Philip Jordan (p.jordan@ed.ac.uk)

Conference Report

Author: Philip Jordan (p.jordan@ed.ac.uk)
Institute of Cell Biology, University of Edinburgh

FASEB Summer Research Conference

Yeast Chromosome Structure, Replication and Segregation
July 10-15, 2004
Pine Mountain, Georgia, USA.
Organisers: John Diffley (Cancer Research UK, London)
Angelika Amon (MIT, Massachusetts)
Tim Stearns (BSG, Stanford)

With the financial assistance of the James Rennie Bequest Travel Grant I was able to attend the FASEB Yeast Chromosome Structure, Replication and Segregation (YCSRS) conference that was held in Georgia, USA in July 2004. In the following I will give a brief report of the conference focussing on one presentation from each of the nine sessions.

The YCSRS conference was held in Callaway Gardens, Pine Mountain a man-made resort in friendly, humid and sunny Georgia. Congratulations to the organisers John Diffley, Angelika Amon and Tim Stearns who ran the conference very well.

The keynote speaker for the YCSRS conference was Kim Nasmyth (IMP), his talk explored the latest knowledge of the cohesin complex and the mechanisms involved in sister chromatid cohesion. The cohesin complex forms a ring structure and his working model is in favour of the complex trapping both of the sister chromatids within its ring structure (1). It is appropriate to note that this model is currently disputed and Douglas Koshland (Carnegie Inst.) presented work that proposes an alternative model. Koshland suggests that the cohesin complex does not trap both sister chromatids within its ring structure rather the cohesin complex binds to single sister chromatids. In this model linkage of sister chromatids is co-ordinated by interactions between the cohesion complexes on each sister chromatid.

The first session of the conference was based on chromatin structure and gene expression. One interesting topic of this session was the regulation of histone levels in the cell. Alain Verreault (Cancer Research UK, Clare Hall) discussed that during DNA synthesis histones are rapidly deposited behind the replication fork. Reduction in the rate of DNA synthesis can cause binding of excess histones, a phenomenon known as histone hypertension, which causes genome instability, transcriptional interference and increased sensitivity to DNA damage. Verreault presented data that showed budding yeast DNA damage checkpoint kinase Rad53p is responsible for regulating degradation of excess histones (2).

The work presented in the second session was based on large-scale and genome-wide experimental screening. Erin O'Shea (U of California, San Francisco) discussed high-throughput analysis of subcellular localisation in budding yeast. By constructing GFP fusion proteins they were able to localise ~75% of the yeast proteome. Among other things protein localisation is extremely useful for functional profiling of uncharacterised genes and supporting predicted protein interactions and functions (3).

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The first session of day two was based on DNA replication control. One interesting talk was presented by Alain Nicolas (Institut Curie, Paris) who discussed the role of budding yeast Rad27p (a 5' flap endonuclease) in minisatellite instability. Minisatellites are tandem repeat arrays with 10-100 base pair repeat tracts that are generally stable during the mitotic cell cycle, however rearrangements of minisatellite sequences during mitosis have been associated with a number of human diseases including type 1 diabetes. Nicolas explained that a rad27Δ mutant displayed increased instability of a human minisatellite sequence (CEB1) in mitosis. It is known that Rad27p contributes to Okazaki fragment maturation. With the aid of RNase H1, Rad27p removes the RNA primer used to initiate lagging strand DNA synthesis. With Rad27p function in Okazaki fragment maturation in mind he proposed a number of possible mechanisms of how minisatellite instability occurs (4).

Session four was based on nuclear architecture and telomeres. Dan Gottschling (FHCRC, Seattle) gave a very interesting presentation; he discussed the link between loss of genome heterozygosity (LOH) with age and how it is related to the development of cancer. Gottschling stated that as an organism ages its nuclear quality control mechanisms become compromised and results in LOH. Currently, much is known about the mechanics of nuclear DNA and RNA quality control but little is known about quality control of nuclear proteins, however recent work has shown that budding yeast protein San1p is involved nuclear protein quality control. When the function of San1p is compromised, aberrant nuclear target proteins that function less efficiently or not at all remain in the cell. The lack of aberrant nuclear protein turnover has been linked with the LOH phenomenon (5).

The mitotic and meiotic spindle was the next sessions focus, and Dean Dawson (Tufts University, Boston) gave a presentation that stood out to me. The topic was the segregation of chromosomes that do not undergo homologous recombination during meiosis. It is known that although errors in meiosis are increased when homologous chromosomes fail to form crossovers they do not segregate randomly. As a model for non-exchange mechanics, Dawson et al. use one copy of the yeast species *Saccharomyces carlsbergenesis* chromosome V in place of one of the copies of native budding yeast chromosome V. Presence of *S.carlsbergenesis* chromosome V allows budding yeast to survive; however it is homeologous to budding yeast chromosome V and prevents recombination. Using a system that is designed to label different regions of chromosome V with green fluorescent protein (GFP), pairing of the homeologous chromosomes during meiosis I was assessed. In meiosis I it was observed that the homeologous chromosomes pair together near the centromere, but not at the arms. Their findings suggest that there is a centromere pairing mechanism that ensures that non-exchange chromosomes attach to the meiosis I spindle in a bipolar orientation (6).

