

Meeting report

Yeast functional genomics and cell biology: no longer in the dark

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A report on the Cold Spring Harbor Laboratory meeting 'Yeast Cell Biology', Cold Spring Harbor, USA, 12-17 August 2003.

Despite a blackout affecting most of the North American eastern seaboard, a bright light shone on genomic research at the Cold Spring Harbor Laboratory's tenth biannual meeting on yeast cell biology. Researchers studying the budding yeast, *Saccharomyces cerevisiae*, have been in the luxurious position of living in a 'post-genomic' era for more than six years, since the complete genome sequence was published in 1996. Functional genomics, proteomics and bioinformatics have become routine parts of the yeast cell biologist's repertoire, and genome-wide analysis featured in many presentations. The rapid incorporation of new technologies into yeast laboratories reflects the open and interactive philosophy of the yeast community, the utility of yeast as a powerful testing ground for new genomic and proteomic concepts, and advances in integrative cell biology. We focus here on a few main themes of the meeting, with an emphasis on studies that have been particularly influenced by genome-wide approaches.

Refining the annotated genome

The *Saccharomyces* Genome Database (SGD [<http://www.yeastgenome.org>]), a publicly funded database encompassing the molecular biology and genetics of yeast, is an enormously valuable online resource. Its curators constantly reannotate the genome as new information becomes available. Recently, the draft genome sequences of several yeast species closely related to *S. cerevisiae* have been published, together with comparative genome analyses. Anand Sethuraman (Stanford University Medical School, USA) from the SGD reported that these analyses have enabled SGD to make

changes to the start sites of 77 open reading frames (ORFs) and to reclassify a large number of ORFs as 'dubious'. The reannotation has provoked a revision of the current number of probable protein-coding genes in *S. cerevisiae* from 6,569 hypothetical ORFs to 5,749 probable ORFs. This fine-tuning of the *S. cerevisiae* genome should aid in the preliminary annotation of the genomes of other organisms.

While some putative genes are being eliminated from the lexicon, others are being added. Although many genes encode proteins shorter than the cut-off of 100 amino acids originally defined for genome annotation in *S. cerevisiae*, they have been difficult to identify within a massive background of potential false positives if short genes are allowed when using gene-finding algorithms. Combined information from several approaches, including RNA analysis by serial analysis of gene expression (SAGE), detection of protein products by lacZ-transposon tagging, and examination of evolutionary conservation, suggests the presence of at least 300 small non-annotated ORFs. James Kastenmayer from the laboratory of Munira Basrai (Center for Cancer Research, NIH, Bethesda, USA) and a consortium of colleagues have initiated a project to generate knockouts of these putative small ORFs. Of course, definitive annotation awaits continued analysis and experimentation.

Yeast screens at the forefront of genomics research

Testing for synthetic lethal interactions, in which the combination of two mutations in the same cell causes death, has been used extensively in classical yeast genetics to identify genes involved in the same biological process or pathway. Amy Tong from the laboratory of Charlie Boone (University of Toronto, Canada) reported on the large-scale mapping of genetic interactions by synthetic genetic array (SGA) analysis. Their approach involves crossing a strain that has a

mutation in a query gene with an arrayed set of viable gene-deletion mutants and scoring the resulting double-mutant progeny for fitness defects. From 130 query strains, Tong and colleagues have mapped around 3,800 synthetic lethal interactions, indicating that each gene has an average of about 30 synthetic interactions with other genes. They predict that the entire network of synthetic lethal interactions in yeast will contain in the order of 80,000 interactions.

The scale of this screen has allowed Tong and colleagues to uncover key principles of genetic networks. For example, their genetic network displays dense local neighborhoods, such that genes interacting with a given query gene tend also to interact with one another. Tong suggested that because synthetic interactions may occur frequently among different alleles of genes in outbred populations, many common human diseases are likely to be caused by interactions between mutant alleles of two or more genes. The widespread use of SGA by yeast researchers is also proving useful in identifying functions for poorly understood or previously uncharacterized genes.

