the expression of genes required for RTS1 activity, fork stalling could be efficiently induced at ura4. We previously reported that: 1) Recombination is required for cell viability in response to RuraR-specific fork stalling, 2) Recombination proteins are recruited at such stalled fork and 3) RuraR-specific fork stalling leads to elevated recombination and Gross Chromosomal Rearrangements (GCR).

In human cells, chromosomal fragile sites are thought to be the signature of stalled forks and are prone to lead to GCR in cancer cells and to chromosomal breakage in response to replication inhibitor treatment. To investigate the consequences of stalled forks on GCR, we have developed various site-specific fork stalling substrates: 1) Rura and uraR which stall the telomere or the centromere proximal replication fork, respectively, 2) RuraR which stall both replication forks leading potentially to ura4 locus unreplicated, and 3) _ura_ which stall both replication forks without leading to ura4 locus unreplicated. Using PFGE following Southern blot analysis, we observed that a one-sided replication fork breakage occurs only at RuraR-specific fork stalling. Moreover, by scoring the loss of ura4 marker and by PCR-based molecular analysis, translocations between chromosome II and chromosome III are observed only at RuraR-specific fork stalling. These results suggest that stalled forks leading to unreplicated DNA regions are prone to fork breakage and to lead to GCR.


Permanently ubiquitinated PCNA and Translesion synthesis

Sharada Ramasubramanyan,
Dr. Catherine Green,
Prof. Alan Lehmann

Genome Damage and Stability Centre, University of Sussex

During Translesion Synthesis (TLS), DNA is synthesized past unrepairable damage in the template DNA by specialized error-prone polymerases that insert correct or incorrect nucleotides opposite the damaged site. However, the mechanism by which the cells switch from replicative polymerases to translesion polymerases at the stalled forks is still unknown. It has been shown recently that after DNA damage, PCNA (Proliferating cell nuclear antigen), the sliding clamp required for DNA replication, is mono-ubiquitinated and this modified form has higher affinity for pol_ (TLS polymerase), suggesting a role for this post-translational modification of PCNA in mediating the polymerase switch. To gain further insights into the role of PCNA modification, we have attempted to mimic permanently ubiquitinated PCNA with a Ubiquitin-PCNA fusion protein, which we have expressed in different Schizosaccharomyces pombe strains. Expressing the fusions did not have any dominant negative effect in wild type cells and expressing the fusion constructs could partially suppressed the UV and 4-NQO sensitivity of rhp18-d and K164R mutants that are unable to mono-ubiquitinate PCNA endogenously. This shows that our fusion construct is able to substitute partially for PCNA ubiquitinated on K164 in repair processes after DNA damage. However, the fusion construct did not suppress the UV sensitivity of rad8-d mutants that are able to mono-ubiquitinate but not poly-ubiquitinate PCNA, suggesting that the fusion construct may not be a substrate for poly-ubiquitination.

To determine whether the Ub-PCNA fusion can substitute for PCNA during replication, the fusion construct was integrated into the genomic Pcn1 locus of diploid wild type S. pombe cells. When the cells were sporulated, only two out of four spores from each ascus were viable, suggesting that Ub-PCNA is not able to substitute for endogenous PCNA in its essential function and that unmodified PCNA may be necessary for cell viability.

We are currently analyzing further the phenotype of cells expressing PCNA-Ub fusions along with localization and interaction studies of the TLS polymerases in association with mono-ubiquitinated PCNA.
Requirement of fission yeast Cid14 in polyadenylation of ribosomal RNAs

Thein Z. Win¹, Chris J. Norbury², and Shao-Win Wang¹

¹Department of Zoology and ²Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, United Kingdom

Polyadenylation in eukaryotes is conventionally associated with increased nuclear export, translation and stability of mRNAs. In contrast, recent studies suggest that the Trf4 and Trf5 proteins, members of a widespread family of non-canonical poly(A) polymerases, share an essential function in Saccharomyces cerevisiae that involves polyadenylation of nuclear RNAs as part of a pathway of exosome-mediated RNA turnover. Substrates for this pathway include aberrantly modified tRNAs and precursors of snoRNAs and rRNAs. Here we show that Cid14 is a Trf4/5 functional homolog in the distantly related fission yeast Schizosaccharomyces pombe. Unlike trf4 trf5 double mutants, cells lacking Cid14 are viable, though they suffer an increased frequency of chromosome mis-segregation. The Cid14 protein is constitutively nuclear, and is required for normal nuclear structure. A minor population of polyadenylated rRNAs was identified. These rRNAs accumulated in an exosome mutant and their presence was largely dependent on Cid14, in line with a role for Cid14 in rRNA degradation. Surprisingly, both fully processed 25S rRNA and rRNA processing intermediates appear to be channelled into this pathway. Our data suggest that additional substrates may include the mRNAs of genes involved in meiotic regulation. Polyadenylation-assisted nuclear RNA turnover is therefore likely to be a common eukaryotic mechanism affecting diverse biological processes.

