Genome Biology

MEETING REPORT

Bright days for yeast research

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Abstract

A report on the British Yeast Group Meeting, Brighton, UK, 23-25 March 2011.

The annual British Yeast Group Meetings form a platform for high quality scientific interactions and have significant impact on the research directions followed. Here, we summarize talks and poster presentations on DNA replication dynamics and evolution, high-throughput screens and phenomics, chromatin biology and DNA damage presented at the meeting.

DNA replication dynamics and evolution

Chromosomes must be correctly replicated before cell division, as aberrant replication can cause genome instability. Therefore, origins of replication must be tightly regulated and appropriately distributed in the genome. Michelle Hawkins (University of Nottingham, UK) discussed the genome-wide measurement of DNA copy numbers from synchronized budding yeast cell populations as they progress through S phase. Using next-generation sequencing, replication profiles were generated. The data obtained were then combined with a mathematical model of DNA replication, which enabled predictions of individual origin properties that largely agree with independent experimental data. The model predicts cell-to-cell variations, the distribution of distances between active origins and the number of replication forks. These studies resulted in a data-rich platform that will be used to understand the mechanisms involved in faithful and precise DNA replication.

Origins of replication fall into three categories - early, late and dormant - because for unknown reasons they do not initiate simultaneously at the beginning of the S phase. Two kinase activities are required for replication initiation throughout S phase: cyclin-dependent kinase (CDK) and Dbf4-dependent kinase (DDK). Philip Zegerman (Gurdon Institute, University of Cambridge, UK) showed that substrates of CDK (Sld2, Sld3 and their binding partner Dpb11) and the DDK subunit Dbf4 are limiting factors for replication initiation in budding yeast. Overexpression of these factors led to early initiation of late origins. When such overexpression was combined with deletion of the histone deacetylase Rpd3 it resulted in firing of dormant origins. When origins of replication fire simultaneously deoxyribonucleotides are depleted. These results demonstrate that competition for limiting factors underlies replication initiation kinetics and S phase length and that a temporal program of replication origin firing is essential.

Stephen Aves (University of Exeter, UK) presented a comprehensive study on the conservation of DNA replication proteins across eukaryotes. The aims of the study were to map the evolution of DNA replication proteins, provide a model for the composition of the replication machinery of the last common eukaryotic ancestor (LCEA) and describe the evolution and diversity of such complexes. Sixty-nine replication proteins from 35 eukaryotes from five different groups (Amoebozoa, opisthokonts (Fungi and Metazoa), excavates, Plantae and chromalveolates) were mapped. Aves showed that replication proteins are widely conserved among sampled eukaryotes, although in some lineages some components differ, and he revealed a model for the LCEA. DNA replication in the LCEA is more complex than in Archaea according to the model, suggesting that gene duplication or multiplication followed by divergence has driven the evolution of eukaryotic DNA replication.

High-throughput approaches and phenomics

Warwick Dunn (University of Manchester, UK) presented methodologies and technologies used in the Manchester Centre for Integrative Systems Biology (http://www.mcisb.org). The Centre does a wide range of experimentation and modeling, including the elucidation of quantitative genome-scale metabolic networks in *Saccharomyces cerevisiae*. An example of how pathway-scale metabolic models are constructed through a combination of experimentally acquired data and mathematical modeling was presented. Other applications discussed included the great efforts of the yeast systems biology community to reconstruct and improve metabolic

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networks. Quantitative studies of carbon flux in continuous cultures of yeast were presented. This included obtaining measurements for the production of biomass, carbon dioxide, ethanol, glycerol, acetate and trehalose and consumption rates of glucose. The experimental data are combined with flux balance analysis modeling to define the areas of metabolism associated with greatest carbon flux and therefore of greatest biological relevance.

Microtubules have pivotal roles in many cellular processes, including mitosis, cytokinesis and vesicular transport. Xenia Studera (Gurdon Institute) presented a genome-wide approach to identifying new factors in cell morphogenesis and specifically new microtubule regulators in fission yeast. Only 25 such interactors are currently known, whereas a few hundred are predicted to exist through Gene Ontology databases. A gene knockout collection is being used, together with a novel high-throughput microscopy screen. Live image acquisition is followed by image processing, feature extraction and analysis of each individual deletion mutant. Using this method, the identification of novel and conserved microtubule regulators of general eukaryotic importance will be obtained.

