

In This Issue

Putting cell polarity on the map

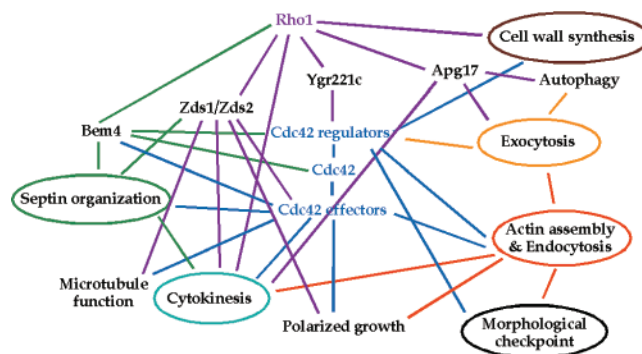
Genetics and biochemistry have been used to map many of the individual pathways that establish and maintain cell polarity in yeast, but Drees et al. (page 549) have now produced the equivalent of an aerial photograph of these processes. Using a high-throughput yeast two-hybrid screen, the authors assayed the universe of likely protein–protein interactions involved in cell polarity development. The resulting protein interaction map provides tantalizing insights and identifies dozens of potential mechanistic connections worth closer examination.

The authors used 68 yeast proteins associated with the actin cytoskeleton, septins, the secretory apparatus, and Rho-type GTPases as baits in parallel two-hybrid screens covering ~90% of the predicted *Saccharomyces cerevisiae* ORFs. The screen uncovered 128 novel protein–protein interactions, including 44 involving previously uncharacterized proteins. The appearance of known interactions in the screen, along with subcellular localization studies, suggests that many of the newly identified interactions are relevant in vivo.

variant in vivo.

Many of the interactions help to explain the phenotypes of previously described yeast mutants. For example, multicopy expression of Msb2 suppresses the phenotype of a Cdc24 mutant, but the function of Msb2 remains unknown. The new analysis identifies a two-hybrid interaction between Cla4 and Msb2,

suggesting that Msb2, like Cdc24, is directly involved in the Cdc42 pathway. Other interactions imply new links between known pathways, such as between the secretory pathway regulated by Rho1 and the Cdc42 pathway, which is essential for establishing and maintaining cell polarity. Still other interactions suggest direct connections between actin assembly and the morphogenesis checkpoint.



Connections in yeast cell polarity.

Because the machinery of cell polarity development is highly conserved from yeast to humans, the newly described interactions merit further study in a variety of cell types. The authors stress that the screen was not exhaustive, so additional interactions certainly await discovery. ■

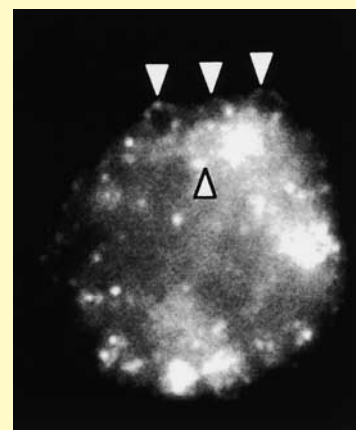
How to survive a macrophage attack

In a classic example of microbiological payback, mycobacteria have evolved the ability to parasitize macrophages, cells that ordinarily digest bacteria circulating in the blood. After a macrophage endocytoses a mycobacterium, the resulting phagosome deviates from the normal maturation process and becomes a safe haven for the pathogen rather than an acidic digestive compartment for the macrophage. Fratti et al. (page 631) used this system to dissect the molecular mechanism of phagosome biogenesis, providing important new insights into phagosomal maturation and mycobacterial pathogenesis.

Phagosomes containing latex beads recruit EEA1, a Rab5 effector and regulator of vesicular trafficking, but phagosomes containing mycobacteria exclude this protein. The authors

attempted to make the latex-bead phagosomes behave like mycobacteria-containing phagosomes, and found that inhibitors of PI-3-kinase activity or microinjection of antibodies against EEA1 and the PI-3-kinase hVPS34 inhibited the acquisition of late endocytic markers. Coating the latex beads with ManLAM, a lipid isolated from *Mycobacterium tuberculosis*, also inhibited EEA1 recruitment to the phagosomes and prevented them from maturing.

EEA1 is believed to be required for trafficking between the trans-Golgi network (TGN) and endosomes. The new data suggest that mycobacteria prevent phagosomal acidification by blocking TGN interactions, which are required for fusion with H⁺ATPase-containing vesicles. The work is also the first demonstration that a phospholipid produced by a pathogen can redirect normal phagosomal trafficking. Results from other studies on EEA1 distribution in infected cells



EEA1 is recruited to normal phagosomes (arrowheads), but is excluded by mycobacteria.

suggest that similar mechanisms may be used by mycobacteria, *Leishmania donovani*, and Human Granulocytic Ehrlichiosis Agent, raising the possibility that therapies targeting this pathway could be useful against several important intracellular pathogens. ■

Deleting keratins to find one

By knocking out the two known keratin 6 (K6) genes in mice, Wojcik et al. (page 619) have discovered a third murine K6 gene, and simultaneously created a promising model system for studying keratin function in more detail.

