

Meeting report

## Dynamic chromosomes

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A report on the sixth biannual FASEB conference on Yeast Chromosome Structure, Replication and Segregation held in Snowmass, Colorado, 19-24 August, 2000.

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Several new themes became apparent at this meeting, which was an outstanding one for researchers interested in any aspect of chromosome dynamics in various yeast species. The first and most striking was that those who study any particular aspect of yeast chromosomes - for example, replication - now increasingly find themselves studying aspects of structure and segregation as well. The traditional cell-cycle-dominated view of chromosomes has been turned on its head (as represented by the non-traditional organization of this meeting, which placed the replication session last). The second thread tying a number of sessions together was the increasing importance of studying events in meiosis, not only in their own right but also to illuminate events in vegetative cells.

### The genomics revolution

The third theme underlying many talks was genomics. Although the *Saccharomyces cerevisiae* genome sequence was completed several years ago, the majority of talks addressing chromosome function still relied on classical yeast biology. Having the genome sequence has not altered the major task of identifying and characterizing mutants. Instead, like PCR, genomics gives us new tools that enormously speed up the process of data collection; it complements, rather than replaces, more classical biological approaches. Mike Snyder (Yale University, USA) described several approaches that are producing valuable data for the community (Yale Genome Analysis Center [<http://ygac.med.yale.edu/>]), including the effort to transposon-tag each budding yeast gene with a hemagglutinin (HA) tag to allow

analysis of subcellular localization; of 1,200 nuclear proteins studied, 600 were found to bind chromosomes. Another project is based on the 'ChIP chip' method: chromatin immunoprecipitation against chip arrays. Transcription factors can be HA tagged, immunoprecipitated while bound to chromatin, and the bound sequence can be hybridized to a microarray of intergenic regions, to identify putative target genes. In an analysis of binding by the Swi4p transcription factor, some binding regions were identified that lacked the canonical Swi4p binding sites. Over 160 binding sites in intergenic regions were identified; 40% of the sites neighbored open reading frames (ORFs) that exhibited G1/S periodicity, and over half of these ORFs were of unknown function.

Snyder also described analysis of 106 of the 122 protein kinases encoded by the budding yeast genome. Microarray wells are coated with a potential substrate and a purified kinase is added to each well for a kinase assay, allowing a fingerprint of substrate specificity. The protein kinases were assayed against various substrates, such as casein, histone H1, and poly(TyrGln). Most kinases appeared to be promiscuous, with only half specific for one or two substrates. These approaches demonstrate the utility of having the genome sequence, but the interpretation of the data obtained requires a return to the organism to determine their relevance using classical means, including analysis of protein or substrate interactions and mutant analyses.

Tim Hughes (Rosetta Inpharmatics, Kirkland, USA) described progress on the use of expression profiles as a method to probe genes of unknown function. In *S. cerevisiae*, roughly 1,800 of the 6,000 genes remain uncharacterized. Rosetta Inpharmatics suggests that the whole genome expression profile is distinct when a given gene or pathway is disrupted, so particular expression profiles can become diagnostic of particular pathways. By cluster

analysis of expression profiles, they can therefore classify novel gene functions as related to known pathways. An unexpected finding was that approximately 8% of deletion mutants exhibited some level of aneuploidy. For example, the deletion strain that has lost the ribonucleotide reductase subunit Rnr1p can apparently survive by maintaining an extra copy of chromosome IX, which harbors *RNR3* (a known dosage suppressor of *rnr1*). M.K. Raghuraman (University of Washington, Seattle, USA) finished this chip-related discussion by describing genome-wide analysis of DNA replication origins in yeast. Defining budding yeast replication origins by combining classical density gradient analysis of replicated DNA and microarray analysis, Raghuraman and colleagues reported the identification of 376 replication origins in the yeast genome and characterized their replication timing. Peter Sorger (Massachusetts Institute of Technology, Cambridge, USA) described an ‘image informatics’ approach that relies upon a large database of biological images, in this case three-dimensional images of live budding yeast cells carrying centromeres tagged with green fluorescent protein (GFP), and uses computational analysis to identify factors affecting kinetochore function. With this technique, Sorger demonstrated that sister chromatids exhibit transient separation during prometaphase in the absence of cohesin proteolysis, and suggested that the yeast kinetochore acted as a tensiometer, detecting the tension between the microtubules and the chromosomes.