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Krapp, Andrea P54
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Kubota, Shunsuke T76
Kumar, Arun P22
Kume, Kazunori P76, P77
Delegate List

Dr Rosa Aigue
University of Barcelona
Casanova 143
Barcelona
08036
ESP
+34934037252
aliquero@ub.edu

Miss Maria Luisa Alonso Nunez
Instituto de Microbiologia Bioquimica
Edificio Departamental. Campus
Unamuno
Lab 236
Salamanca
7 007
ESP
+34923294400
luanalui@yahoo.es

Dr Beatriz Alvarez
Instituto de Biologica Molecular y Celular del Cancer, CSICUniversidad de Salamanca
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34923294810
balavez@usal.es

Dr Victor Alvarez Tallada
Paterson Institute
Wilmslow Road
Withington
Manchester M20 3DT
GBR
+44 (0)161 446 3167
vattallada@piacr.man.ac.uk

Dr Andreas Anders
University of Edinburgh
Swann Building, Mayfield Road
Edinburgh
EH9 3JH
GBR
+44 (0)131 650 7074
Andreas.Anders@ed.ac.uk

Mr Keita Aoki
Kyoto University
Yoshida-Honmachi, Sakyo-ku
Kyoto
606-8317
JPN
+075-753-4206
aoki@kozo.lif.kyoto-u.ac.jp

Dr Benoit Arcangiololo
Pasteur Institute
25-28 rue du Dr Roux
Paris
75015
FRA
+33 1 45 68 8454
barcan@pasteur.fr

Dr John Armstrong
University of Sussex
Falmer
Brighton
BN1 9QG
GBR
+44 (0)1273 678576
j.armstrong@sussex.ac.uk

Mr Sheran Attanapola
University of Kent
Mulvihill Lab
Department of Biosciences
Canterbury
Kent
CT2 7NJ
GBR
+44 (0)1227 764 000
sla6@kent.ac.uk

Dr Stephen Aves
University of Exeter
The Henry Wellcome Building for Biocatalysis
Stockwell Road
Exeter
EX4 4XD
GBR
+44 (0)1392 264675
S.J.Aves@exeter.ac.uk

Dr Jose Ayte
Universitat Pompeu Fabra
Doctor Aiguader, 80
Barcelona
08003
ESP
+34-93-542-2891
Jose.ayte@upf.edu

Dr Jurg Bahler
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
Cambridge
CB10 1SA
GBR
+44 (0)1223 494861
jurg@sanger.ac.uk

Dr Mohan Balasubramanian
Temasek Lifesciences Laboratory
1 Research Link
The National University of Singapore
Singapore
117604
SGP
+65-6872-7000
mohan@ttl.org.sg

Dr Daniel Barker
University of St Andrews
School of Biology
University of St Andrews
Sir Harold Mitchell Building
St Andrews
Fife
KY16 9TH
GBR
+44 (0)1334 476161
db60@st-andrews.ac.uk

Mr Tim Beck
University of Sussex
Falmer
Brighton
BN1 9QG
GBR
+44 (0)1273 674341
t.j.beck@sussex.ac.uk

Mrs Samia Ben Hassine
Pasteur Institute
25-28 rue du docteur Roux
Paris
75015
FRA
+33 140613686
miled@pasteur.fr

Mr Ron Ben-Yosef
Technion-Israel Institute of technology
Faculty of Biology
Technion
Haifa
32000
ISR
+972-8293456
byron@tx.technion.ac.il

Mr Fredrik Berglund
University of Dundee
Level 5
Ninewells Hospital and Medical School
Dundee
DD1 9SY
GBR
+44 (0)1382 660 111 ext 32566
f.berglund@dundee.ac.uk

Ms Saeeda Bhatti
University of Glasgow
University Avenue
Glasgow
G12 8QO
GBR
+44 (0)141 339 8855 x0417
saeeda04@yahoo.com

