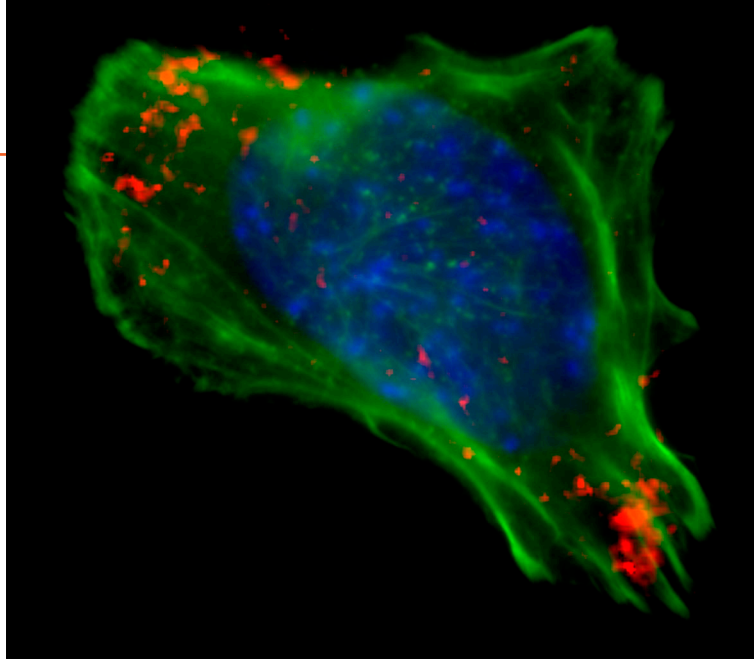
In This Issue



Late endosomes (red) scatter to the cell periphery when the p14 adaptor is missing.

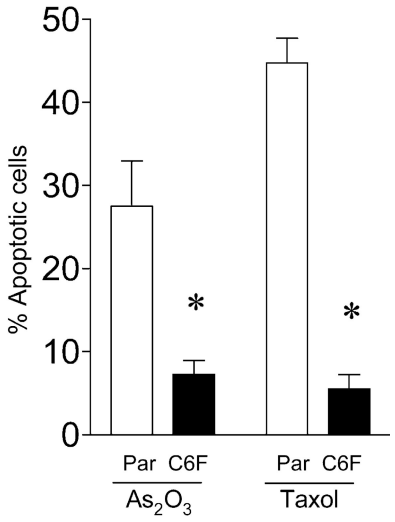
Essential adaptor

Receptors keep signaling long after they are endocytosed thanks to a variety of adaptor proteins. Now, Teis et al. show on page 861 that the loss of p14, a protein that helps attach active MAP kinase to endosomes, results in endosome positioning defects, cell cycle problems, and death.

Mice lacking p14 died as embryos. Fibroblasts from the embryos had normal early endosomes but, compared with wild-type cells, twice as many of their late endosomes and lysosomes were located far from the nucleus, and degradation of internalized EGF receptor was half as efficient.

Epidermal-specific deletion of p14 resulted in mice that were born alive but died soon after from dehydryation. EGF receptors, normally found only in basal cell layers, were not degraded properly and were therefore expressed even in suprabasal cell layers. The mice had thin skin, apparently because of the reduced proliferation that was evident in vitro for harvested keratinocytes. This defect could not be rescued by p14 forced to localize to the plasma membrane, suggesting that the endosome localization is necessary to build a fully comptetent signaling complex.

A paper in press at *Nature Medicine* (authored in collaboration with Teis et al.) identifies a hypomorphic allele of p14 as the explanation for a familial immunodeficiency. Here again, endosome dynamics are disturbed, possibly because one of the motors that brings adaptor proteins to endosomes also helps localize endosomes. Teis et al. now want to see whether inhibition of endosome-localized MAP kinase signaling might be more effective against proliferation-related diseases and have fewer side effects than inhibition of all MAP kinase signaling. JCB



Cells die less readily if they have mitochondrial mutations (black bars).

Surviving on low energy

Many cancer cells resort to inefficient glycolysis for their energy but can nevertheless survive, even when competing with their more energy-efficient noncancerous neighbors. One explanation, say Pelicano et al. (page 913), is that the altered metabolism can turn on an Akt survival pathway.

Most cells rely primarily on the rich energy harvest that comes from oxidative phosphorylation. But a switch to glycolysis can be induced by hypoxia, the loss of the tumor suppressor p53, expression of tumor inducers such as Myc and Ras, or mutation of certain mitochondrial enzymes.

Mitochondrial mutation is particularly common in cancer cells, which are under metabolic stress that generates mutagenic oxidants. Mitochondrial DNA is a prime target for these mutations as these organelles lack many of the safegard mechanisms that prevent and repair damage of nuclear DNA.

Pelicano et al. mutated mitochondrial DNA. The resultant cells grew better under hypoxic conditions and were less sensitive to common anticancer drugs. The cells produce high levels of NADH, which is normally consumed by oxidative phosphorylation, and this altered redox environment inactivated the PTEN phosphatase. With PTEN out of action, its target, Akt, was activated. This kinase is known to increase cell survival. In hypoxic or toxic conditions, this survival pathway may be used in normal cells to keep the cells healthy, but cancer cells have used it to survive despite their unorthodox metabolism.

