

In This Issue

Microtubules get swept up in the excitement of mitosis

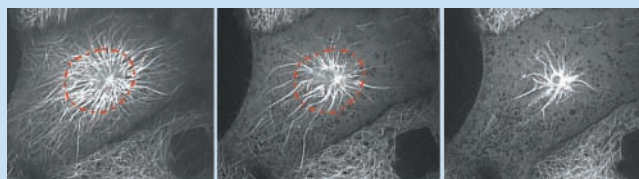
Microtubules (MTs) undergo a dramatic suctioning at the onset of mitosis, according to a report by Rusan et al. on page 997. The process places MTs in the vicinity of the growing mitotic spindle and may prevent nonspindle MTs from interfering with chromosome segregation.

Visualizing mitotic MT rearrangement has not been easy, because the process is so rapid. The new confocal videos of living cells expressing GFP-tubulin catch prophase in a way that imaging of fixed or microinjected cells never did. Rearrangement began in prophase with the depolymerization of cytosolic MTs and the formation of bundles and foci.

Bundles were then pulled along other MTs extending from the centrosome. They continued to disassemble along the way, and eventually appeared to join with the forming spindle. A video of the entire process (which was completed within several minutes) is available at <http://www.jcb.org/cgi/content/full/jcb.200204109/DC1/1>. Astral MTs continued to scan the cytoplasm even as late as metaphase, drawing in errant MTs.

Dynein may regulate the timing of MT influx. Pulling was dependent on cytoplasmic dynein

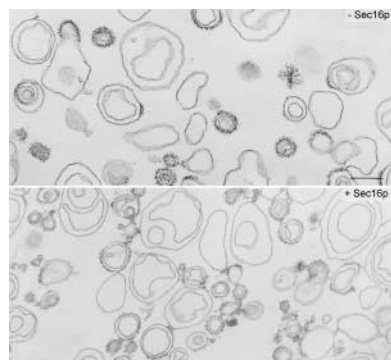
activity, indicating that MTs are both a track and cargo for this motor. Previously, nuclear envelope-associated dynein was shown to tear the envelope by pulling outward along microtubules. Possibly, the switch from dynein's interphase vesicular cargo to its mitotic cargoes are coordinately regulated, although the relevant signals to dynein are not yet known. ■



Microtubules are pulled inward (left to right) in early mitosis.

COPs are held up by Sec16p

On page 1029, Supek et al. illustrate how a peripheral membrane protein organizes a coat protein complex involved in secretory vesicle formation.



More vesicles bud from liposomes when Sec16p is present (bottom).

The protein in question, yeast Sec16p, is an ER resident required *in vivo* for COPII-dependent vesicle budding. *In vitro*, Sec16p is not necessary for budding from liposomes reconstituted with pure cytosolic COPII proteins. However, this *in vitro* reaction depends on a nonhydrolyzable form of GTP, probably because the COPII coat

falls apart when Sar1p (the initiator of coat assembly) hydrolyzes GTP. Until now, the function of Sec16p in liposome budding could not be tested, because the protein was difficult to purify.

Supek et al. report conditions that stabilize Sec16p and have purified enough protein for *in vitro* studies. Microsomal membranes stripped of endogenous Sec16p were stimulated in vesicle budding by the purified protein, but only in the presence of hydrolyzable GTP. Thus, the *in vivo* function of Sec16p may be either to slow GTP hydrolysis during budding or to stabilize the COPII coat even after GTP is hydrolyzed by Sar1p.

The authors used liposomes to show that Sec16p recruited Sar1p-GTP and another coat component, Sec23/24p, to membranes. However, this interaction did not inhibit GTP hydrolysis. Thus, the function of Sec16p appears to be to tether the COPII coat on membranes. ■

Pinosomes help a virus escape

Many virus species are taken into their host via receptor-mediated endocytosis, after which the virus particle is temporarily contained within endosomes. For genome propagation, most viruses must escape into the cytosol. New results from Meier et al. (page 1119) explain how Adenovirus type 2 (Ad2), one of the most rapidly escaping viruses known, tricks the cell into helping it break free.

Meier et al. first show that Ad2 enters in clathrin-dependent endosomes. But subsequent escape from the endosomes is dependent on pinocytosis, a process by which extracellular material is taken in within enclosed lamellipodia at ruffling membranes. Ad2 initiates integrin signaling and thus pinocytosis at the plasma membrane. It also triggers pinosomal lysis, again apparently from the cell surface, as internalized viral particles were not required.