So far, the SGA project has taken advantage of an incredible genetic resource, the array of haploid strains that carry deletions of all the nonessential yeast genes. A different approach is required to allow manipulation of the 1,000 or so essential genes in *S. cerevisiae* in similar genomic projects. Armaity Davierwala and colleagues from Tim Hughes's laboratory (University of Toronto, Canada) have generated integrated tetracycline-regulatable promoter-replacement alleles for more than 60% of all essential genes, providing a conditional allele of each. These strains are being analyzed under a variety of different growth conditions, and are being assayed for morphology, cell size and gene-expression defects (Figure 1). This set of strains will be a useful resource for identifying functions of uncharacterized essential genes.

Two new sets of reagents for looking at both essential and nonessential genes were introduced to the genomic community at the meeting. Bhupinder Bhullar from Joshua LaBaer's group (Harvard Medical School, Boston, USA) reported the construction of a set of arrayed yeast strains bearing plasmids with ORFs under the control of the strong inducible *GAL* promoter. Richelle Sopko from our laboratory (University of Toronto, Canada) has created a similar array of yeast strains containing plasmids under the control of the *GAL* promoter expressing glutathione-*S*-transferase (GST)-tagged ORFs (the plasmids were constructed in Michael Snyder's laboratory, Yale University, New Haven, USA). Both groups are carrying out similar assays as a first pass: screening for genes that are toxic when overexpressed.

The Harvard group is also screening for sensitivity to DNA-damaging agents, to identify genes whose overexpression inhibits normal DNA-damage checkpoint function. Sopko and colleagues are using a variation of SGA to delve further

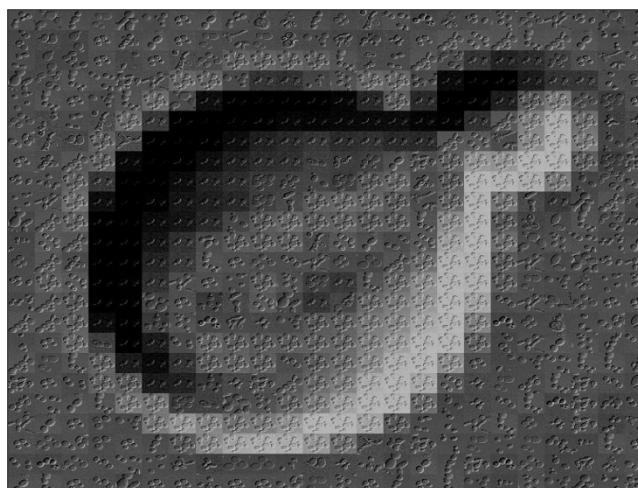


Figure 1

A photo-mosaic showing Nomarski images of 28 strains of *S. cerevisiae* containing different tetracycline-regulatable promoter-replacement alleles. Expression of essential genes is repressed by treatment with the tetracycline analog, doxycycline. Armaity Davierwala and colleagues have constructed such alleles for more than 60% of all essential genes and have identified a number of strains with morphological defects - visible as deviations from the typical budding shapes of wild-type cells shown in the dark-shaded region of the montage. Image courtesy of Tim Hughes and Jennifer Haynes, University of Toronto, Canada.

into dosage-dependent genetic relationships, screening for suppressors and synthetic-lethal interactions that are dependent on the dosage of the overexpressed gene, using query genes containing point mutations. The combination of overexpression with genetic or chemical sensitivity assays should be useful in identifying negative regulators and dominant-negative effectors. The essential gene and overexpression arrays will nicely complement the deletion set and augment the arsenal of reagents available for high-throughput yeast genetics.

Combining genomic approaches

Combining different genomic techniques can extend the findings from a single approach. Ainslie Parsons from Charlie Boone's lab has screened the set of gene-deletion mutants for sensitivity or resistance to a variety of chemicals and drugs to generate a 'synthetic chemical' profile of gene-deletion mutants that are sensitive to each compound. Her poster compared the synthetic chemical profiles of a variety of compounds with a compendium of synthetic genetic profiles. Proof-of-principle experiments with well-characterized drugs and their targets suggest that this approach will be useful for identifying pathways and targets affected by drug treatment.

