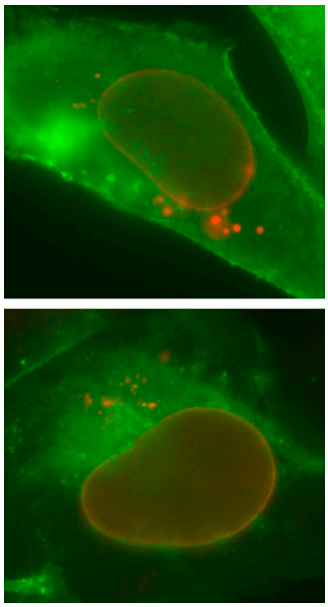
In This Issue



HB-EGF (green) on the plasma membrane (top) departs for the nuclear envelope (red) in cells that are reactivating the cell cycle (bottom).

Trafficking from cell surface to nuclear envelope

A newly identified trafficking pathway takes a cell surface protein to the inner nuclear membrane (INM), reveal Hieda et al. In its nuclear envelope locale, the protein can reactivate cell cycle genes.

Activation of the cell cycle is an important job for EGF family members. These transmembrane proteins start out on the plasma membrane but are cleaved when cells receive mitogenic cues, thereby generating a soluble extracellular growth factor. Although the cytoplasmic portion remains hitched to the transmembrane domain, recent evidence indicates that this portion of one EGF member, HB-EGF, inactivates transcriptional repressors of cell cycle genes.

The new work suggests that this transcriptional function requires HB-EGF relocation from the plasma membrane to the INM. Upon cleavage, trafficking of the remaining protein to the INM depended on Rab GTPases that are required in the endocytic pathway. It also depended on an ER retrieval signal within the HB-EGF. The protein thus seems to be recycled in endosomes to the Golgi, where the ER retrieval signal operates. Once at the ER, the protein is small enough to diffuse freely within the membrane through the nuclear pore to the INM.

In the absence of mitogenic cues, the ER retrieval signal must be stymied for HB-EGF to pass from the Golgi to the plasma membrane. The authors imagine that the signal—a 5-aa sequence—is structurally hidden within HB-EGF but exposed by a mitogen-activated phosphorylation event.

Hooked to the INM, HB-EGF is probably in close proximity to its repressor targets, since sites of gene repression are thought to reside near the nuclear envelope. The group is now examining whether genes that are repressed by HB-EGF's targets lie near the envelope. JCB

Hieda, M., et al. 2008. *J. Cell Biol.* 180:763–769.

Quick hops on and off plus ends

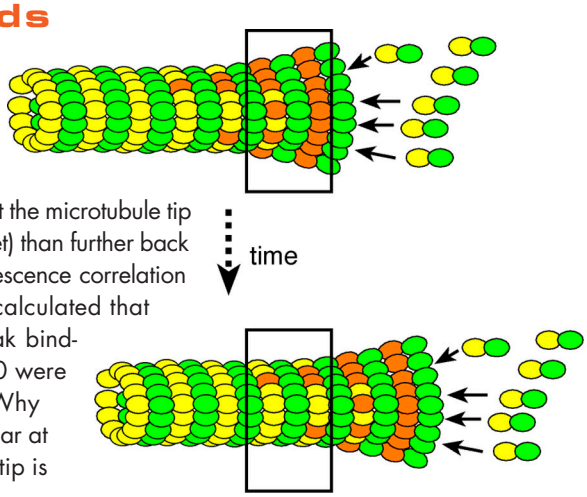
Microtubule tips are a hotbed of weak binding sites, say Dragestein et al. The authors suggest a new model to describe the behavior of microtubule plus end–binding proteins.

The plus end of a microtubule—its growing end—attracts a variety of binding proteins, including EB1 and CLIP-170, which are thought to influence microtubule dynamics. Viewed on a growing microtubule, fluorescently labeled CLIP-170 appears as a comet: a bright dot at the tip trailed by a progressively fainter tail.