In an effort to study the function of K6, the authors generated a mouse line lacking both K6a and K6b, and found that plaques develop on the tongues of these mice and cause the majority to die of starvation within two weeks of birth. Surprisingly, and in contrast to a previously described mouse K6a/b knockout line, ~25% of the mice survive to adulthood and grow normal hair and nails. Further analysis uncovered a previously undescribed murine K6 gene, an ortholog of the K6hf gene from human

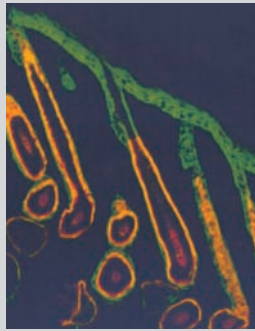
hair follicles, which the authors have named MK6hf.

While the presence of MK6hf helps explain why the knockout mice develop normal hair and nails, some mysteries remain. MK6hf is not

expressed in oral epithelia, and all of the mice exhibit ultrastructural abnormalities in their tongues, so it is unclear why some survive to adulthood while others die. Wojcik and colleagues are now performing backcrosses to search for unknown genetic modifiers involved in epithelial integrity.

The results also suggest that inducible K6 expression may be less important in wound healing than was previously

believed, since the knockout mice heal wounds normally. Rather than helping in the initial events of wound healing such as proliferation and migration, keratins may be induced to strengthen the tissue once it has been repaired. ■



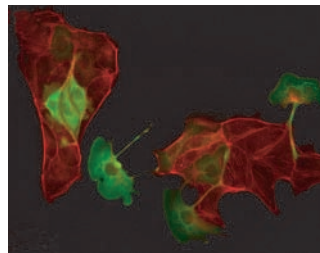
Expression from another K6 gene (yellow) explains hair growth in double knockout mice.

ARNO gets things moving

Stationary cells sometimes undergo a dramatic morphological change and begin to migrate, a transition that is essential for processes like wound healing, development, and tumor metastasis. Beginning on page 599, Santy and Casanova provide important new mechanistic insights into this transition, and also describe a novel assay that should be useful in future studies of the ARF family of GTPases.

Because of the importance of ARF proteins in modulating actin assembly, Santy and Casanova reasoned that ARNO, a guanine nucleotide exchange factor for ARFs, might control changes in the actin cytoskeleton. They found that when ARNO is expressed in MDCK cells, many of the cells change dramatically, forming fan-shaped lamellipodia and becoming migratory. A novel pull-down assay, in which an immobilized ARF-binding protein is used to isolate ARFs, shows that ARNO expression selectively activates ARF6 in the MDCK cells. In turn, ARNO-induced activation of ARF6 causes increased activation of both Rac1 and phospholipase D in the cells, apparently by independent pathways that function together to induce cell migration.

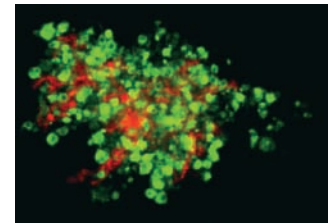
The work is the first demonstration of Rac activation through an ARF-mediated pathway, and localization experiments suggest a model in which ARF6 activates Rac through a GIT/Pkl-paxillin-PIX complex. Intriguingly, ARNO expression only causes migration in MDCK cells that have a free edge exposed at the outside of a cell cluster or wound. The authors suggest that the free edge creates a novel membrane and cytoskeletal environment where ARNO can be recruited to induce migration. ■



ARNO (green) activates cell movement.

The hidden power of platelets

Platelets get no respect. Traditionally, these anucleate cells have been acknowledged as critical mediators of blood clotting, but they were considered metabolically challenged drones, incapable of signal-dependent gene expression. Now, Lindemann et al. (page 485) demonstrate that platelets are not only able to translate preformed mRNAs in response to environmental signals, they may also provide an important link between the coagulation and inflammatory cascades via regulated production of an inflammatory cytokine.



Platelets (green) produce IL-1β (red) during a clotting reaction.

Using an arrayed cDNA library, the authors identified a variety of mRNAs present in resting platelets, including one encoding the interleukin-1β (IL-1β) precursor. Activation of the platelets as in clot formation triggers IL-1β production; the IL-1β then induces adhesiveness of endothelial cells for neutrophils, an inflammatory reaction. The platelet-produced IL-1β accumulates over a period of several hours, indicating that platelets can exert long-term influence over an inflammatory response. Previous work had shown that activated platelets could synthesize new proteins, but the new study is the first demonstration that the cells can inducibly produce physiologically relevant levels of cytokines.

The results suggest that platelets may be more important in mediating pathological responses than was previously recognized. Myocardial infarction, for example, begins with platelet deposition near a ruptured atherosclerotic plaque, followed by leukocyte infiltration. If the synthesis of IL-1β by the platelets is facilitating leukocyte accumulation, this signal might be a good therapeutic target. ■