### The nuts and bolts of cell division

Amongst the highlights of the classical biological approach was analysis of mechanisms of spindle positioning during the cell cycle. David Pellman (Dana Farber Cancer Institute, Boston, USA) presented a mechanism for the cortical capture process, which defines how the spindle finds the bud. Bim1p, a budding yeast homolog of human EB1, may provide the bridge between the spindle microtubules and Kar9p, which is localized to the bud cell cortex. Tim Huffaker (Cornell University, Ithaca, USA) suggested that Myo2p might bind to the cytoplasmic microtubules through interaction with Kar9p, thereby linking spindle orientation to actin cables.

The telomere session focused on mechanisms by which telomeres are maintained in the absence of telomerase enzyme. Mundy Wellinger (Université de Sherbrooke, Canada) suggested that a mechanism independent of both telomerase and *RAD52* can maintain telomeric repeats; surprisingly, overproduction of the nucleolar protein NET1 in a *tlc1 rad52* double mutant leads to survivors that persist over 200 generations. Virginia Zakian (Princeton University, USA) reported that the telomere-binding proteins Rif1p and Rif2p function to inhibit telomere lengthening in wild-type cells. She proposed that when telomeres are very short and Rif proteins no longer bind, Rad50p can gain access and promote recombination events to lengthen the telomere. The

rate-limiting event in generating a long telomere may be the availability of the template; Zakian posited that extrachromosomal circles may provide the template for the elongation of telomeres via a rolling-circle mechanism. Michael McEachern (University of Georgia, Athens, USA) presented evidence for such a rolling-circle replication event for telomere elongation in *Kluyveromyces lactis*: he transformed the yeast with plasmids and showed that the lengthened telomeres included plasmid sequence.

The mitotic exit network in budding yeast was described by Angelika Amon (Massachusetts Institute of Technology, Cambridge, USA). The analogous pathway in fission yeast was described by Dan McCollum (University of Massachusetts Medical School, Worcester, USA) and Viesturs Simanis (Swiss Institute for Experimental Cancer Research (ISREC), Epalinges, Switzerland); in one of the many differences between fission and budding yeast, it appears that the *Schizosaccharomyces pombe* homolog of the nucleolar Cdc14p phosphatase is not an essential gene. Interestingly, however, despite the apparently symmetrical division of a fission yeast cell compared with the asymmetry of the budding yeast, there is substantial similarity between the genes involved in septation initiation (*S. pombe*) and mitotic exit (*S. cerevisiae*), even including asymmetric association of the cognate proteins with the spindle poles.

### Linking replication to chromosome structure

Michael Christman (University of Virginia, Charlottesville, USA) described a new essential DNA polymerase, Pol kappa, which may be involved in linking replication to chromosome cohesion during the S phase in vegetative cells. Evidence for a link between replication and subsequent events is also provided by analysis of meiosis; Valerie Borde (National Cancer Institute, National Institutes of Health, Bethesda, USA) and Alain Nicolas (Institut Curie, Paris, France) each had data suggesting that replication during meiosis is required for recombination to occur. We showed evidence that the initiation of meiotic replication requires different initiation factors from those used in vegetative S phase.

Susan Gasser (ISREC, Epalinges, Switzerland) presented microscopy data that emphasized the dynamic nature of origins of replication within the budding yeast nucleus, and showed that late-firing origins may preferentially localize to the nuclear periphery. Bruce Stillman (Cold Spring Harbor Laboratory, USA) linked the activity of proliferating cell nuclear antigen (PCNA) to epigenetic inheritance by demonstrating that PCNA interacts with Chromatin Assembly Factor 1 (CAF-1) and that PCNA mutants are defective in the inheritance of silenced chromatin. But in contrast to previous data, Ann Kirchmaier (University of California, Berkeley, USA) suggested that although silencing of chromatin is established during S phase, neither initiation of DNA replication nor replication fork passage is actually required for

silencing to occur. This result indicates that the precise mechanism to establish silencing during S phase remains to be identified. Kirchmaier suggested that the PCNA remaining on replicated chromatin may recruit CAF-1 to establish silencing. Biochemical approaches to understanding the complexities of replication were provided by John Diffley (Imperial Cancer Research Fund Clare Hall Laboratories, UK) who described an *in vitro* assay for the assembly of the pre-replicative complex in budding yeast extracts that appears to recapitulate events *in vivo*. Steve Bell (Massachusetts Institute of Technology, Cambridge, USA) analyzed the *cis*-acting elements in the autonomously replicating sequence ARS1 that regulate positioning of nucleosomes around the origin, and found that the origin-recognition complex (ORC) and the transcription factor Abf1 position the nucleosomes on ARS1 *in vitro*, thus affecting regulation and loading of replication factors.

As at previous meetings in this series, those attending the meeting left with the picture of a vibrant community doing state-of-the-art research, directed towards understanding an elegant element of every cell: the chromosome.