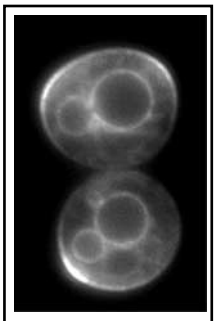
Text By William A. Wells
wellsw@rockefeller.edu



Cdc42 (white) polarizes even without a cue.

Li/AAAS

Polarity feeds back

Polarizing cells must first pick a direction. The external cues that guide this choice are obvious: bud scars or mating partners for budding yeast, and chemoattractant gradients for *Dictyostelium* and neutrophils. But polarization still occurs, albeit in a random direction, when these cues are either removed or made uniform.

Now, Roland Wedlich-Soldner, Rong Li (Harvard Medical School, Boston, MA), and a group of mathematical modelers have come up with an explanation for this intrinsic polarization in budding yeast. In wild-type situations, the intrinsic mechanism may be used to solidify the direction originally dictated by the external cue.

Normally, the bud scar acts as a site for activating Cdc42. Somehow, expression of activated Cdc42 is by itself sufficient to polarize cells. This polarization is now shown to involve the formation of a cap of Cdc42 on the plasma membrane. The cap's location is independent of any obvious cue, including bud scars, microtubule arrays, and lipid localization. The polarization does depend on transport apparatus—actin cables, a type V myosin motor, and vesicle exocytosis—and Cdc42 cofractionates with a secretory vesicle marker.

The researchers suggest that an initial stochastic grouping of Cdc42 on the plasma membrane stimulates the formation of actin cables and thus the vesicle-based delivery of more Cdc42. A similar positive feedback is seen in neutrophil chemotaxis, where lipids stimulate Rho GTPases to produce more lipids.

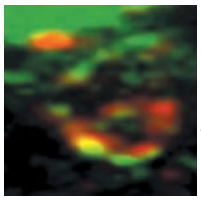
Thus, says Li, there are "intrinsic mechanisms [that] are sufficient to break symmetry without contributions from external cues." Yeast cells lacking this feedback can still respond to a normal external cue, but their inefficient polarization results in secretion in both mother and daughter cells making the cells fat. ■

Reference: Wedlich-Soldner, R., et al. 2003. *Science*. 10.1126/science.1080944.

Linking spindle to furrow

Once it gets going, cytokinesis is a simple squeeze. The complicated machinery is devoted instead to localizing the cytokinesis furrow. Now, Gregory Somers and Robert Saint (Australian National University, Canberra, Australia) have provided a link from mitotic spindle to contractile furrow that may explain how one positions the other.

The group started with the furrow-localized protein Pebble (PBL), a guanine nucleotide exchange factor (GEF) and thus activator for Rho1 and actin remodeling. Two-hybrid and coimmunoprecipitation experiments with PBL turned up RacGAP50C. The worm homologue of RacGAP50C, CYK-4, is essential for cytokinesis completion and binds a kinesin-like protein that bundles microtubules in the central spindle.



Saint/Elsevier

Rings of RacGAP50C (red) and PBL (green) link spindle and furrow.

Consistent with these interactions, RacGAP50C was found in an inner ring near central spindle microtubules, abutting an outer ring of PBL. How these rings affect each other is unclear. PBL and RacGAP50C, despite being an activating GEF and an inhibitory GTPase-activating protein (GAP), interact synergistically rather than antagonistically. Somers and Saint find that the GAP activity is not directed at Rho1 but is required for cytokinesis. Perhaps the logic behind the activity will be tied up in the need to regulate the timing of PBL activation. ■

Reference: Somers, W.G., and R. Saint. 2003. *Dev. Cell.* 4:29–39.

Exocytosis in action

Geri Kreitzer, Enrique Rodriguez-Boulan (Cornell University, New York, NY), and colleagues have provided the first visualization of targeted exocytosis in polarized epithelial cells.

Exocytic events can be seen by specialized microscopy techniques that selectively illuminate the bottom of cells. But to see exocytosis on the lateral side of a polarized cell, the researchers had to comb through many confocal images looking for events in which fluorescence intensity diminished due to emptying rather than movement of a vesicle or tubule. One clue was the spread of fluorescence visible only after release of an exocytic cargo.

"Technologically this is not so difficult," says Kreitzer. "But the analysis was very labor intensive." The reward was a direct readout of fusion events. Basolateral cargoes were located in the most apical two thirds of the cytoplasm and fused with the corresponding region of basolateral membrane. Apical cargoes were concentrated in approximately the top 4 μm of cytoplasm. Their fusion was not observed in polarized cells, and thus was presumed to be restricted to the apical membrane. Neither cargo fused with the basal membrane.

Kreitzer/Macmillan

Lateral vesicles dump their cargo as they fuse.

Microtubule depolymerization is known to result in mislocalization of apical membrane proteins, and Kreitzer and colleagues saw fusion of apical cargoes with basal membranes of nocodazole-treated cells. This correlated with syntaxin 3—a fusion machinery protein normally restricted to apical membranes—mislocalizing to basolateral membranes. Thus, syntaxin localization may direct delivery of apical and basolateral cargoes. But it is also possible that syntaxin 3 is itself an apical cargo, and that the real regulation is at the level of loading cargoes onto the correct motors or arrays of microtubules. ■

Reference: Kreitzer, G., et al. 2003. *Nat. Cell Biol.* 10.1038/ncb917.