Systems cell biology of the mitotic spindle

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Cell division depends critically on the temporally controlled assembly of mitotic spindles, which are responsible for the distribution of duplicated chromosomes to each of the two daughter cells. To gain insight into the process, Vizeacoumar et al., in this issue (Vizeacoumar et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200909013), have combined systems genetics with high-throughput and high-content imaging to comprehensively identify and classify novel components that contribute to the morphology and function of the mitotic spindle.

When, in the mid-1930s, Professor Øjvind Winge at the Carlsberg Laboratory in Denmark discovered the sexual practices of brewer's yeast (Winge, 1935), he set in motion an era of scientists exploiting *Saccharomyces cerevisiae* as an experimental model system for biological research. For generations, geneticists and cell biologists alike have used the "awesome power" of yeast genetics to reveal the fundamental mechanisms of eukaryotic cell behavior. Over time, the traditional genetics approaches have been augmented by reverse genetic screens—searches for gene functions starting from the mutated gene, not the phenotype—and undertakings such as the yeast deletion project, in which each gene is systematically perturbed, has left the biological community with thousands of genes with unannotated functions. One approach for discovering these gene functions has been to combine mutant alleles of interest with arrayed gene yeast deletion libraries, and to ask whether the combination of alleles leads to a dramatic change in the ability of the cells to grow. These synthetic lethal or synthetic genetic arrays have contributed a great deal to our understanding of genetic interactions and how they relate to physical network structure (Meluh et al., 2008; Dixon et al., 2009). However, scoring yeast colony size as a phenotype is somewhat removed from the mechanisms that lead to the observed changes in growth characteristics of the organism. As the field of cell biology well appreciates, intermediate phenotypes that reflect more directly the nature of subcellular structures, complexes, and dynamics can yield insights into the molecular programs and networks that underlie such gross phenotypic changes. Thus, in this issue, Vizeacoumar et al. demonstrate the power of a systems cell biology approach, wherein they integrate high-throughput imaging and functional genomics with computation-based data analyses and modeling to identify and place into context >120 genes newly implicated in mitotic spindle function.

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In eukaryotic cells, duplicated chromosomes must be symmetrically partitioned to opposite ends of the cell by the activities of the mitotic spindle. During mitosis, spindles are assembled, chromosomes are partitioned, and the spindles are then disassembled. The fidelity of this process is critical to ensure equal chromosome segregation during division and maintenance of proper chromosome number. In higher eukaryotes, structures called centrosomes serve as central organizers of the mitotic spindle. In yeast, spindle pole bodies are structurally distinct from centrosomes, but perform an analogous function. At the start of the cell cycle, cells have a single spindle pole body embedded in the nuclear envelope. The spindle pole body is duplicated early in the cell cycle, and microtubules associate with and radiate from the structure (Byers and Goetsch, 1975). As the cell cycle progresses, the microtubules associate with the cortices of the mother and the budding daughter cell, pulling one of the spindle pole bodies into the bud and retaining one in the mother. The spindles thus become oriented parallel to the main cell axis to segregate chromosomes to the poles of the dumbbell-shaped dividing cell (Carminati and Stearns, 1997). After chromosome segregation, the spindle breaks down as the cell cycle reaches completion.

The approach to characterize the networks governing spindle morphology taken by Vizeacoumar et al. (2010) was to take advantage of the yeast deletion library and the synthetic genetic array (SGA) methodology pioneered by the Boone and Andrews laboratories (Tong et al., 2001). In the newest adaptation, they systematically introduced a GFP-tagged version of tubulin (GFP-TUB1) into each of the ∼4,700 strains of the haploid deletion set (Fig. 1). This provided a library of single deletions in which they could monitor spindle morphology. In a second iteration, they used the genetic gymnastics of the SGA method to generate two haploid libraries of GFP-spindle–labeled double mutants, each containing a "query" allele compromised in spindle function in combination with the other mutants of the deletion set. The query alleles chosen for the double mutant combinations were *bni1Δ* and *bim1Δ*. Each mutant alone has a subtle defect in spindle function, and each respective protein has a well-characterized, yet distinct role. Bni1p is a formin protein that functions in polarized actin assembly, which is required for polarized cell growth and spindle orientation. Bim1p is a plus-end microtubule-binding protein that together with Kar9p

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 188 No. 1 7–9
www.jcb.org/cgi/doi/10.1083/jcb.200912028