Current explanations for the comet postulate that CLIP-170 hops onto the tip, perhaps together with tubulin, hangs on for a couple seconds as the microtubule grows, and then falls off behind the tip. But the new work reveals that CLIP-170 hops on and off much faster.

Measurements of exchange rates using fast FRAP revealed that CLIP-170 only held on for ~0.3 seconds before hopping off a microtubule. Measurements were limited by the speed with which CLIP-170 can diffuse to the tip, so true exchange rates might be even faster. CLIP-170 was also equally capable of hopping on at spots that were further back than just the very tip of the plus end, in contrast to the proposals of previous models.

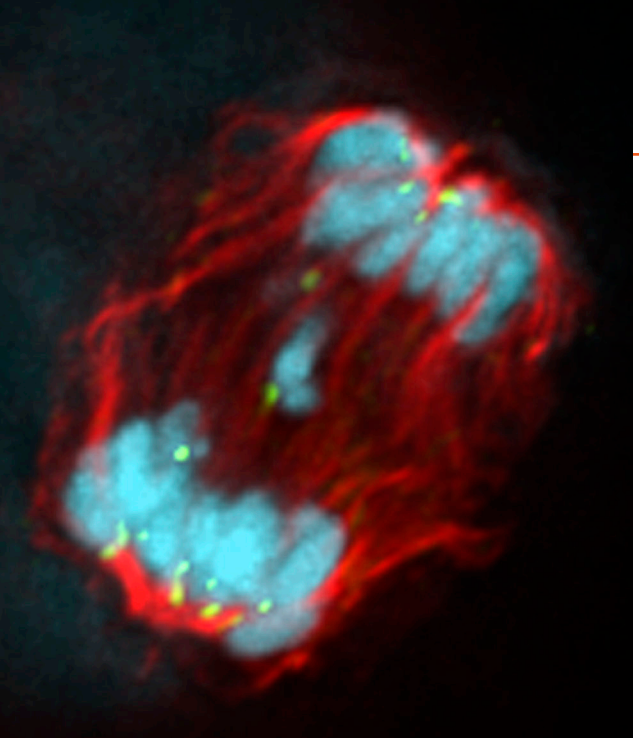
To explain the fluorescent comet, the group now proposes that binding sites for CLIP-170 are more abundant at the microtubule tip (the head of the comet) than further back (the tail). Using fluorescence correlation spectroscopy, they calculated that ~100 relatively weak binding sites for CLIP-170 were found at the tip. Why binding sites disappear at a distance from the tip is not clear. Perhaps tubulin undergoes structural changes as it is incorporated into the filament.



Binding sites (orange) for CLIP-170 are abundant at microtubule plus ends (top) but disappear as the filament grows (bottom).

Exchange rates for EB1 were similar to those of CLIP-170, suggesting that plus end–binding proteins are generally transient binders. The authors wonder whether quick changes in the structure at plus ends might relay information to other cellular components. They are now aiming to do single-molecule imaging on microtubule plus ends inside cells. JCB

Dragestein, K.A., et al. 2008. *J. Cell Biol.* 180:729–737.



A chromosome (blue) whose kinetochore (green) is attached to spindle microtubules (red) from both poles lags behind during mitosis and creates aneuploidy.

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Losing odd chromosomes

Cancer cells need a little something extra to propagate with unusual chromosome numbers, based on evidence from Thompson and Compton.

Many tumors are packed with cells that have too many or too few chromosomes—a state known as aneuploidy. In theory, aneuploidy is the result of chromosome segregation errors during mitosis. The authors now identify one mechanism behind these errors: anaphase starts up while chromosomes are wrongly attached to both poles.

For proper segregation, sister chromatids of each chromosome should attach to opposite poles. But in several cancer cell lines, chromatids were often hitched to both poles during division, resulting in daughter cells with either both copies of a chromosome or neither.