Dr Hasanuzzaman Bhuiyan
University College Sodertorns
Alfred Nobel s Alle 3
Huddinge
S-14152
SWE
+486084000
hasan.bhuiyan@sh.se
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution/Position</th>
<th>Address</th>
<th>Phone Number</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms Claudia Bicho</td>
<td>National Institute for Medical Research</td>
<td>The Ridgeway Mill Hill London NW7 1AA GBR +44 (0)20 8959 3666 <a href="mailto:cbicho@igc.gulbenkian.pt">cbicho@igc.gulbenkian.pt</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Perry Blackshear</td>
<td>NIH/NIHS</td>
<td>111 T.W. Alexander Dr. P.O. Box 1223 MD A2-05 Research Triangle Park North Carolina 27709 USA +1 919-541-4899 <a href="mailto:black009@niehs.nih.gov">black009@niehs.nih.gov</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Cathrine Arnason Boone</td>
<td>The Norwegian Radium Hospital Ullerchsauen 70 Montebello Oslo 0310 NOR +4722935945 <a href="mailto:c.a.boe@medisin.uio.no">c.a.boe@medisin.uio.no</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neil Bone</td>
<td>University of Sussex Falmir 8001 Bergen Norway +44 (0)1235 687308 <a href="mailto:baafv4@sussex.ac.uk">baafv4@sussex.ac.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Carolina Bonilla</td>
<td>University College Sodertorns Alfred Nobel s Alle 7 Huddinge S-14152 SWE +4686084000 <a href="mailto:carolina.bonilla@sh.se">carolina.bonilla@sh.se</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miss Katalin Boros</td>
<td>CRUK Paterson Institute for Cancer Research Wilmotso Road Manchester M20 4BX GBR +44 (0)1614463156 <a href="mailto:Kboros@picr.man.ac.uk">Kboros@picr.man.ac.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Rafael Edgardo Carazo Salas</td>
<td>Cancer Research UK 44 Lincolns Inn Fields London WC2A 3PX GBR +44 (0)207 242 0200 <a href="mailto:carazo01@cancer.org.uk">carazo01@cancer.org.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Stefania Castagnetti</td>
<td>Cancer Research UK 44 Lincolns Inn Field London WC2A 3PX GBR +44 (0)927 2693244 <a href="mailto:S.Castagnetti@cancer.org.uk">S.Castagnetti@cancer.org.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Sandra Codlin</td>
<td>University College London Gower Street London WC1E 6BT GBR +44 (0)2076921222 <a href="mailto:s.codlin@ucl.ac.uk">s.codlin@ucl.ac.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Philippe Collin</td>
<td>ISREC Ch. des Boveresses 155 Epalinges Vaud 1066 CHE +41216925858 <a href="mailto:philippe.collin@isrec.ch">philippe.collin@isrec.ch</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Julia Cooper</td>
<td>Cancer Research UK 44 Lincolns Inn Field London WC2A GBR +44 (0)207 2693415 <a href="mailto:julie.cooper@cancer.org.uk">julie.cooper@cancer.org.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miss Karen Crawley</td>
<td>London Research Institute, Cancer Research UK Cell Regulation lab, Room 329 Cancer Research UK 44 Lincolns Inn Fields London WC2A 3PX GBR +44 (0)207 2693415</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. Attila Ciskas-Nagy</td>
<td>Budapest University of Technology and Economics Gellert ter 4 Budapest 1521 HUN +3614632910 <a href="mailto:ciskasz@mail.bme.hu">ciskasz@mail.bme.hu</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Jason Cullen</td>
<td>Medical Research Council Harwell Dicot OX11 0RD GBR +44 (0)1235 841000 <a href="mailto:j.cullen@har.mrc.ac.uk">j.cullen@har.mrc.ac.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miss Heledd Dafydd</td>
<td>Prifysgol Cymru Bangor Lab G30 Adelaid Coffa Ffodd Deiniol Bangor LL57 2UW GBR +44 (0)1248 382362 <a href="mailto:bsp234@bangor.ac.uk">bsp234@bangor.ac.uk</a></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Miss Sonya Hartmuth**  
University of Manchester  
Michael Smith Building  
Oxford Road  
Manchester  
M13 9PT  
GBR  
+44 (0)161 275 5512  
sonya.hartmuth@postgrad.manchester.ac.uk

**Dr Edgar Hartsuiker**  
GDSC  
University of Sussex  
Falmer  
Brighton  
BN1 9RQ  
GBR  
+44 (0)1273 873118  
e.hartsuiker@sussex.ac.uk

**Dr Jacqueline Hayles**  
Cancer Research UK, London Research Institute  
44, Lincolns Inn Fields  
London  
WC2A PX  
GBR  
+44 (0)20 7269 2072  
j.hayles@cancer.org.uk

**Miss Anna Hebden**  
Cancer Research UK  
44 Lincolns Inn Fields  
London  
WC2A 3PX  
GBR  
+44 (0)20 7269 3212  
anne.hebden@cancer.org.uk

**Mr Dom Helmlinger**  
Harvard Medical School  
77 avenue Louis Pasteur  
New Research Building  
room 239  
Boston  
MA  
02215  
USA  
+1-617-432-7557  
dom@genetics.med.harvard.edu

**Dr Damien Hermand**  
FUND-P-GEM  
rue de Bruxelles, 61  
Namur  
5000  
BEL  
+3281724241  
Damien.Hermand@fundp.ac.be

**Dr Ana B Herrero**  
Center for Cancer research  
Campus Miguel de Unamuno  
Salamanca  
37007  
ESP  
+34923294810  
anah@usal.es

**Prof Dai Hirata**  
Hiroshima University  
1-3-1 Kagamiyama  
Higashi-Hiroshima  
739-8530  
JPN  
+81 82 424 7764  
dhirata@hiroshima-u.ac.jp

**Miss Cassandra Hogan**  
The Babraham Institute  
Babraham Research Campus  
Cambridge CB2 4AT  
GBR  
+44 (0)1223 496000  
cassandra.hogan@bbsrc.ac.uk

**Dr Christian Holmberg**  
Department of Genetics, University of Copenhagen  
Oester Farimagsgade 2A  
DK-1353 Copenhagen K  
DK  
holm@my.molbio.ku.dk

**Mrs Johanna Hoog**  
EMBL  
Meyerhofstrasse 1  
Heidelberg  
69117  
DEU  
+49-6221-387620  
hoog@embl.de

**Dr Tim Humphrey**  
MRC Humphrey  
Radiation and Genome Stability Unit  
Harwell  
Oxford  
OX11 0RD  
GBR  
+44 (0)1235 841114  
T.Humphrey@har.mrc.ac.uk

**Dr Chiho Ikebe**  
Cancer Research UK, London Research Institute  
44 Lincolns Inn Fields  
London  
WC2A 3PX  
GBR  
+44 (0)20-269-3330  
chiho.ikebe@cancer.org.uk

**Ms Anja Irmisch**  
University of Sussex  
Science Park Road  
East Sussex  
Falmer, Brighton  
BN1 9RQ  
GBR  
+44 (0)1273 678123  
a27@sussex.ac.uk