The virus is not taken in by pinocytosis, so what is the function of the pinosomes? Pinosomes in maturing dendritic cells leak their contents into the cytoplasm; perhaps Ad2 uses integrin signaling to stimulate the formation of similar leaky compartments. The group is now examining whether



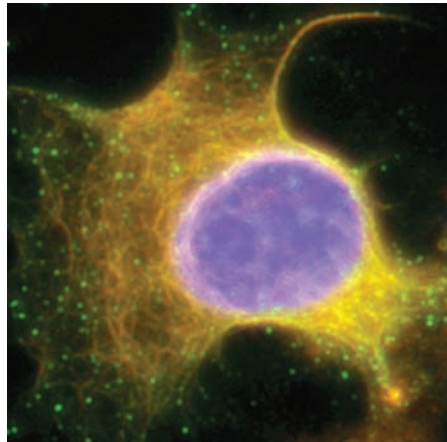
Pinosomes (arrow) form in Ad2-infected cells.

virus-loaded endosomes and pinosomes fuse, thereby shuffling virus into a compartment that can be sufficiently destabilized for viral escape. In any case, efficient gene expression from many viral vectors used for gene therapy will require more than just vector endocytosis. ■

DEDD spells death to caspase substrates

The major apoptotic effector protein caspase-3 holds the power to destroy a huge number of proteins in a cell—over 34 thousand human proteins with a caspase-3 cleavage motif have been sequenced. Nevertheless, apoptosis proceeds in an ordered fashion; only specific substrates are cleaved at the proper time. On page 1051, Lee et al. describe a new function of the apoptosis regulator DEDD as a scaffolding protein that directs this orderly destruction.

DEDD works by bringing together the important participants, much like scaffolding proteins that control signaling cascades. DEDD resides mainly in the cytosol, despite its previous identification based on its DNA-binding death effector domain (DED). Localization studies by Lee et al. revealed that, even in nonapoptotic epithelial cells, DEDD associated with the keratin intermediate filament network. Once apoptosis was induced, an apoptosis-related epitope within the DED of DEDD was exposed in a caspase-3-dependent manner.



DEDD (red) brings active caspase-3 (green) to its substrates.

Activated DEDD returns the favor by bringing caspase-3 to its keratin substrate. Active caspase-3 localized almost entirely to keratin filaments, suggesting that keratin is its main substrate in epithelial cells. Later in apoptosis, DEDD, caspase-3, and fragments of keratin filaments formed inclusion bodies that eventually moved into apoptotic blebs.

Ubiquitination of DEDD at apoptosis may regulate its scaffolding activity, as keratin and caspase-3 only associated with diubiquitinated DEDD. When DEDD could not be ubiquitinated, keratin filaments remained intact. Caspase-3 also remained largely inactive, indicating that DEDD further activates the protease and thereby regulates the destruction of substrates beyond keratin. The lag between the initial activation of caspase-3 by cytochrome *c* and later

caspase-3 activation by DEDD may ensure that only small amounts of the protease are active until it reaches its main cytoskeletal target. ■

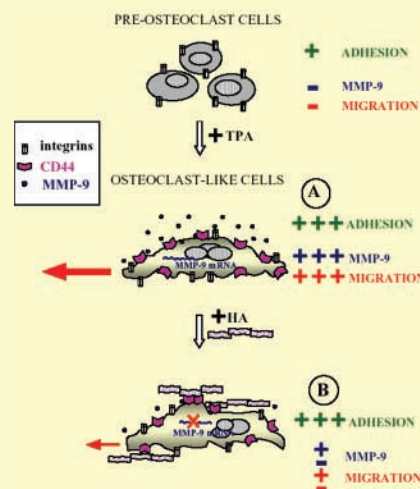
Gnawing at the bone

The destructive ability of osteoclasts (OCs) to weaken bones is reduced by the interaction between an extracellular matrix (ECM) ligand and an adhesion receptor, according to results from Spessotto et al. on page 1133.

The correct balance between bone-destroying OCs and bone-generating osteoblasts maintains bone mass in adults. However, too much OC activity can cause diseases like osteoporosis and rheumatoid arthritis. OCs are derived from hematopoietic precursors that leave the bloodstream and migrate toward the bone surface. Once they reach their destination, the precursors stop migrating and differentiate into OCs, which secrete cysteine proteases that chew up the bone.

Other proteases, namely matrix metalloproteases (MMPs), are thought

to help mobilize OCs by degrading small pieces of the ECM. As such, Spessotto et al. were not surprised to find that reducing MMP-9 expression



OCs adhere when HA/CD44 binding inhibits MMP-9 expression.

in OCs led to a shift from OC migration to adherence.

What they did not anticipate was what causes MMP-9 inhibition *in vivo*. MMP-9 expression was reduced and migration ceased when cells encountered hyaluronic acid (HA), an ECM component. The cells bind HA via CD44, and this engagement normally promotes cell motility, as seen in melanoma cells. But OCs migrating *in vitro* on various other ECM components, including laminin and fibronectin, stopped when they encountered HA. They also stopped when MMP-9 activity was inhibited, either chemically or using antisense techniques. The group does not yet know how HA binding elicits MMP-9 down-regulation, but they have initial evidence that HA also inhibits motility in certain tumor cells. ■