

Meeting report

## Fission yeast enters a joyful new era

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A report on the Second International Fission Yeast Meeting, Kyoto, Japan, 25-30 March 2002.

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The Second International Fission Yeast Meeting was a celebration of two significant milestones for the community that works on *Schizosaccharomyces pombe*. First, the award of the 2001 Nobel Prize to Paul Nurse (together with Leland Hartwell and Tim Hunt) provided worldwide recognition for research in fission yeast and its relevance to understanding cell-cycle controls and cancer. Second, the recent announcement of the completion of the *S. pombe* genome extended the value of this experimental system into the fields of genomics and proteomics. The cherry trees of Kyoto bloomed early for the occasion.

The keynote speakers at the meeting provided historical context in this most historical of cities, as they reflected on the past few decades that lifted fission yeast from obscurity: Paul Nurse (Cancer Research UK, London, UK) recalled the history of cell-cycle research in *S. pombe*; Tony Hunter (The Salk Institute, La Jolla, USA), a new convert to working with *S. pombe*, described the evolution of the 'kinome', the complete complement of protein kinases; and Tim Hunt (Cancer Research UK) reviewed the cell cycle from the perspective of cyclins and stressed the importance of comparing multiple models in advancing research. The eleven sessions that followed provided experimental proof of the progress of fission yeast research, particularly in the areas of genomics and cell-cycle biology.

### Cell cycle and checkpoints

The long history of fission yeast cell-cycle research continues into the new millennium. Among the significant findings reported at the meeting, Anthony Carr (University of Sussex, Brighton, UK) proposed that the cyclin Cdc13, which

functions with the Cdc2 protein kinase in cell-cycle control, directs the pathway by which DNA damage (such as double-strand breaks) is repaired during G2 phase of the cell cycle. Carr identified and characterized a *cdc13* mutant that is radiation-sensitive yet proficient for the DNA-damage checkpoint. This provides a unique link between the cell cycle and the DNA-repair machinery. Along similar lines, Tim Humphrey (Medical Research Council Radiation and Genome Stability Unit, Harwell, UK) described the cellular response to a single, site-specific double-strand DNA break in *S. pombe*. By adapting use of the *Saccharomyces cerevisiae* HO mating-type endonuclease to *S. pombe*, Humphrey was able to induce a single, unique, double-strand break in a nonessential minichromosome. Repair of this double-strand break required homologous recombination and, surprisingly, the Rad16 nucleotide-excision repair gene product.

Cell-cycle studies have now merged with broader questions of genome dynamics, including replication and segregation. Thus, for example, Stephen Kearsey (University of Oxford, UK) spoke on monitoring the binding of replication factors to chromatin *in vivo*. By using a heat-inducible 'degron' mutation (which drives protein degradation in a temperature-sensitive manner) of the *cdc23* gene, along with fluorescently tagged proteins, Kearsey showed that Cdc23 is needed for Sna41 protein to bind to chromatin but not for the minichromosome maintenance (MCM 2-7) complex to bind chromatin; and one of us (S.F.) spoke in the replication session on a novel role for the Hsk1/Cdc7 kinase in sister-chromatid cohesion that involves interaction with heterochromatin.

### DNA replication

The existing model for the structure of the sites at which DNA replication begins - the replication origins - was tested by evidence from several groups. Tom Kelly (Johns Hopkins University, Baltimore, USA) presented a 'quasi-random' model for how certain stretches of DNA are chosen for use as

replication origins. Kelly analyzed a 70 kilobase region of *S. pombe* chromosome II for origin activity and found that origin sites are not determined by primary DNA sequence, although they do tend to be intergenic and A-T-rich. This supports studies on previously defined individual origins such as *ars1*. Interestingly, the proteins that make up the origin recognition complex (ORC) bind to multiple sites within *ars1*. Hisao Masukata (Osaka University, Japan) presented evidence that ORC binds to two sites within another previously defined origin, *ars2004*. Surprisingly, another component of the prereplicative complex (preRC), namely the MCM 2-7 complex, localizes to a site within the origin distinct from the ORC-binding site. This molecular organization has interesting implications for how origin sequences are chosen and how the preRC is assembled.

The control of replication-origin firing (the initiation of replication at an origin) was addressed by Joel Huberman (Roswell Park Cancer Institute, Buffalo, USA). Huberman used two-dimensional gel analysis to demonstrate that the activity of a late origin of replication is suppressed in a checkpoint-dependent manner when cells are treated with hydroxyurea. Furthermore, Huberman suggested that checkpoint proteins such as Cds1 contribute to the structure of stalled replication forks, since *cds1* mutants show aberrant replication intermediates, and *cds1* cells released from hydroxyurea replicate their DNA much more slowly than wild-type cells.

### Kinetochores structure and function

The structure and regulation of the *S. pombe* kinetochore was addressed by several groups. Kohta Takahashi and Ei Sin Chen, working in Mitsuhiko Yanagida's laboratory (Kyoto University, Japan), reported the identification and characterization of Ams2, a novel GATA-family transcription factor that regulates loading of the kinetochore protein CENP-A onto centromere sequences. Gohta Goshima of Yanagida's group described the regulation of kinetochore chromatin structure by the interaction of Ppe1 phosphatase and Gsk3 kinase with the Mis12 kinetochore protein. Six new *mis* genes, encoding novel components of the kinetochore, were also presented by Yanagida's group. Robin Allshire (The Wellcome Trust Centre for Cell Biology, ICMB, Edinburgh, UK) also reported the identification of new kinetochore components, using a screen for mutants defective in gene silencing, since efficient silencing of centromeric genes is linked to a functional kinetochore.

