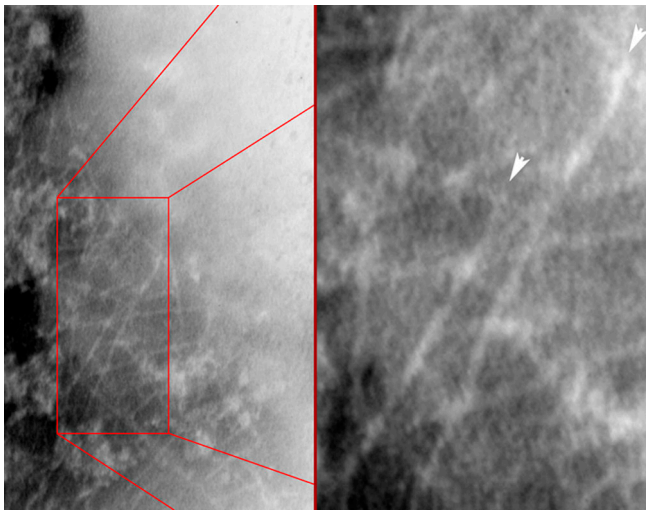
Actin branches out in yeast

Many of the same proteins control actin polymerization in budding yeast and mammalian cells. But budding yeast researchers—hampered by an emphasis on genetics and an inaccessible yeast cytoplasm dense with ribosomes and glycogen granules—have often lagged behind in providing structural descriptions based on electron microscopy (EM). This has fueled suspicions about whether the study of yeast actin is relevant to human systems.

Now, Young et al. (page 629) get the structural ball rolling with an EM characterization of yeast cortical actin patches, which are the most prominent actin structures in budding yeast. They find significant structural parallels between the yeast and mammalian systems, suggesting that the smaller yeast structures may be a handy model for tackling problems of actin dynamics.

The team partially purified patches from cells expressing GFP-labeled capping protein. Once cells were lysed, the researchers stabilized and cross-linked the actin patches and used correlated fluorescence and electron microscopy to visualize them.

Actin filaments were arrayed in a branched fashion, with the branch placement and angles characteristic of Arp2/3 complex–induced branching seen in mammalian cells. The authors conclude that a modified form of the dendritic nucleation model, which is used to explain actin dynamics at the leading edge of motile mammalian cells, does apply to yeast actin dynamics. The relevance of this model to yeast had been questioned because the concentration of actin protein in yeast appeared to be too low to support dendritic nucleation.



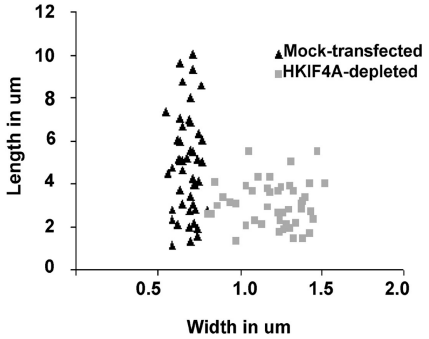
Yeast actin patches show filaments at angles suggestive of Arp2/3 action.

The yeast actin patches are known to move as they drive endocytosis. In the isolated patches, the branching was equal in all directions, but patches isolated directly from the cortex of living cells (both wild type and mutant) may reveal what process provides directionality. ■

Less kinesin, more condensation

Kinesin motors drag cargos, including chromosomes, but do not normally reshape those cargos. But on page 613, Mazumdar et al. demonstrate that a human chromokinesin HKIF4A is needed to establish the correct condensation state of chromosomes.

Chromokinesins are localized along chromosome arms and are thought, at least in some cases, to act as part of the polar wind: they walk along microtubules away from centrosomes, thus dragging their chromosome cargos toward the middle of the mitotic spindle. The authors depleted HKIF4A from human fibroblast cells using antibodies and RNAi. They observed numerous mitotic defects including misaligned chromosomes, incomplete chromosome separation during anaphase, and disorganized spindles. The resulting daughter cells had a high rate of aneuploidy.



Without HKIF4a, chromosomes are shorter and fatter.

When the authors visually examined the chromosomes in RNAi-treated cells, they saw that chromosomes were hypercondensed relative to those in control cells. This was not due to an extended mitosis, as chromosomes of cells just entering mitosis were also hypercondensed. Furthermore, they found that HKIF4A interacts with the condensin complexes responsible for chromosome condensation.

Cells lacking HKIF4A had a diffuse rather than the normal axial localization of some condensin complex proteins. Thus, HKIF4A, having localized to chromosomes so that it can perhaps act as part of a polar wind, may use that localization to recruit or otherwise organize the condensins so that they can do their job. The more provocative possibility is that the kinesin motor itself is used to power a condensation event—a possibility that can be tested by injecting a kinase-dead mutant. The group also hopes to understand whether the segregation errors are triggered by the condensation problems, lack of motor function, or both. ■

Text By Rabiya Tuma
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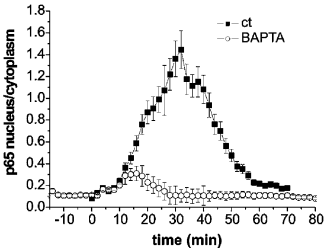
Calcium lights up cell cycle

Calcium biologists have long suggested that there was a fundamental role for the cation in the cell cycle, but the supporting data have been largely circumstantial. Now, Sée et al. (page 661) have direct evidence for a transient Ca^{2+} trigger in the initiation of the cell cycle upon serum stimulation. They also find that the transcription factor NF-κB links the ion flux to a well-known cell cycle control gene, cyclin D1.