Paul Cullen from George Sprague's group (University of Oregon, Eugene, USA) has used a combined approach to study a developmental fate change: the transition from the rounded yeast form to the elongated filamentous form. He

first screened the gene-deletion set for mutants that displayed enhanced or reduced filamentous growth, and classified mutants by the use of markers specific to filamentous growth. He then compared the data with expression profiles from strains undergoing filamentous growth. In this way he was able to identify key regulatory points controlling the decision to undergo filamentous growth.

SGA analysis is impaired by the use of query genes that confer a growth defect, resulting in identification of non-specific synthetic lethality. To overcome this difficulty when using a deletion of the gene encoding the kinase *Sch9p*, David Bellows from the laboratory of Mike Tyers (University of Toronto) and colleagues have used a query strain carrying an analog-sensitive version of *Sch9p*, which contains an amino-acid substitution rendering the kinase sensitive to a specific nucleoside-analog inhibitor. By adding the nucleoside inhibitor only at the last selection of the SGA screen, a number of interactions with *SCH9* were observed that were otherwise obscured using traditional methods.

Identifying components of large multiprotein complexes

A variety of large-scale projects, including functional genomics, genome-wide two-hybrid screens, mass spectrometry-based proteomics, and large-scale protein localization projects, have led to the compilation of an extensive list of parts for several key cytoskeletal and other cellular structures in yeast. For example, concentrated efforts from a number of groups are providing a comprehensive view of the spindle-pole body, the kinetochore, the nuclear pore, and organelles such as the nucleolus.

Several speakers described genomic approaches to elucidating the molecular architecture of the kinetochore - the protein complex assembled on centromeric DNA that attaches chromosomes to the mitotic or meiotic spindle. Kristin Baetz from Phil Hieter's laboratory (University of British Columbia, Vancouver, Canada) reported the use of the nonessential gene deletion set to identify new genes or pathways affecting chromosome transmission. Baetz and colleagues screened for deletion mutants that were sensitive to either mutation or overexpression of various genes encoding kinetochore components. They have also screened the deletion set for mutants with defects specifically in the fidelity of chromosome transmission, using an elegant chromosome-loss assay. Stefan Westermann of Georjana Barnes's laboratory (University of California, Berkeley, USA) described his use of affinity chromatography with kinetochore proteins, and the identification of interacting proteins by mass spectroscopy to generate a map of kinetochore protein interactions. Andrew McAinsh from Peter Sorger's group (Massachusetts Institute of Technology, Cambridge, USA) described a proteomic approach involving immunoprecipitation of tagged proteins and identification of

co-immunoprecipitated proteins, to identify interactions between kinetochore components and reported the identification of six novel kinetochore proteins. Biochemical evidence from the Sorger lab suggests that kinetochores are made up of many small independent scaffolds that recruit different microtubule-binding proteins. Information generated from these various post-genomic approaches is allowing researchers to embark on a comprehensive exploration of the mechanism of kinetochore function and regulation.

A report of this meeting would be incomplete without acknowledging the loss to our community earlier this year of Ira Herskowitz, a leading figure in yeast genetics. Appropriately, the opening session was a celebration of his science, with outstanding retrospective talks by David Botstein (Princeton University, USA) and Jeff Strathern (National Cancer Institute, Bethesda, USA). They both highlighted aspects of Ira's scientific philosophy and explained the broad influence of his science on fields as diverse as chromatin remodeling and gene expression, lineage determination and developmental biology. Among Ira's prescriptions for good research were to choose a well-established paradox; to look at your cells; to never let one fact stand in the way of a good model; and to believe good genetics. Layered on top of these guiding philosophies was an extraordinary ability to reduce a huge amount of literature to testable models. These models were typically illustrated with so-called 'Ira-grams', often composed of simple arrows (designating activation) or T-bars (illustrating repression) as well as showing an enthusiasm that is impossible to capture in more complex drawings. Ira's approach to yeast cell biology perhaps explains the continued prominence of yeast as a model system: he, and other leaders in the community, encouraged the cross-fertilization of ideas, insights and methods that will continue to lead us to a more integrated view of eukaryotic cell structure and function.