**Miss Alessa Jaendling**  
University of Wales, Bangor  
Memorial Institute  
Deiniol Rd  
Bangor  
Gwynedd  
LL57 2UW  
GBR  
+44 (0)1248 382369  
bsp032@bangor.ac.uk

**Professor Nic Jones**  
Paterson Institute for Cancer Research  
Christie Hospital NHS Trust  
Wilmslow Road  
Manchester  
M20 4BX  
GBR  
+44 (0)161 446 3101  
NJones@picr.man.ac.uk

**Dr Nimesh Joseph**  
Paterson Institute for Cancer Research  
Wilmslow Road  
Withington  
Manchester  
M20 4BX  
GBR  
+44 (0)161-4463156  
Njoseph@picr.man.ac.uk

**Mrs Viv Junker**  
Swiss Institute of Bioinformatics  
CMU, 1 Michel-Servet  
Geneva  
CH-1211  
CHE  
+41-22-379 58 22  
viv.junker@isb-sib.ch

**Mr Mune Yoshi Kanai**  
Hiroshima University  
Kagamiyama1-3-1  
Higashihiroshima  
Hiroshima  
739-8530  
JPN  
+81-82-424-7764  
kanai@hiroshima-u.ac.jp

**Mrs Orsolya Kapuy**  
Budapest University of Technology and Economics  
Budapest  
H-1111  
HUN  
+3614632910  
okapuy@mail.bme.hu

**Dr Jim Karagiannis**  
Temasek Life Sciences Laboratory  
1 Research Link  
National University of Singapore  
Singapore  
117604  
SGP  
+65-6872-7000  
jim@ttl.org.sg
Dr Andrea Krapp
ISREC
Boveresses 155
Epalinges
1066
CHE
+41 21 692 58 58
andrea.krapp@isrec.ch

Ms Marit Krohn
The Norwegian Radium Hospital
Montebello
Oslo
0310
NOR
+47 22935945
markro@labmed.uio.no

Mr Shunsuke Kubota
Hiroshima University
Kagamiyama 1-3-1
Higashi Hiroshima
Hiroshima
739-8530
JPN
+81 82 424 7764
hero326@hiroshima-u.ac.jp

Mr Arun Kumar
Institute of Genomics and Integrative Biology
Opposite Jubilee Hall ,
Delhi university North campus
Mall Road
Delhi
India
110007
IND
+91-011-27666156, 27666157, 276
arunigib@gmail.com,
arun.kumar@igib.res.in

Mr Kazunori Kume
Hiroshima University
Kagamiyama 1-3-1
Higashi Hiroshima
Hiroshima
739-8530
JPN
+81 82 424 7764
kume513@hiroshima-u.ac.jp

Mr Daniel Lackner
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
Cambridge
CB10 1SA
GBR
+44 (0)1223-494862
dh@sanger.ac.uk

Mr Ronak Lakhani
University of Sussex
Falmer
Brighton
BN1 9QG
GBR
+44 (0)1273 678308
rl43@sussex.ac.uk

Mrs Eva Lambea
University of Barcelona
C Casanova 143
Barcelona
08036
ESP
+34934037252
eva_lambea@ub.edu

Mrs Sarah Lambert
Institut Curie, CNRS
Institut Curie, UMR2027
bat 110 Centre universitaire
Orsay
91405
FRA
+1 69 86 71 91
sarah.lambert@curie.u-psud.fr

Dr Clare Lawrence
Paterson Institute for Cancer Research
Christie Hospital NHS Trust
Wilmslow Road
Manchester
M20 4BX
GBR
+44 (0)161 446 3171
clarence@picr.man.ac.uk

Mr Minho Lee
Korea Advanced Institute of Science and Technology
Protein Bioinformatics Laboratory
Department of BioSystems
Korea Advanced Institute of Science and Technology
373-1, Guseong-dong, Yuseong-gu, Daejeon
305-701
KOR
+82-42-869-4357
MinhoLee@kaist.ac.kr

Mr Ioannis Legouras
University of Patras
Rio, Patra
Achaia
26500
GRC
+30-2610-99182
ioannislegouras@gmail.com

Miss Li Phing Liew
University of Oxford
South Parks Road
Oxford
OX1 3RE
GBR
+44 (0)1865 285510
lliew@path.ox.ac.uk

Dr Luis Lopez
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
CB10 1SA
GBR
+44 (0)1223494862
lfl@sanger.ac.uk

Mrs Sandra Lopez-Aviles
University of Barcelona
C Casanova 143
Barcelona
08036
ESP
+34934037252
sandralopezaviles@ub.edu
Dr Zoi Lygerou
University of Patras, School of Medicine
University Campus
Rio, Patras
26500
GRC
+30 2610 997621
lygerou@med.upatras.gr

Dr Yasmine Mamnun
Cancer Research UK, LRI
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44(0)207 269 3330
yasmine.mamnun@cancer.org.uk

Ms Ryoko Mandeville
The Rockefeller University
1230 York Avenue
New York, NY
NY
USA
+1-212-327-8472
mandevr@rockefeller.edu

Prof Haim Manor
Technion-Israel Institute of Technology
Technion City
Haifa
32,000
ISR
+972-4-8293456
manor@tx.technion.ac.il