Changes in the composition of protein complexes, the operational units within cells, may be a driving force of evolutionary innovation and improved organismal fitness. Yeast natural hybrids are useful models for studying protein complexes. The lager yeast *Saccharomyces pastorianus* is a natural hybrid between *S. cerevisiae* and *Saccharomyces bayanus*. Previous data suggest extensive gene loss that varies between strains of *S. pastorianus*. Sarah Hewitt and Daniela Delneri (University of Manchester, UK) presented data from genome sequencing of three different *S. pastorianus* strains that enable the construction of a database of genes retained from one or both parents in each strain. This database will be used to determine whether protein complexes derive from the same or both of the parental species. Future studies will include the targeted formation of distinct chimeric protein complexes in laboratory hybrids of *S. pastorianus*. The significance of chimeric protein complex retention will be examined through fitness measurements of such strains.

Deciphering chromatin biology

Histone variant H2A.Z has important roles in the regulation of gene expression, formation of heterochromatin boundaries and the DNA damage response. In many organisms, though not in budding yeast, H2A.Z is an essential gene and it has been associated with cancer in humans. Catherine Millar (University of Manchester) is interested in the role of post-translational modifications of H2A.Z and the poorly understood mechanistic details of H2A.Z function. Millar has constructed a library of randomly mutated budding yeast H2A.Z alleles and used it to identify novel functional residues in the protein. She found a number of point mutations that are essential for viability in the absence of the Asf1 histone chaperone. Notably, these mutated sites mostly corresponded to serine and lysine residues in the histone tails, suggesting that post-translational modifications are involved.

Next-generation sequencing technologies have revolutionized many aspects of biology and new applications keep emerging. Nicholas Kent (Cardiff University, UK) and colleagues have developed a new method for analyzing chromatin features, such as nucleosome positioning and transcription factor binding sites. Unlike most current protocols, their approach relies on paired-end sequencing of DNA derived from a mixture of mono- and oligonucleosomes prepared by partial micrococcal nuclease digestion. The sequencing data can be used to extract information on the size of various nuclease-protected species in the samples. This can allow the identification of putative unstable nucleosomes at transcription start sites of highly expressed genes and shows changes in transcription factor occupancy modulated by SWI/SNF complex activities. By comparing profiles from over 100 mutants of chromatin remodelers and other DNA-binding proteins, the Kent laboratory now aims to define a dictionary of 'chromatin landscapes' in budding yeast.

Tackling DNA damage

Heat stress triggers multiple cellular responses, an example being the activation of the pathway involving the p38-related mitogen activated protein kinase Sty1 in fission yeast. Curiously, human cells turn off DNA repair following heat shock and heat is used as radiosensitizer in cancer therapy. Rad9 is part of the 9-1-1 protein complex that is loaded to sites of damaged DNA, and it activates the $Rad3^{ATR}$ checkpoint kinase, which in turn activates the downstream kinase Chk1 that can block cell cycle progression. Thomas Caspari (University of Wales, Bangor, UK) referred to a shorter Rad9 isoform that his group identified in cycling fission yeast cells. The truncated protein, resulting from alternative initiation codon usage, is upregulated following heat shock in a Sty1-independent manner. The short Rad9 variant can delay mitotic progression in response to heat stress by targeting the Chk1 kinase subpopulation at the spindle pole body (fission yeast centrosome). Chk1 subsequently triggers the Mad2-dependent spindle checkpoint to block exit from mitosis.

DNA double-strand breaks (DSBs) pose a serious threat to genome integrity that can potentially result in tumorigenic chromosomal rearrangements if not repaired properly. To identify genes required for DSB repair, Timothy Humphrey (University of Oxford, UK) used the

fission yeast genome deletion library in a screen for mutants sensitive to the radiomimetic bleomycin or the mutagen methylmethane sulfonate. Besides known homologous recombination and DNA checkpoint genes, Humphrey's screen hit additional genes involved in ubiquitylation, transport and metabolism, the majority of which promote efficient repair by homologous recombination. Similar to previously identified HR genes, the novel genes are also required for the suppression of break-induced chromosomal rearrangements and loss of heterozygosity, which can arise by extensive end processing at the site of DSBs. Importantly, the novel genes include homologs of tumor suppressors from animals, suggesting functional conservation of this mechanism of generating and suppressing chromosomal rearrangements.

Overall, the meeting highlighted advances on the main avenues of yeast research. The next British Yeast Group Meeting will be held in Edinburgh in 2012.

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