A Profusion of SNAREs

Three SNARE proteins are known to be in a complex involved in vacuole–vacuole fusion. Ungermann et al. identify two additional SNAREs in this complex, suggesting that SNARE complexes can be larger and more complicated than previously thought (page 1435).

Other researchers have determined the crystal structure of a complex of purified neuronal SNARE proteins implicated in synaptic exocytosis. The structure is built around a core of four parallel coiled-coil domains contributed by three proteins. The established complex of vacuolar SNARE proteins was consistent with this model (although it seemed that each protein only contributed a single coiled-coil), but Ungermann et al. use immunoaffinity purification and mass spectrometry to add two more SNAREs, Vti1p and Ykt6p, bringing the total to five. The authors use immunoprecipitation to confirm that the proteins define a single complex, and antibodies and mutants to show that both of the new proteins are required for fusion.

Senior author William Wickner says the new results do not contradict the structural information. “A dditional SNAREs and other proteins may build on and modify the known structural core that was revealed by x-ray crystallography,” he says.

Chromosome Segregation

Building Spindles with Tension

Spindles are usually constructed using microtubules emanating from two microtubule-organizing centers; these microtubules then capture chromosomes. In mouse oocytes, however, the microtubules polymerize from multiple microtubule organizing centers around the condensing chromosomes and then coalesce into a bipolar spindle. Woods et al. suggest that tension from the early bipolar attachment of chromosomes is essential for the construction of a robust version of such a meiotic spindle (page 1395).

Woods et al. base their conclusion on an unexpected source: the phenotype of mice lacking the mismatch repair protein Mlh1. The bacterial homologue of Mlh1, MutL, is known to be a DNA-stimulated ATPase, but the exact function of these proteins in the repair cycle, or in meiotic recombination (see below), is not known.

Mutation of MLH1 results in a paucity of recombination and a low level of monopolar cells in budding yeast. Biggins et al. find that the ATP effect is mediated by Ipl1p. The missing link is a broader function for the Ipl1p kinase. The authors use an in vitro assay, Sassoon et al. show that kinetochore–microtubule binding is reduced by addition of ATP, but restored by G1c7p (the only type I phosphatase in budding yeast). Biggins et al. find that the ATP effect is mediated by Ipl1p. The missing link is a broader function for this kinase/phosphatase pair. These proteins could be regulating any one of a number of dynamic interactions between the kinetochore and microtubules including the attachment process and the direction or speed of movement along microtubules.

Woods et al. suggest that bivalent chromosome pairs normally tether the spindle poles, whereas the univalent chromosomes provide no counterforce to the expansion forces of the spindle.

The Function of a Transforming Kinase


A s ipl1 mutants pass through mitosis, they suffer massive chromosome missegregation without delaying the cell cycle. Kim et al. note that up to 50% of the sister chromatid pairs separate to some degree, suggesting that sister separation is not the primary defect. Biggins et al. obtain similar results, and go on to show that even more sister separation is seen when sisters are allowed to drift apart in the presence of a microtubule-depolymerizing drug. Furthermore, a sister-cohesion protein leaves the sister chromatids on schedule.

If sister separation is not the problem, what is? Here the two papers diverge. Biggins et al. find that spindles elongate when ipl1 cells are arrested before anaphase onset, suggesting that sister chromatid pairs cannot tether the poles (analogous to the situation in the mlh1 oocytes described above), and that kinetochores may not be functioning correctly. Sure enough, a mutation in NDC10 (a gene that encodes one of the kinetochore components) interacts genetically with ipl1.

Using an in vitro assay, Sassoon et al. show that kinetochore–microtubule binding is reduced by addition of A TP, but restored by G1c7p (the only type I phosphatase in budding yeast). Biggins et al. find that the A TP effect is mediated by Ipl1p. The missing link is a broader function for this kinase/phosphatase pair. These proteins could be regulating any one of a number of dynamic interactions between the kinetochore and microtubules including the attachment process and the direction or speed of movement along microtubules.

Kim et al. do a synthetic lethal screen and find Sli5p, a unique protein that binds directly to Ipl1p. They also note a low level of monopolar cells in ipl1 mutant cultures (which Biggins et al. do not see), and find that Nuf2p is unequally distributed between the two spindle poles. Nuf2p localization could be explained by its association with the aberrantly segregating kinetochores (or the kinetochore microtubules) that cluster at the poles, especially as integral components of the spindle pole body are not per-
Shuttling On and Off the Membrane

A stoul et al. use a fusion to green fluorescent protein to see protein kinase B (PKB) participating in a remarkably rapid cycle of translocation to and from the plasma membrane (page 1511). PKB was known to be recruited to the membrane where it is activated by phosphorylation. But the brevity of its stay there was unexpected.

The authors examine the activation of B cells caused by triggering of the B cell receptor (BCR). One result of BCR activation is the production of phosphatidylinositol-3,4,5-phosphate (PIP$_3$) by PI3 kinase. The pleckstrin homology (PH) domain of PKB binds the PIP$_3$ in the membrane, and PKB is activated by nearby kinases. A stoul et al. find that PKB translocates to the membrane within 10 s of BCR stimulation, but recycles to the cytosol and nucleus within 40–60 s. This is despite the fact that PIP$_3$ levels remain elevated for at least 15 min, and PKB activation is sustained for at least 1 h. A fusion protein with only the PH domain remains localized at the membrane; in full-length PKB the activating phosphorylations may override the increased affinity for PIP$_3$.

Once antigen has stimulated B cells to produce sufficient antibodies, the B cells must be shut down. That task falls to the FcyRIIB receptor. A ntigen-antibody complexes bind to both the BCR and FcyRIIB, with the ligation of FcyRIIB recruiting a phosphatase that degrades PIP$_3$. Mice that lack this negative feedback loop are prone to inflammatory diseases and anaphylaxis. A stoul et al. show that, after coligation of BCR and FcyRIIB, PKB is neither localized to the membrane nor activated. These data identify PKB as a target for FcyRIIB feedback control.

Large-Scale Chromatin Unfolding

Transcriptional activators can induce local changes around promoters by altering nucleosome binding and structure. On page 1341, Tumbar et al. show that a massive recruitment of the transcriptional activator VP16 can also induce large-scale chromatin unfolding.

The chromatin that unfolds is an amplified, heterochromatic region of ~90 mbp, which contains arrays of binding sites for the lac repressor near the dihydrofolate reductase (DHFR) gene. Lac repressor–VP16 hybrid proteins are recruited to the region, and this causes the region to spread out as a fibrillar structure that occupies up to one-half of the cross-section of the nucleus. Transcription is not needed for the maintenance or formation of the extended structure. In some nuclei the region forms a large, hollow, ball-like structure, whose genesis remains unexplained.

VP16 is known to interact with at least one histone acetyltransferase, and histones in the expanded region are hyperacetylated. This may displace heterochromatin proteins and attract chromatin remodeling complexes.

"Our hypothesis is that we are seeing a magnified version of what would be present with normal genes," says senior author Andrew Belmont. "The next step is to see what is happening at a single-gene level." These experiments will involve visualizing chromatin condensation by electron microscopy, to see if single-gene activation involves changes not just in local protein interactions, but also in large-scale structure.