In This Issue

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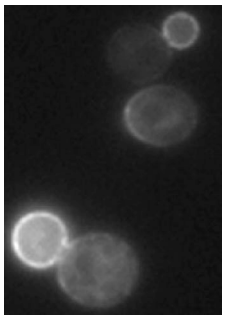
Cdc55 keeps Rho1 focused on growth



Constitutively active Rho1 localizes to the cortex in a yeast bud.

The GTPase Rho1 can spur a yeast cell to grow or to repair damage and resist stress. Jonasson et al.reveal how Rho1 switches between these different roles.

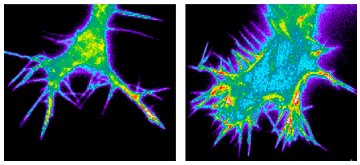
When conditions are good, Rho1 promotes cell growth and budding. It stimulates the synthesis of glucan, a building block of the cell wall. When the cell wall is damaged, Rho1 activates Pkc1 and switches on the cell wall integrity pathway, which curbs growth and turns on genes that repair the injury and protect the cell from stress.

To determine how Rho1 changes its function, Jonasson et al. screened for proteins that enable cells to survive the loss of three Rho1 activators. The researchers identified the protein Cdc55, which forms the regulatory portion of the PP2A phosphatase. The PP2A–Cdc55 complex hooks up with Rho1 and two other proteins, Zds1 and Zds2, at the bud cortex.

Jonasson et al. found that the PP2A–Cdc55 complex targets two RhoGAPs, Lrg1 and Sac7, that control Rho1's activity. Lrg1 blocks glucan synthesis, whereas Sac7 prevents Pkc1 activation. PP2A–Cdc55 inhibited Lrg1 but stabilized Sac7, thus suppressing the cell wall integrity pathway while permitting growth. After cell wall damage, however, activated Pkc1 dislodged PP2A–Cdc55 from the cortex. Rho1's function therefore depends on whether it binds to Pkc1 or the PP2A–Cdc55 complex, but researchers still need to figure out how Rho1 makes that choice.

Jonasson, E.M., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201508119

All-access coverage for neurites showcases their subtleties



Longer filopodia sprout from the growth cone of a neurite after depletion of DLC1 (left) than after depletion of ARHGAP5 (right).

The signaling network that controls neurite growth is much more complex than researchers thought, Fusco et al. reveal.

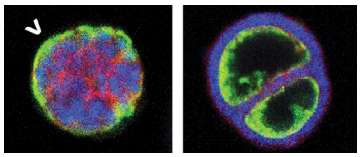
Neurites, the extensions that sprout from neuronal cell bodies, are highly dynamic. Within a few hours, a neurite can go through initiation, elongation, branching, and retraction phases. Within a few minutes, filopodia at the tips of neurites can change position. Neurites contain more than 200 proteins that might affect or be affected by Rho GTPases. Rac1 and Cdc42 GTPases spur neurite growth, whereas RhoA causes them to shrink. However, previous studies focused on neurites' final lengths rather than tracking their changes over time.

Fusco et al. used siRNAs to deplete 219 proteins that might interact with Rho GTPases. The researchers captured even subtle alterations in neurite dynamics by photographing a large number of cells every 12 minutes for nearly 20 hours. To make sense of this vast amount of data, the team created a computer vision system, NeuriteTracker, that registers changes in the nuclei, cell bodies, and neurites of neurons from frame to frame and translates them into geometric features. Statistical analyses can then determine which features change upon different siRNA perturbations.

Because RhoA triggers neurite retraction, the researchers expected that knocking down GTPase activating proteins (GAPs) that inhibit RhoA would lead to short neurites. Although depleting one GAP, ARHGAP5, produced short neurites by promoting shrinkage, depleting another GAP, DLC1, caused neurites to elongate. The two GAPs may control different functions of RhoA, indicating that researchers need to systematically analyze Rho GTPases and their interacting proteins to tease out the circuits that manage neurite growth.

Fusco, L., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506018

Epithelia prefer their ECM firm and easily digested



High RGD peptide levels prompt lumen formation (right), but the lumen is absent when levels are low (left).

By engineering synthetic gels, Enemchukwu et al. identify extracellular matrix (ECM) properties that control epithelial development.

Epithelial cells arrange themselves into an assortment of tubes, ducts, sacs, and spheres. Researchers have used natural gels containing collagen and laminin to probe how the ECM shapes these structures. But scientists can't manipulate the properties of these matrices to determine which factors are crucial for normal development.

Enemchukwu et al. developed synthetic polyethylene glycol gels whose biochemical and biophysical properties can be modulated. The researchers first tested the effect of altering polymer density, which dictates gel elasticity. At moderate gel stiffness, individual kidney cells proliferated to form spheres, with the cells' apical surfaces facing a central lumen. The cells' polarity was often reversed at low gel densities, however, and at high densities no spheres formed.

ECM proteins carrying RGD peptides stimulate integrins and help steer epithelial development. Increasing RGD levels in the gels enhanced the formation of hollow spheres with normal polarity.

Before clusters of epithelial cells can grow or extend protrusions, they need to clear space by dissolving the surrounding ECM. Enemchukwu et al. varied how susceptible the gels were to digestion by proteases and determined that enzyme-resistant ECM disrupted lumen formation and polarization. Thus, ECM with moderate elasticity, abundant RGD peptides, and at least a low level of digestibility promotes normal epithelial development. The researchers say their tunable gel system can be used to study morphogenesis in various normal and pathological contexts.

Enemchukwu, N.O., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506055