The structure of the *S. pombe* kinetochore was analyzed by Karl Ekwall's group (Karolinska Institute, Stockholm, Sweden), using electron microscopy. Ekwall proposed a multi-layered kinetochore structure, in which the structure around the central core of the centromere sequence is distinct from the structure in the heterochromatin-containing regions, and that these two are joined by an anchor structure that

may include the Ndc80 protein. It will be important to understand how these different domains interact, given their vital role in proper chromosome segregation. The plethora of new mutants affecting the kinetochore suggests that many more of the approximately 4,800 fission yeast genes will have their functions determined soon.

### Genomics and proteomics

The sequence of the *S. pombe* genome was published in February 2002. Valerie Wood, heading the genome project from The Wellcome Trust Sanger Institute (Hinxton, UK), reported that 12.5 Mb of the 13.8 Mb *S. pombe* genome has been sequenced; the few remaining gaps are limited to the rDNA and some portions of the centromeres, which are problematic because of the repeats within centromeric DNA. Thus, *S. pombe* is now officially 'post-genomic'. The GeneDB project [<http://www.genedb.org>] organized by the Sanger Institute is one of the first resources for the *S. pombe* community; it aims to maintain and update a database of genome-related information.

The new potential to analyze *S. pombe* at the genomic level has allowed the development of new scientific approaches and technologies. Minoru Yoshida's group (University of Tokyo, Japan) has constructed a library of *S. pombe* open reading frames (ORFs), using a new Gateway™ cloning system developed by Invitrogen Corp (Carlsbad, USA). This system allows the ORFs to be expressed from multiple vectors, including production of their products as fusion proteins with green fluorescent protein (GFP). Yoshida and colleagues used these expression vectors to characterize the subcellular localization pattern of many *S. pombe* proteins. Other groups, such as those of Hyang S. Yoo (Korea Research Institute of Bioscience and Biotechnology, Taejeon, Korea) and Jianhua Liu (University of Singapore) described early progress towards the systematic deletion of *S. pombe* genes. Yoo and colleagues aim to delete each gene individually, with the goal of aiding drug discovery. Lui and colleagues are in the process of systematically deleting each of the 500-600 genes predicted to be involved in cytokinesis, in order to improve the characterization of that process.

Ramsay McFarlane (University of Wales, Bangor, UK) described preliminary studies of the fission yeast proteome. Advances in the area of two-dimensional gel electrophoresis and mass spectrometry, in combination with the information from the genome project, are expanding the capacity for comparative analysis of the *S. pombe* proteome under different conditions. High-throughput analysis is planned as these methods mature.

Yoshuda Hiraoka (Kansai Advanced Research Center, Kobe, Japan) who has already developed a GFP fusion library of *S. pombe* proteins, available at Hiraoka's website [<http://www-karc.crl.go.jp/bio/CellMagic/index.html>],

reported the construction of a DNA microarray of the fission yeast genome and its use in monitoring gene expression during the process of telomere clustering in meiosis. Interestingly, other groups are also developing fission yeast DNA microarrays. Jurg Bahler (The Wellcome Trust Sanger Institute, Hinxton, UK) is using this technology to study gene expression under various conditions. Bahler demonstrated one such analysis of gene-expression profiling during meiosis and sporulation. He found that more than 2,000 genes are regulated in meiosis. There are at least four successive waves of transcription during meiosis corresponding to nitrogen starvation (used to induce meiosis), pre-meiotic S-phase and prophase, the meiotic nuclear division, and sporulation.

The use of DNA microarrays has become commonplace for the analysis of other organisms with sequenced genomes. With the near completion of the *S. pombe* genome project, DNA microarrays should also become commercially available for use in the fission yeast scientific community. The Belgian company Eurogentec (Seraing, Belgium) [<http://www.eurogentec.com>], in collaboration with the Södertörns University College in Sweden and the Max Planck Institute in Germany, has developed an *S. pombe* DNA microarray representing 99.8% of the annotated genes; this microarray is now for sale and available for immediate use.

The need for a community-wide facility for *S. pombe* functional genomics has encouraged John Armstrong (University of Sussex, Brighton, UK) to create a community resource called FYSSION [<http://pombe.biols.susx.ac.uk/>]. This site has already made two mutant libraries available to the *S. pombe* community: a collection of temperature-sensitive strains, and a collection of strains randomly mutagenized by insertional mutagenesis. In addition, the FYSSION team has offered to serve as strain curators and distributors.

Although not strictly part of the genomics/proteomics discussion, Kathy Gould (Vanderbilt University, Nashville, USA) presented a picture of how the developing technology will lead to fascinating new approaches in cell biology. Using tandem affinity-purification (TAP tagging) of the spliceosome, followed by sophisticated mass spectrometry using genome data, the protein components of the spliceosome were identified. The actual shape of this purified complex is now being determined using advanced electron microscopic methods. Ultimately, the positions of the different subunits will be determined using tags and antibodies.

Since the Pombe 2000 workshop, London, UK, October 2000, where the first pombe genomics discussion was held, substantial progress has been made. Fission yeast has truly entered the 21st century and takes its rightful position with a handful of other species as a major experimental system. *S. pombe* aficionados anticipate much progress with this 'model eukaryote'. Gaudeamus igitur!