The other side of kinetochore fibers

On page 671, Khodjakov et al. uncover a new mechanism of spindle morphogenesis that involves the capture of microtubule minus ends.

In the established model of spindle formation in vertebrate cells, spindle microtubule minus ends are focused at spindle poles in association with centrosome-nucleated microtubules. Kinetochore fibers, which pull chromosomes to opposite poles, form when the plus ends of centrosome-associated microtubules capture kinetochores. A second mechanism must also form kinetochore fibers, however, because functional spindles form in the absence of centrosomes.

In their new article, the authors got a close look at this second mechanism by focusing on unobstructed kinetochores. They avoided the obtrusive mass of non-kinetochore microtubules that usually blocks imaging by inducing monopolar spindle formation and examining kinetochores that faced away from the centrosome. Fully formed bundles of microtubules occasionally appeared near the kinetochores. These bundles became attached at their plus ends to the kinetochore in the end-on arrangement typical of kinetochore fibers, but their minus ends remained oriented away from the centrosomes. The growing minus ends looped back as if captured by spindle microtubules and were incorporated into the spindle. This strategy is not limited to monopolar spindles, as looping and capture of kinetochore-anchored microtubules was also seen during bipolar spindle formation.

The loop-and-capture behavior depended on NuMA, a minus end–localized protein that associates with dynein. Thus, dynein may be the motor that pulls kinetochore fiber minus ends toward centrosomes along tracks provided by centrosome-induced microtubules. Centrosomes, therefore, may be critical to orient the axis of division by drawing in microtubule minus ends, irrespective of the direction the kinetochores face. This function is consistent with results from centrosome removal experiments, which often result in misorientation of the division plane.

Phosphorylation cycles on vesicles

Clathrin-coated vesicles (CCVs) that traffic between the trans-Golgi network and endosomes are nucleated by the adaptor protein complex-1 (AP-1). AP-1 sorts and packages cargo into CCVs and then falls off the vesicles before they arrive at their destination. On page 699, Ghosh and Kornfeld demonstrate that two of the four AP-1 subunits are opposingly phosphorylated and dephosphorylated. The regulatory cycles control vesicle trafficking by turning on cargo recruitment and clathrin coating at the appropriate time.

Cargo recruitment is mediated by the χ1 subunit of AP-1. The authors demonstrate that χ1 is phosphorylated on the membrane and dephosphorylated in the cytosol. Phosphorylation induced a conformational change in χ1 that increased its affinity for cargo ligands. Addition of a phosphate group also alters the conformation of the χ2 subunit of AP-2, which regulates vesicle formation at the plasma membrane.

Dephosphorylation by protein phosphatase 2A (PP2A) reversed the shape change and allowed χ1 to release the cargo. As a result, AP-1 disembarks from the vesicle and returns to the cytosol. PP2A is recruited to vesicles by Hsc-70, which is also required for uncoating. In combination, PP2A and Hsc-70 released both AP-1 and AP-2 from vesicles in vitro, suggesting that dephosphorylation is a common uncoating mechanism.

In addition to χ1, PP2A had another target in the AP-1 complex. But unlike χ1, this second substrate, the β1 subunit, was dephosphorylated on the Golgi rather than on vesicles. As β1 phosphorylation impairs its ability to interact with clathrin, PP2A activity is necessary for CCV formation. The opposing preference for substrates based on location (Golgi-associated PP2A preferred β1, whereas vesicle-associated PP2A favored χ1) thus restricts the activity of each subunit to the desired location.
**PDE5 says NO to cGMP**

A short burst of nitric oxide (NO) is remembered by a phosphodiesterase long after NO levels decline, according to results on page 719 by Mullershausen et al. The memory of this enzyme, PDE5, may be responsible for the tolerance that patients develop to nitrovasodilators like nitroglycerin. Nitrovasodilators are NO-releasing compounds that are used to treat coronary heart disease. NO decreases cGMP levels, which lowers blood pressure by both relaxing blood vessels and inhibiting platelet aggregation. But platelets rapidly decrease NO-induced cGMP and thus become desensitized to later NO exposure. Circumstantial evidence suggests that the cGMP is degraded upon phosphorylation of the phosphodiesterase PDE5 by a cGMP-dependent kinase. The new article describes a more direct route of cGMP self-limitation.

As expected, NO-induced PDE5 phosphorylation required cGMP increases and the cGMP-dependent kinase cGKI. Yet phosphorylation was not necessary for PDE5 activation by cGMP, as shown using cGKI-deficient mice. Instead, cGMP was sufficient to activate PDE5. Others recently showed that cGMP binds to and activates PDE5. This interaction probably provides the direct mechanism for NO-induced PDE5 activation. Low concentrations of cGMP, however, stimulated phosphorylation of the phosphodiesterase PDE5 by a cGMP-dependent kinase. The new article describes a more direct route of cGMP self-limitation.

A small, transient NO stimulus dampened subsequent cGMP production in response to NO ≤ 1 h later, at which time PDE5 was still active. Inhibition of this active PDE5 may be necessary to achieve lasting nitrovasodilator therapy.

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**A protease inhibitor unsticks cells**

PAI-1 is unique among protease inhibitors because it binds to the matrix protein vitronectin (VN). PAI-1 binding blocks VN’s binding site for the cell surface receptor uPAR and for integrin family members. Czekay et al. (page 781) now show that PAI-1 is also able to detach cells through a less direct approach.

Unlike the direct competition method, PAI-1 also disrupted integrin-mediated adhesion without ever contacting VN. Instead, PAI-1 bound to another uPAR ligand, the protease uPA. Binding of uPA to uPAR causes integrin recruitment into complexes with uPA and uPAR. PAI-1 disrupted adhesion by inactivating these complexes and triggering their endocytosis. Endocytosis required the low density lipoprotein receptor-related protein, but how PAI-1 triggers integrin inactivation has yet to be determined.

Integrins interact with other matrix molecules in addition to VN. So far, the authors have shown that PAI-1 also detaches cells from fibronectin and collagen, again by promoting integrin endocytosis. In each case, cells expressing high levels of uPAR were more susceptible to PAI-1–induced detachment, because more of their surface integrins were complexed with uPAR. This association could explain why a high level of PAI-1 indicates a poor prognosis for many metastatic cancers—unusual for a protease inhibitor, since proteases normally promote cell invasion.

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**Ran sticks a GEF to chromatin**

GTP-bound Ran is produced by the GEF RCC1, which binds only weakly to histones. Using a GFP-tagged version, the authors show that RCC1 is highly mobile and exchanges rapidly between free and chromatin-bound states. As RCC1 has strong GEF activity in the presence or absence of chromatin, the authors were interested in determining how RanGTP production is limited to chromosomes. They found that Ran-bound RCC1 had a stronger chromosome association. Locking RCC1 to chromosomes because the complex of Ran and its guanine nucleotide exchange factor (GEF) binds strongly to chromatin.

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Ran, using a mutant version of Ran, immobilized RCC1 on chromosomes. Ran also enhanced RCC1 binding to chromatin in vitro. Only nucleotide exchange, which displaces RCC1 from Ran, released the GEF and RanGTP from chromosomes. The increased affinity of the complex for chromosomes can be explained by its binding geometry. RCC1 binds histones H2A and H2B, whereas Ran binds weakly to histones H3 and H4. The complex is therefore well suited to bind to nucleosome octamers. Thus, exploiting the geometry of nucleosomes is a simple way to couple GTP exchange to chromatin-bound Ran.