Session six was based on DNA replication mechanisms, and Stephen Bell (MIT, Cambridge) described the Origin Recognition Complex (ORC) of budding yeast and it's role in recruitment of additional replication factors. The ORC comprises of six subunits and is essential for the selection of origins of DNA replication and the recruitment of additional replication factors that together form the pre-Replicative Complex (pre-RC). From Bell's presentation it was apparent that ATP (adenosine triphosphate) hydrolysis by ORC is required for assembly of part of the pre-RC to the origin of replication, namely the minichromosome maintenance complex (Mcm2-7p) (7).

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Nancy Kleckner spoke during session seven dedicated to chromosome structure and segregation (Harvard University, Cambridge). During meiosis I double strand breaks allow homologous chromosomes to recombine, resulting in either crossover or non-crossover events. It is known that crossovers occur less frequently than non-crossover events and are the sites of chiasmata formation. Chiasmata physically connect the two homologous chromosomes and ensure their correct disjunction. Kleckner described the distribution of chiasmata to be non-random and when two or more sites are present along a chromosome they exhibit a phenomenon known as 'interference', meaning that if a crossover occurs at one site, there is a reduced probability of another event to occur nearby. In her presentation she describes a mechanical explanation for interference based on the redistribution of stress placed upon the DNA/chromatin fibre proceeding a crossover event and the formation of the chiasmata (8).

Checkpoints and Genome Stability was the title of session eight and Rodney Rothstein (Columbia University, New York) gave an exciting talk on the choreography of the DNA damage response. Rothstein and colleagues constructed a series of fluorescent fusion proteins of known DNA repair proteins of budding yeast. With the fluorescent DNA repair proteins he was able to analyse the cellular response to DNA double-strand breaks (DSBs) and thus establish the timing of recruitment to a site of DNA damage for the DNA repair proteins. When budding yeast was subjected to multiple DSBs the DNA damage checkpoint and repair proteins redistribute from being diffuse in the nucleus to form distinct subnuclear foci. Rothstein called these subnuclear foci centres of recombinational repair that are able to repair multiple DSBs (9).

The final session of the conference was based on the control of chromosome segregation initiation. In this session Vincent Guacci discussed the role of budding yeast Smt4p and sumolyated Pds5p in controlling the dissolution of chromosome cohesion. Sumolyated Pds5p is an essential budding yeast protein known to be required for sister chromatid cohesion, condensation and chromosome segregation, its localisation to the chromosome has been shown to require the cohesin complex. Guacci showed that Smt4p alters the sumolyation state of the Pds5p to regulate its association to cohesin and in-turn the dissolution of chromosome cohesion (10).

Refenences:

1. Nasmyth, K., (2002). Segregating Sister Genomes: The Molecular Biology of Chromosome Separation. *Science*, Vol 297, Issue 5581, 559-565.
2. Gunjan A. and Verreault A., (2003). A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in *S. cerevisiae*. *Cell* 115(5):537-49.
3. Huh W.K., Falvo J.V., Gerke L.C., Carroll A.S., Howson R.W., Weissman J.S., O'Shea E.K., (2003). Global analysis of protein localization in budding yeast. *Nature*. 425(6959):686-91.
4. Lopes J., Debrauwere H., Buard J., Nicolas A., (2002). Instability of the human minisatellite CEB1 in rad27Delta and dna2-1 replication-deficient yeast cells. *EMBO J* 21(12):3201-11.
5. McMurray M.A., Gottschling D.E., (2003). An age-induced switch to a hyper-recombinational state. *Science* 301(5641):1908-11.

6. Kemp B., Maxfield Boumil R., Stewart M.N., and Dawson D.S., (2004). A role for centromere pairing in meiotic chromosome segregation. *Genes and Dev*. 18:1946-1951.
7. Kleckner N., Zickler D., Jones G.H., Dekker J., Padmore R., Henle J., Hutchinson J., (2004) A mechanical basis for chromosome function. *Proc Natl Acad Sci U S A*. 101(34):12592-7.
8. Klemm R.D., Bell S.P., (2001). ATP bound to the origin recognition complex is important for preRC formation. *Proc Natl Acad Sci U S A*. 98(15):8361-7.
9. Lisby M., Barlow J.H., Burgess R.C., Rothstein R., (2004). Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell*. 118(6):699-713.
10. Stead K., Aguilar C., Hartman T., Drexel M., Meluh P., Guacci V., (2003). Pds5p regulates the maintenance of sister chromatid cohesion and is sumoylated to promote the dissolution of cohesion. *J Cell Biol.* 163(4):729-41.