Ms Madelaine Marchin
Stowers Institute for Medical Research
1000 E 50th St
Kansas City
MO
64110
USA
+1 816 926 4454
mcm@stowers-institute.org

Dr Samuel Marguerat
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
Cambs
CB10 1SA
GBR
+44 (0)1223 834244
sbm@sanger.ac.uk

Prof Ian Marshall
University of Kent
Computing Laboratory
Canterbury
CT2 7NF
GBR
+44 (0)1227 827753
i.w.marshall@kent.ac.uk

Miss Rebecca Martin
Universidad de Salamanca
Edificio Departamental
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34923121589
a56547@usal.es

Dra Cristina Martin-Castellanos
Instituto de Biología Molecular y Celular del Cán cer
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34 923 29 48 10
cmartin@usal.es

Dr Juan Mata
The Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
Cambs
CB10 1SA
GBR
+44 (0)1223 494862
jmk@sanger.ac.uk

Mr Orestis Mavroudis-Chocholis
Paterson Institute for Cancer Research
Christie Hospital
Wilmslow Road
Withington
Manchester
M20 4BX
GBR
+44 (0)161 446 3171
OMavroudisChocholis@PfCR.man.ac.uk

Dr Karen May
Edinburgh University
Kings Buildings
Mayfield Road
Edinburgh
EH9 3JL
GBR
+44 (0)131 650 8087
karen.may@ed.ac.uk

Ramsey McFarlane
NWCRF, UWB
Memorial Building
Deiniol Road
Bangor
Gwynedd
LL57 2UW
+44 (0)1248 382360
GBR
ramsay@sbs.bangor.ac.uk

Mr John Meadows
National Institute for Medical Research
The Ridgeway
Mill Hill
London NW7 1AA
GBR
+44 (0)20 8959 3666
jmeadow@nimr.mrc.ac.uk

Dr Margaret Migocki
MRC National Institute of Medical Research
The Ridgeway
Mill Hill
London
NW7 1AA
GBR
+44 (0)20 8959 3666
margaret.migocki@gmail.com

Dr Jonathan Millar
National Institute for Medical Research
The Ridgeway
Mill Hill
London NW7 1AA
GBR
+44 (0)208 959 3666
jmillar@nimr.mrc.ac.uk

Ms Vicky Miller
University of Edinburgh
The Wellcome Trust Centre for Cell Biology
Swann Building
Mayfield Road
Edinburgh EH9 3JR
GBR
+44 (0)131 650 7063
vicky.miller@ed.ac.uk

Miss Laura Milne
University of Edinburgh
Michael Swann Building
King’s Buildings
Mayfield Road
Edinburgh EH9 3JR
GBR
0131 650 7083
l.milne-1@sms.ed.ac.uk

Dr Izumi Miyabe
Genome Damage and Stability Centre
University of Sussex
Brighton
BN1 9RQ
GBR
+44 (0)1273 873118
I.Miyabe@sussex.ac.uk

Dr Kenichi Mizuno
Genome Damage and Stability Centre
University of Sussex
Falmer
East Sussex
BN19RQ
GBR
+44 (0)1273 877521
K.Mizuno@sussex.ac.uk
Dr Sara Mole
University College London
MRC Laboratory for Molecular Cell Biology
University College London
Gower Street
London
WC1E 6BT
GBR
+44 (0)20 7679 37806
s.mole@ucl.ac.uk

Dr Hiroshi Murakami
Nagoya City University,
1 Kawasumi, Mizuho-cho, Mizuho-ku,
Nagoya
467-8601
JPN
+81-52-853-8145
hmura@med.nagoya-cu.ac.jp

Dr Jo Murray
Sussex University
Falmer
Brighton
BN1 9RQ
GBR
+44 (0)1273 678123
j.m.murray@sussex.ac.uk

Ms Lindsay Murrells
European Molecular Biology Laboratory
Meyerhofstrasse 1
Heidelberg
D-69117
DEU
+49 0 6221 387-0
murrells@embl.de

Dr Koji Nagao
Initial Research Project, Okinawa
Institute of Science and Technology
Suzuki 12-22
Uruma
Okinawa
904-2234
JPN
+81 98 921 3985
nagao@irs.ist.jp

Dr Kentaro Nakano
University of Tsukuba
1-1-1 Tennohdai
Tsukuba
Ibaraki
305-8572
JPN
+81-29-853-6642
knakano@biol.tsukuba.ac.jp

Miss Szu Shien Ng
University of Glasgow
Davidson Building, IBLS
Glasgow
G12 8QQ
GBR
s.ng.1@research.gla.ac.uk

Prof Olaf Nielsen
University of Copenhagen
Farimagsgade 2A
Copenhagen K
DK-1353
DNK
+45 3532 2000
onigen@my.molbio.ku.dk

Dr Osami Niwa
Kazusa DNA Research Institute
2-6-7 Kazusa-kamata
Kisarazu
Chiba
292-0818
JPN
+81-438-52-3923
niwa@kazusa.or.jp

Mrs Chiaki Noguchi
Drexel University College of Medicine
245 N 15th Street
MS497
Philadelphia
PA
19102
USA
+1 215-762-4424
cn43@drexel.edu

Dr Eiishi Noguchi
Drexel University College of Medicine
245 N 15th Street
MS497
Philadelphia
PA
19101
USA
+1 215-762-4424en34@drexel.edu