Chromosome attachment is monitored by the spindle checkpoint, but this mechanism only stalls anaphase when it recognizes unattached chromosomes; it does not sense chromatid attachment to both poles. How they are prevented in normal cells is not yet known but probably involves microtubule detachment by aurora kinase.

Even with high segregation error rates, diploid cancer cell lines failed to maintain abnormal chromosome numbers. In two diploid lines, newly formed aneuploid cells were rapidly lost over subsequent generations. Perhaps aneuploid cells undergo apoptosis or senescence or are simply too slow-growing to compete with their diploid neighbors.

The authors now want to determine how aneuploid tumor cells keep their mismatched chromosome sets. Once their secret is identified, it might prove to be a therapeutic target that is unique to the tumor. JCB

Thompson, S.L., and D.A. Compton. 2008. *J. Cell Biol.* 180:665–672.

An adhesion kinase on centrosomes

New results from Fielding et al. place a kinase found in focal adhesions on centrosomes. There, the kinase helps arrange microtubules rather than actin.

Focal adhesions contain a mass of proteins that link matrix-bound integrins to the actin cytoskeleton. Within that mass is integrin-linked kinase (ILK), which phosphorylates other focal adhesion proteins during cell spreading and migration. While searching for ILK binding partners, Fielding and colleagues fished out an unexpected class of cytoskeletal proteins—tubulins.

In addition to α- and β-tubulin, several centrosomal microtubule-binding proteins were identified. The localization of active ILK and its partners to centrosomes was necessary for proper spindle formation during mitosis. The absence of ILK disrupted a complex of centrosomal proteins that includes Aurora A. In these cells, microtubules polymerized from only one pole and did not reach the DNA.

Although it is not clear how ILK creates a bipolar spindle, the authors hypothesize that it might be needed for centrosome duplication before mitosis. They are now using a proteomics approach to identify centrosomal targets of the kinase. JCB

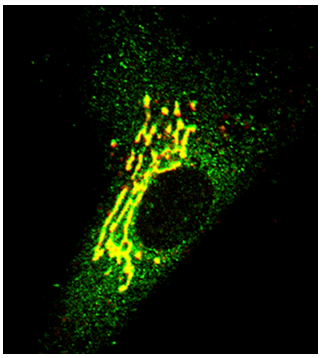
Fielding, A.B., et al. 2008. *J. Cell Biol.* 180:681–689.

Matching traffic and growth

The shuttling of a phosphatase keeps membrane trafficking in tune with cell growth, say Blagoveshchenskaya et al. The enzyme's removal from the Golgi increases trafficking when cells need it most.

Growing cells need new proteins and lipids delivered to the expanding plasma membrane. The outward transport of these components from the Golgi is driven in part by a pool of PI4P phospholipid, which is thought to help create a suitable lipid environment for the formation of Golgi-derived carriers. The new findings reveal that Golgi PI4P levels rise when cells get a taste of extracellular growth factors.

In dormant cells, PI4P levels at the Golgi were kept low by a phosphatase called SAC1, which turns PI4P to PI. To reach the Golgi, SAC1 formed oligomers that seem to uncover a binding motif that recruits it into ER-to-Golgi transport vesicles.



SAC1 (green) limits membrane trafficking from the Golgi (red) until cells are stimulated into proliferation.

The oligomers were disrupted when cells were given growth factors to jolt them out of dormancy. Both FGF and PDGF activated the ERK1/2 and p38 MAPK pathways, which led to disassembly of SAC1 oligomers. Collapse of the oligomers caused SAC1 relocation to the ER, thereby allowing Golgi PI4P levels to rise.

Constitutive activation of p38 was enough to nudge cells out of quiescence and back into proliferation, as occurs during early stages of cancer. The group will soon test whether SAC1 is necessary for this rejuvenation. JCB

Blagoveshchenskaya, A., et al. 2008. *J. Cell Biol.* 180:803–812.