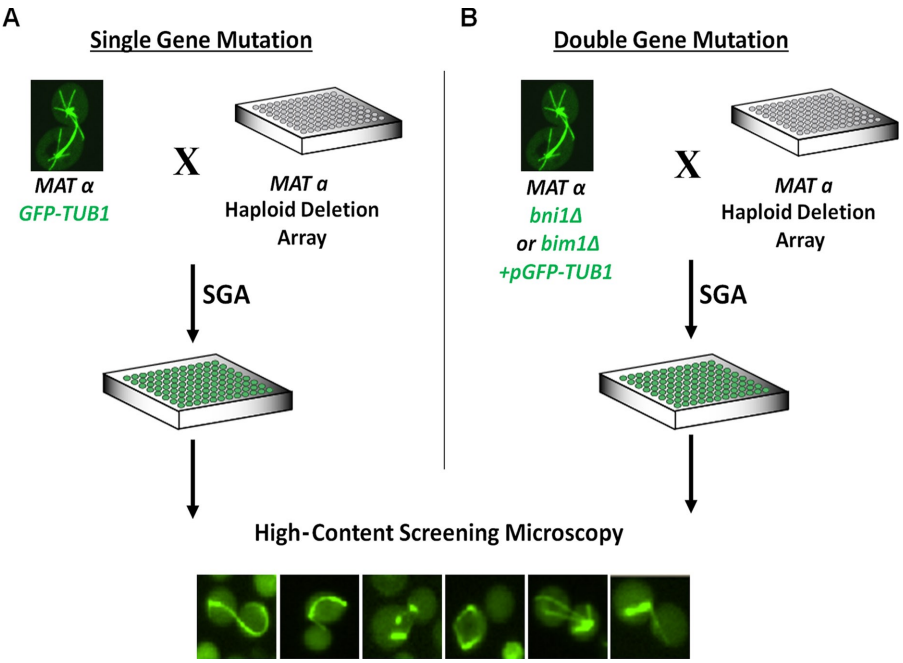

Figure 1. **Systems cell biology: SGA and high-content imaging studies of the mitotic spindle.** Spindles are critical for cell division, but are relatively challenging to study because they show dynamic morphology at different stages of the cell cycle. Two approaches were taken to reveal functional genetic interactions regulating spindle biology. (A) A query strain expressing a GFP-tagged version of *TUB1*, which allows for visualization of the mitotic spindle, was mated to each strain in the haploid deletion library, generating an array of single deletion mutants in which the mitotic spindle can be visualized. High-content screening was then used to monitor the morphological variations of the growing mitotic spindle. (bottom) Microscopic images of the different types of spindle morphologies that were detected are shown. (B) A combinatorial genetic approach was taken to study spindle morphology. In addition to the *GFP -TUB1* chimera, the query strain also carried one of two mutations, *bni1Δ* or *bim1Δ*. These mutations sensitize the yeast to spindle pole morphological defects. After mating across the yeast deletion library, the resulting arrays were studied by high-content screening microscopy.

links the actin cytoskeleton to microtubules at the cortical capture site (Pruyne et al., 2004).

Armed with close to 15,000 single and double mutant strains, Vizeacoumar et al. (2010) then had the challenge of imaging and analyzing the dynamics of the fluorescently labeled spindles. The key to this analysis was to generate sufficient high-quality images such that quantitative measurements could be made on multiple aspects of cell and spindle morphology. Once these morphological attributes are converted to numbers, they can be analyzed by machine learning or other computational approaches to reveal spatiotemporal defects associated with each mutant allele combination. Such multiparameter analyses are typical of systems biology approaches, and can reveal multiple, often subtle, phenotypes and identify classes of proteins that are likely to work together in specific aspects of cellular function. Indeed, high-content screens have been applied in other systems using single mutants or RNAi perturbations (Krausz, 2007), but what makes the Vizeacoumar et al. (2010) approach so valuable is the use of a sensitized background; the number of genes identified in this study was easily doubled over single mutant screens.

As with many such screens, the list of newly identified genes include those for which links to spindle regulation are not immediately clear, and those that reveal tantalizing new connections. The former group includes genes involved in metabolism and ribosome biogenesis, and although perhaps difficult to understand mechanistically, they underscore the complexity of the fully integrated cellular system. Perhaps one of the most exciting examples from the latter category is the identification of three classes of mutants characterized by the appearance of hyperextended fish hook spindles. These included kinetochore components of the CTF19 complex, components of the mitotic exit network (MEN) and fourteen early anaphase release (FEAR) network, and a SUMO ligase component (Mms21). Further analyses of these mutants revealed that sumoylation of Mcm21 (a component of the CTF19 complex) is required to localize components (Ipl1p and Sli15p) of the chromosomal passenger complex (CPC) to the spindle midzone. *MCM21* also shows genetic interactions with members of the FEAR complex, which is required for the release of Cdc14p from the nucleolus (D'Amours and Amon, 2004). Cdc14p, in turn, is required for dephosphorylation and correct localization of Sli15p to the spindle midzone (Pereira and Schiebel, 2003). These data, and the phenotypes of deletion mutants of the MEN, led the authors to propose a model in which the CTF19 complex acts as a scaffold at the kinetochore Vizeacoumar et al. (2010). Furthermore, sumoylation of Mcm21p and Cdc14p-dependent dephosphorylation are proposed to cooperate to signal the movement of CPC to the midzone.

The era of systems cell biology is upon us. High-content screening has traditionally been limited to the domain of large-budget drug discovery companies; however, the development of next-generation high-throughput microscopy has made this technology available to the scientific community as a whole. Moreover, "off the shelf" and open-source software further enable relatively sophisticated feature analyses (Carpenter et al., 2006) and the conversion of morphological phenotypes into quantitative, continuous data. Such data are readily amenable to computational analyses, and when combined with systems and chemical genetics, can reveal new interactions, expose network structure (Fiedler et al., 2009) and information flow (Carter et al., 2007), and identify potential drug targets (Parsons et al., 2004).

The authors acknowledge support from the National Institutes of Health (grants GM075152, RR02222, and GM076547) and have no commercial interests or conflicts of interest.

Submitted: 4 December 2009
Accepted: 16 December 2009

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