Dr Chris Norbury
University of Oxford
South Parks Road
Oxford
OX1 3RE
GBR
+44 (0)1865 275500
chris.norbury@path.ox.ac.uk

Prof Bela Novak
Budapest University of Technology and Economics
Gellert ter 4
Budapest
1111
HUN
+3614631364
bnovak@mail.bme.hu

Paul Nurse
The Rockefeller University
1230 York Avenue, Box 257
New York
NY 10021
USA
+1 212-327-8080
nurse@rockefeller.edu

Mr Yong-Chin Oei
University of Cambridge
Cavendish Laboratory
Madingley Road
JJ Thompson Avenue
Cambridge
CB3 0HE
GBR
+44 (0)1223 337200
yungkin@molphys.leidenuniv.nl

Dr Koei Okazaki
Kazusa DNA Research Institute
2-6-7 Kazusa-Kamata
Kisarazu
Chiba
292-0818
JPN
+81-438-52-3925
kokazaki@kazusa.or.jp
Ms Olivia Rissland
University of Oxford
South Parks Road
Oxford OX1 3RE
GBR
+44 (0)1865 275500
olivia.rissland@path.ox.ac.uk

Mr Ofer Rog
Cancer Research UK
44 Lincolns Inn Fields
London WC2A 3PX
GBR
+44 (0)2072693212
offer.rog@gmail.com

Mr Assen Roguev
BIOTEC TU-Dresden
Tatzberg 47-51
Dresden
01307
DEU
+4935146340107
roguev@biotec.tu-dresden.de

Mrs Laura Roseaulin
Pasteur Institute
25-28 rue du docteur Roux
Paris
75015
FRA
+33 140613686
lroseaulin@pasteur.fr

Mrs Maja Rothenberg
University of Bern
Baltzer-Strasse 4
Bern
CH-3012 Bern
+41-31-631-4654
maja.rothenberg@gmx.de

Mrs Ana Elisa Rozalén
Instituto de Biología Molecular y Celular
de Cáncer CSIC-USAL
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34 923 29 48 10
anae@usal.es

Prof Paul Russell
The Scripps Research Institute
10550 North Torrey Pines Road
MB3
La Jolla
CA
92037
USA
+1 858 784-8273
prussell@scripps.edu

Mr Cagri Sakalar
BIOTEC TU Dresden
Biotechnologisches Zentrum
TU Dresden
Dresden
01062
DEU
+4935146340107
sakalar@biotec.tu-dresden.de

Dr Itaru Samejima
University of Edinburgh
Michael Swann Building
Mayfield Road
Edinburgh
EH9 3JF
GBR
+44 (0)131 650 7063
itaru.samejima@ed.ac.uk

Dr Yolanda Sanchez
Universidad de Salamanca-CSIC
Edificio Departamental, lab 231
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34-923-121589
ysm@usal.es

Dr Beatriz Santos
CSIC University of Salamanca
Aved. Campo Charro sn Hab.223
Salamanca
37007
ESP
+34-923-121644
bss@usal.es

Dr Masamitsu Sato
Cancer Research UK, London Research Institute
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44 (0)2072693330
masamitsu.sato@cancer.org.uk

Dr Kenneth Sawin
University of Edinburgh
Swann Building, Mayfield Road
Edinburgh
EH9 3JF
GBR
+44 (0)131-650-706
ken.sawin@ed.ac.uk

Dr Shelley Sazer
 Baylor College of Medicine
One Baylor Plaza
Houston
77030
713 798-4531
ssazer@bcm.tmc.edu

Ms Christine Schmidt
CRUK - London Research Institute
Lincoln s Inn Fields Laboratories
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44 (0)2072693229
christine.schmidt@cr.ac.uk

Dr K. Anke Schürer
Leibniz Institute for Age Research - Fritz Lipmann Institute
Beutenbergstrasse 11
Jena
D-07745
DEU
+49-3641-656137
aschuer@gwdg.de

Miss Matylda Szczygiecka
University of Edinburgh
Swann Building S5.08
Kings Buildings
Mayfield Road
Edinburgh
EH9 3JF
GBR
+44 (0)131 650 7083
matylda.szczlkecka@ed.ac.uk

Mr Nadeem Shaikh
National Institute for Medical Research
The Ridgeway
Mill Hill
London
NW7 1AA
GBR
+44 (0)20 8955 3666
nshaikh@nimr.mrc.ac.uk

Professor Kazuhiro Shiozaki
University of California, Davis
One Shields Ave.
Davis
95616
USA
+1530752-7467
kshiozaki@ucdavis.edu

Mr Indranil Sinha
Karolinska Institutet
S\&246;der\&246;msg 1 H\&246;skola, Alfred
Nobels Alle 7,
Huddinge
14189
SWE
+4686084000
indranil.sinha@sh.se

Dr Srivani Sistla
Institute for Molecular and Cell Biology
61, Biopolis Drive
05-01, PROTEOS
Singapore
138673
SGP
+65-6858 95 57-58
srivanis@imcb.a-star.edu.sg
Miss Kate Sloan  
CRUK Paterson Institute for Cancer Research  
Christie Hospital NHS Trust  
Wilmslow Road  
Manchester  
M20 4BX  
GBR  
+44 (0)1614463156  
Ksloan@picr.man.ac.uk