When cells with mitochondrial mutations were treated to prevent the Akt activation, they once more became sensitive to anticancer drugs. Inhibition of the Akt pathway may also be a promising approach in the clinic, although it is not yet clear which protein in the Akt pathway would make the best target, or which anticancer drugs would make the best follow-up treatment. JCB

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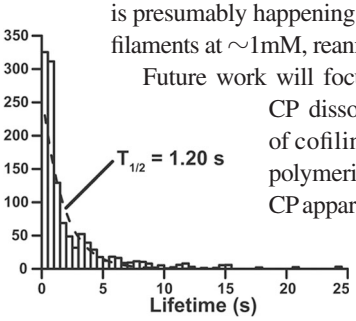
Dynamic uncapping

The surge of actin growth toward the cell periphery is stopped abruptly by capping protein (CP). In vitro, this protein sticks like glue, but in vivo it comes off the ends of those filaments rapidly, say Miyoshi et al. (page 947). Cycles of severing and annealing may thus be more important than expected in creating a dynamic actin array.

The researchers had previously tracked the lifetimes of actin filaments in the lamellipodia of spreading *Xenopus* fibroblasts. They sought explanations for the range of lifetimes by looking at actin regulatory proteins such as CP. Surprisingly, the binding half life of CP in vivo was barely more than a second, compared with almost half an hour in vitro. The uncapped filaments appeared to resume their growth.

Although actin turnover is ~20-fold slower than CP turnover, a further reduction in actin turnover by adding a cofilin inhibitor significantly reduced CP turnover. The authors suggest that cofilin-mediated filament severing may cause the dissociation of CP attached to a small actin oligomer. Such severing is presumably happening throughout the actin arrays, but with actin filaments at ~1mM, reannealing would be a very favorable reaction.



Capping protein is removed rapidly from the ends of actin filaments.

Future work will focus on the factors that may be promoting CP dissociation and the in vivo cleavage activity of cofilin. Dendritic nucleation models of actin polymerization may also need to be revised, with CP apparently effecting a temporary and quantitative rather than qualitative change in actin polymerization. The new, more dynamic model might explain how motile cells can rapidly remodel an actin mesh to achieve a change in direction. JCB

Making an autophagosome

An understanding of de novo organelle construction comes one step closer thanks to He et al. (page 925), who find that Atg11 leads Atg9 to the preautophagosomal structure (PAS).

The PAS is intriguing because it is the site where fragments of membrane coalesce to form a new organelle: the autophagosome. In yeast, two flavors of this process exist. Bulk autophagy is induced by starvation and is essentially a cell nondiscriminately eating itself. The cytoplasm-to-vacuole targeting (Cvt) pathway, however, is constitutive and picks selected cargoes for delivery to the vacuole (the yeast equivalent of the lysosome).

Nobody knows what protein gets to the PAS first, but Atg9, as the first characterized transmembrane protein required for both pathways, is a good starting point. It cycles between the PAS and other sites, including mitochondria, probably as a way of collecting membrane fragments to build an autophagosome. It is not yet clear, however, what targets Atg9 to the PAS.

He et al. find that Atg11 uses one of its coiled coil regions to bind to Atg9, and this interaction and an intact actin cytoskeleton are required for Atg9's anterograde transport to the PAS. This transport mechanism is only required for the Cvt pathway; during bulk transport, another mechanism somehow ensures Atg9 cycling.

Both Atg9 and Atg11 have multiple binding partners, but further efforts will be needed to identify where in the autophagy pathway these interactions occur. An assay using semipermeabilized cells should help determine which complexes are most important for creating an autophagosome. JCB

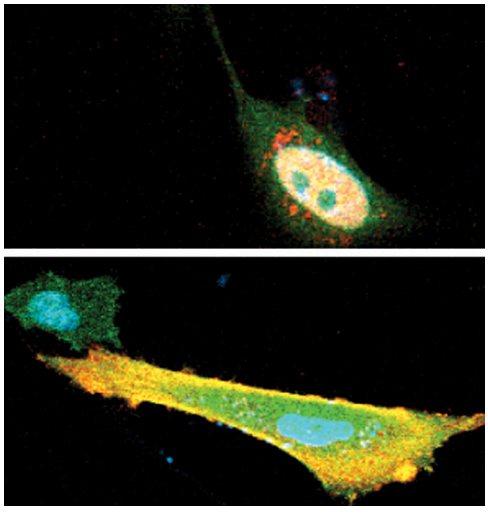
Rac controls nuclear entry

Rac proteins are most familiar in the context of cytoskeletal regulation. Now, Kawashima et al. (page 937) find that Rac1 and its regulator help STAT transcriptional activators to get into the nucleus.

STAT proteins signal downstream of cytokine receptors. These researchers previously reported an association between STAT3 and the GTPase-activating protein MgcRacGAP. Here they report that Rac1 and MgcRacGAP bind STAT5A, and that the association between MgcRacGAP and STAT5A is enhanced by IL-3 signaling. Both Rac1 and MgcRacGAP were necessary for efficient entry of STAT5A into the nucleus, and in semipermeabilized cells a dominant-negative Rac1 prevented binding of STAT5A to importin-α, and thus nuclear entry.

Others have previously shown, using armadillo proteins as import substrates, that Rac1 has a nuclear localization sequence (NLS) that is active when Rac1 is in its active, GTP-bound form. Unpublished data suggest, however, that it is an MgcRacGAP NLS that is required for STAT5A import.

MgcRacGAP is also found at the midbody, where it is phosphorylated to convert it into a Rho-GAP that helps complete cytokinesis. At the nuclear envelope it may undergo a different modification or activation, leading to release of STAT5A after nuclear import, as the group detected this dissociation event. The larger remaining question is whether cytoskeletal changes, such as those occurring when cells reach confluence, change Rac's regulation of nuclear entry and thus proliferation. JCB



STAT5A stays out of the nucleus (blue) when Rac1 is removed (bottom).