Preventing a Ca^{2+} transient blocks NF-κB activation and cell cycle entry

Using Ca^{2+}-sensitive dyes, the authors saw that serum-starved fibroblasts responded to serum stimulation with a rise in intracellular Ca^{2+} that lasted for only 30 s. Serum addition also triggered NF-κB activation, which is required for cell cycle entry and transcription of cyclin D1, a rate-limiting G1 cyclin.

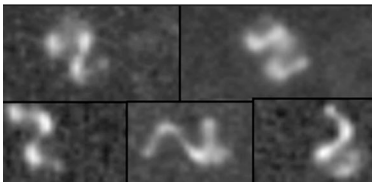
To determine whether the brief Ca^{2+} flux was crucial for NF-κB activation, the investigators introduced a caged Ca^{2+} scavenger into the cells. Photoactivation of the Ca^{2+} scavenger prevented the Ca^{2+} flux that would naturally occur in response to serum stimulation and blocked NF-κB activity. However, the scavenger was only effective when activated immediately after serum stimulation.

The Ca^{2+} flux is necessary but not sufficient to induce NF-κB activity. The researchers find that p42/p44 MAPK activity, which is dependent on both Ca^{2+} and probably other signals, is also required for NF-κB activation and cyclin D1 transcription. ■

Flagellar microtubules do the twist

In many organisms, the central pairs (CPs) of 9+2 cilia and flagella spin. Mitchell and Nakatsugawa (page 709) now claim that this spinning is an effect, not a cause, of flagellar bend propagation.

Motile 9+2 cilia and flagella owe their whip-like movement to motors in the outside barrel of fused doublet microtubules, with motors anchored to one doublet pushing on a neighboring doublet. But the more enigmatic part of this structure is the CP. This doublet of microtubules is connected to the outside barrel via radial spokes that are thought to modulate motor action.

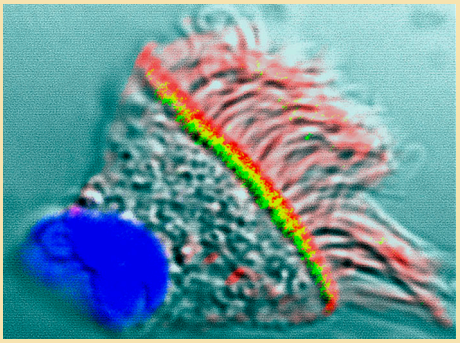


Flagellar central pair complexes are helical when extruded.

That modulation requires a constant relationship between a particular face of the CP and those microtubule motors that are active at any one time—which is where spinning and twisting come in. Looking at electron micrographs of wild-type *Chlamydomonas*, Mitchell and Nakatsugawa see that the CP is twisted in straight, quiescent flagella. In mutants that lack the radial spoke heads, and therefore lack a physical connection between the outer and inner microtubules, the CP remains twisted, suggesting that the twist is inherent to the CP structure.

When this twisted structure is forced to bend, as during the beating of the flagella, the CP curves along with the bend. The C1 tubule (half of the CP) is always on the outside of the curve, as if it is longer and this greater length must be accommodated by either helical twisting or being on the outside of a curve. As the bend propagates down the length of the flagellum, it recreates this helix-to-curve transition in the CP and thus yields the rotation. The researchers speculate that the twist helps orient certain CPs: in flagella that change their bend direction the CP can rotate freely to accommodate the new direction, whereas in fixed-direction flagella such as sperm tails the CP is not twisted and does not rotate. ■

Pericentrin pivotal in primary cilia



Pericentrin (green) is needed to build primary cilia (red).

The centrosome protein pericentrin acts as an anchor for primary cilia formation in human cells, according to a report by Jurczyk et al. (page 637). The finding further unites the centrosome and primary cilia, both of which are based around centrioles, and both of which use pericentrin as an anchor for other proteins.

The authors show that pericentrin is located not only at centrosomes but also around the centrioles at the base of primary and motile cilia. After depletion of most pericentrin using RNAi, primary cilia failed to assemble, but centrosomes could still nucleate microtubules. The divergent outcomes may result from selective depletion of pericentrin's large isoform by RNAi, with remaining smaller isoforms perhaps acting in centrosomes.

Depletion of pericentrin also disrupted the localization of both the intraflagellar transport (IFT) proteins that help build cilia and PC2, a cation channel important in ciliary signaling. Furthermore, depletion of IFT proteins with RNAi caused pericentrin mislocalization and disrupted primary cilia formation.

Endogenous pericentrin copurifies and coimmunoprecipitates with IFT proteins. Additional studies are needed to understand exactly how this complex acts to direct primary cilia formation. The association is certainly reminiscent of pericentrin's function in centrosomes, where it is needed to anchor the γ-tubulin ring complexes that nucleate microtubules. ■