Dr Hilary Snaith  
Wellcome Trust Centre for Cell Biology  
University of Edinburgh  
Swann Building  
Mayfield Road  
Edinburgh  
EH9 3JY  
GBR  
+44 (0)131 650 7063  
hilary.snaith@ed.ac.uk

Mrs Hela Soltani  
Institute of Cancer Research, DNR Ullelunch. 70  
Oslo  
0310  
NOR  
+47 22 93 40 00  
hela.soltani@klinmed.uio.no

Dr Nathalie Spieweloy  
Cancer Research UK  
44 Lincolns Inn Field Institute  
London  
WC2A 3PX  
GBR  
+44 (0)207 061 8080  
nathalie.spieweloy@cancer.org.uk

Mr Peter Stansfield  
University of Dundee  
Level 5  
Ninewells Hospital and Medical School  
Dundee  
DD1 9SY  
GBR  
+44 (0)1382 660 111 ext. 32566  
p.j.stansfield@dundee.ac.uk

Miss Abigail Stevenson  
University of Oxford  
South Parks Road  
Oxford  
OX1 3PS  
GBR  
+44 (0)1865 271234  
abigail.stevenson@queens.ox.ac.uk

Ms Geetanjali Sundaram  
Guha Centre for Genetic Engg. and Biotechnology  
35 Ballygunje Circular Road  
Ballygunje Science College  
University of Calcutta  
Kolkata  
700019  
IND  
+91-033- 24614983  
geetanjali.rajee@gmail.com

Prof Sasa Svetina  
Faculty of Medicine, University of Ljubljana  
Lipiceva 2 Ljubljana  
1000  
SVN  
+s386 1 5437600  
sasa.svetina@biofiz.mf.uni-lj.si

Miss Virginia Tajadura  
Universidad de SalamancaCSIC  
Edificio Departamental, lab. 231  
Campus Miguel de Unamuno  
Salamanca37007  
ESP  
+34-923-121589  
virginia.tajadura@gmail.com

Dr Kayoko Tanaka  
Graduate School of Science, University of Tokyo  
7-3-1 Hongo, Bunkyo-ku  
Tokyo  
113-0033  
JPN  
+81-3-5841-4388  
katanaka@biochem.s.u-tokyo.ac.jp

Dr Zhaohua Irene Tang  
Claremont Colleges  
925 N. Mills Ave.  
Claremont Colleges  
Claremont  
California  
CA 91711  
USA  
+1 909-607-9067  
ztang@claremont.edu

Miss Helen Tinline-Purvis  
Medical Research Council  
Harwell  
Didcot  
OX11 0RD  
GBR  
+44 (0)1235 841000  
h.tinline-purvis@har.mrc.ac.uk

Dr Takashi Toda  
Cancer Research UK  
London Research Institute  
44 Lincolns Inn Fields  
London  
WC2A 3PX  
GBR  
+44 (0)207269 3535  
toda@cancer.org.uk

Dr Iva Tolic-Norrellykke  
MPI-CBG Max Planck Institute of Molecular Cell Biology and Genetics  
Pfotenhauerstr. 108  
Dresden  
01307  
DEU  
+493512102020  
tolic@mpi-cbg.de

Dr Kazunori Tomita  
Cancer Research UK  
44 Lincolns Inn Fields  
London  
WC2A 3PX  
GBR  
+44 (0)207-269-3415  
kazunori.tomita@cancer.org.uk

Dr Yuko Tonami  
Nagoya City University Graduate School of Medicine  
1 Kawasumii, Mizuho-cho, Mizuho-ku  
Nagoya  
Aichi  
467-8601  
JPN  
+81-52-853-8146  
ytonami@med.nagoya-cu.ac.jp

Dr Sylvie Tournier  
UMR5088  
118 route de Narbonne  
TOULOUSE  
31062  
FRA  
+33 05 61 55 69 10  
tournier@cict.fr

Dr Mika Toya  
Cancer Research UK, London Research Institute  
44 Lincolns Inn Fields  
London  
WC2A 3PX  
GBR  
+44 (0)207 269 3330  
mika.toya@cancer.org.uk

Dr Phong Tran  
University of Pennsylvania  
421 Curie Blvd, Room 1009  
Philadelphia  
PA  
19104  
USA  
+1 215 746 2755  
trang@mail.med.upenn.edu

Dr Michelle Trickey  
Marie Curie Research Institute  
Limpfield Chart  
Oxted  
Surrey  
RH8 0TL  
GBR  
+44 (0)1883 722306  
m.trickey@mcri.ac.uk
Miss Ellen Tsang
Genome Damage and Stability Centre
Science Park Road
University of Sussex
Falmer
BN1 9RQ
GBR
+44 (0)1273 873118
et39@sussex.ac.uk

Ms. Tonje Tvegåard
Institute for Cancer Research, DNR
Montebello
Oslo
0310
NOR
+47 22 93 40 00
tonje.tvegard@klinmed.uio.no

Dr Frank Uhmann
Cancer Reserach UK London Research Institute
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44 (0)207 269 3024
frank.uhmann@cancer.org.uk

Dr Makoto Umeda
Baylor College of Medicine
One Baylor Plaza
Houston
77030
USA
+1 713-798-5486
mumeda@bcm.tmc.edu

Mrs Amy Unsworth
Cancer Research UK
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44 (0)20 72693330
amy.unsworth@cancer.org.uk

Dr Noelia Valbuena
Instituto de Biología Molecular y Celular
del Cáncer
Campus Miguel de Unamuno
Salamanca
37007
ESP
+34923294810
noeliav@usal.es

Miss Henar Valdivieso
Universidad de Salamanca CSIC
Edificio Departamental. Campus
Unamuno
Salamanca
37007
ESP
+34923121589
henar@usal.es

Mr Luis Valente
Cancer Research UK
Telomere Biology Laboratory
44 Lincolns Inn Fields
London
WC2A 3PX
GBR
+44 (0)20 7242 0200
luis.valente@cancer.org.uk

Dr Vincent Vanoosthuyse
Wellcome Trust Centre for Cell Biology
University of Edinburgh
King's Buildings
Mayfield Road
Edinburgh
Ecosse
EH9 3JR
GBR
+44 (0)131 650 7083 lab0131 650 7
vanoost@staffmail.ed.ac.uk

Dr Usha Vijayraghavan
Indian Institute of Science
C. V. Raman Avenue
Bangalore
Karnataka
560012
IND
+ 91 80 23600168
uvr@mcbli.iisc.ernet.in

Mr. Sven K. Vogel
Max Planck Institute
Pfotenhauer Str. 108
Dresden
D-01307
DEU
+49-351-210-0
vogel@mpi-cbg.de

Miss Huan Wang
Queen Mary, University of London
333 Mile End Road
London
E1 4NS
GBR
+44 (0)2078824784
huan.wang@qmul.ac.uk

Dr Shao-Win Wang
University of Oxford
South Parks Road
Oxford
OX1 3PS
GBR
+44 (0)1865 271212
shao-win.wang@zoo.ox.ac.uk

Mr Stephen Watt
Wellcome Trust Sanger Institute
Hinxton
Cambridge
CB10 1SA
GBR
+44 (0)1223 834244
sbw@sanger.ac.uk

Dr Felicity Watts
University of Sussex
School of Life Sciences
Falmer
Brighton
E Sussex
BN1 9QG
GBR
+44 (0)1273 678257
f.z.watts@sussex.ac.uk

Mr Boon-Yu Wee
MRC Radiation and Genome Stability Unit
Harwell
Oxford
OX11 0RD
GBR
+44 (0)1235 841114
B.Wee@har.mrc.ac.uk

Mr Jikai Wen
The University of Birmingham
Room 601, School of Biosciences
The University of Birmingham
Edgbaston
Birmingham
B15 2TT
GBR
+44 (0)121 414 5406
wxj580@bham.ac.uk

Dr Simon Whitehall
University of Newcastle
Catherine Cookson Building
Framlington Place
Newcastle upon Tyne
NE2 4HH
GBR
+44 (0)191 222 6000
s.k.whitehall@ncl.ac.uk

Dr Brian Wilhelm
WTSI
Wellcome Trust Genome Campus
Hinxton
CB10 1SA
GBR
+44 (0)1223 834244
btw@sanger.ac.uk

Dr Caroline Wilkinson
Paterson Institute for Cancer Research
Wilmslow Road
Manchester
M20 4BZ
GBR
+44 (0)161 446 3171
cwilkinson@pirc.manch.ac.uk
Dr Jo Wixon
John Wiley and Sons Ltd
4 Pieces Court
Waterbeach
Cambridgeshire CB5 9QL
GBR
+44 (0)1223 479052
jwixon@wiley.co.uk

Ms Valerie Wood
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Hinxton
Cambridge CB10 1SA
+44 (0)1223 494954
val@sanger.ac.uk

Prof Anthony Wright
Sodertorn
Alfred Nobels Alle 7
Huddinge
SE-141 89
SWE
+46 86084708
anthony.wright@sh.se

Dr Pei-Yun Jenny Wu
The Rockefeller University
1230 York Avenue
New York NY
10021
USA
+1-212-327-8472
mandevr@rockefeller.edu

Dr Jerome Wuarin
University of Dundee
Level 5, Ninewells Hospital
Dundee DD1 9SY
GBR
+44 (0)1382 632566
jerome.wuarin@cancer.org.uk

Mrs Yongtao Xue-Franzén
Sodertorns University College &
Karolinska Institute
Alfred Alle 3
Huddinge
147 70
SWE
+6084000
yongtao.xue@sh.se

Dr Hiro Yamano
Marie Curie Research Institute
The Chart
Oxted, Surrey
RH8 0TL
GBR
+44 (0)1883 722306
h.yamano@mcri.ac.uk

Dr Akira Yamashita
University of Tokyo
7-3-1, Hongo, Bunkyo-ku
Tokyo
113-0033
JPN
+81-3-5841-4387
ymst@biochem.s.u-tokyo.ac.jp

Prof Mitsuhiro Yanagida
Kyoto University
Yoshida-Honmachi
Sakyoku
Kyoto
606-8501
JPN
+81 75 753 4205
yanagida@kozo.if.kyoto-u.ac.jp

Dr Paul G. Young
Queen’s University
R.2443 Biosciences Complex
Kingston
Ontario
K7L 3N6
CAN
+1 613-533-6148
youngpg@biology.queensu.ca

Dr Xuefeng Zhu
Karolinska Institutet
Stockholm
SE-141 86
SWE
+46-8-58583761
xuefeng.zhu@ki.se

Notes