In This Issue

Text by Ben Short
bshort@rockefeller.edu

Budding yeast do the Cdc42 two-step

Two different proteins sequentially activate Cdc42 to ensure yeast form a bud at the right place, Kang et al. reveal.

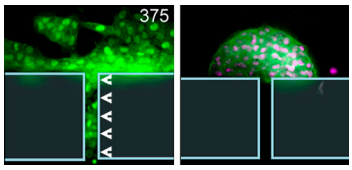
Haploid *S. cerevisiae* cells form new buds immediately adjacent to the division site from the previous cell cycle. The new bud's position is determined by a protein complex called the axial landmark, which assembles at the mother–bud neck of yeast cells during mitosis. An axial landmark protein called Bud3 is required for bud site selection, but whether this function involves the protein's Dbl homology (DH) domain, a module commonly found in nucleotide exchange factors that activate Rho family GTPases, was unknown.

Kang et al. found that yeast expressing a Bud3 variant lacking part of its DH domain failed to assemble the axial landmark and formed buds in the wrong position. A mutant allele of the GTPase Cdc42 produced an identical phenotype, which could be rescued by Bud3 overexpression. Bud3 might therefore control bud site selection by activating Cdc42. However, the only known Cdc42 exchange factor is Cdc24, which stimulates the GTPase downstream of the axial landmark to promote cell polarization and bud formation.

Bud3 stimulated nucleotide release from Cdc42 in vitro and activated Cdc42 in Cdc24-deficient cells. Live imaging of wild-type cells revealed that Cdc42 normally undergoes two waves of activation: a Bud3-dependent wave in late mitosis/early G1 followed by a Cdc24-dependent activation phase in late G1. Senior author Hay-Oak Park thinks that this biphasic activation is necessary for spatial cue–directed polarity establishment in haploid budding yeast.

Kang, P.J., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201402040.

The fluid dynamics of collective cell migration



Control neural crest cells flow into a 25-µm-wide tunnel (left), whereas cells lacking LPAR2 congeal at the tunnel entrance (right).

Kuriyama et al. describe how the phospholipid lysophosphatidic acid (LPA) stimulates N-cadherin endocytosis, allowing groups of neural crest cells to collectively migrate through *Xenopus* embryos.

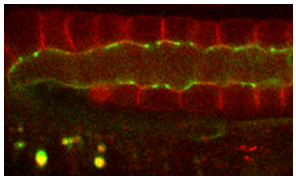
During development and tumor metastasis, cells prepare to migrate by undergoing an epithelial-to-mesenchymal transition that loosens their connections to neighboring cells. Subsequently, however, many cell types, including neural crest cells, migrate en masse to their final destinations, indicating that their intercellular adhesions aren't disrupted completely. Indeed, totally abolishing cell–cell contacts inhibits neural crest migration. Kuriyama et al. investigated the role of LPA and its receptor, LPAR2, in regulating neural crest motility.

Neural crest cells lacking LPAR2 failed to migrate out of the neural plate in *Xenopus* embryos. But LPAR2 knockdown had no effect on neural crest cell chemotaxis in vitro, unless the cells had to migrate through a narrow tunnel to reach the chemoattractant, recreating the physical constraints the cells experience in vivo. Under these conditions, groups of LPAR2-deficient cells failed to enter the tunnels, whereas wild-type cells rearranged their intercellular contacts so that they could flow through the tunnel like water in a pipe.

Kuriyama et al. found that endocytosis of the adhesion receptor N-cadherin was inhibited in LPAR2-deficient neural crest cells, raising the protein's surface levels and increasing intercellular adhesion. Partially inhibiting N-cadherin, or enhancing the protein's endocytosis, restored the fluid-like behavior of LPAR2-depleted cells and rescued their ability to migrate through in vitro tunnels and embryonic tissues. Senior author Roberto Mayor now wants to investigate whether groups of metastasizing tumor cells show similar fluidity as they invade surrounding tissues.

Kuriyama , S., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201402093.

A short Anillin opens the way for germline development



ANI-2 (green) is enriched at the intercellular bridges connecting germ cells (membranes labeled red) to the central rachis.

Amini et al. describe how the Anillin protein ANI-2 promotes the opening of intercellular bridges that allow *C. elegans* germ cells to resist mechanical stress during oogenesis.

The germ cells of worm gonads form a syncytium in which each cell is connected to the others via small intercellular bridges that feed into a central cytoplasmic core called the rachis. Two Anillin proteins localize to the rachis and intercellular bridges. ANI-1 is an actomyosin scaffold protein that promotes cytokinesis. ANI-2, on the other hand, lacks the actin- and myosin-binding domains found in ANI-1, yet its depletion disrupts gonad organization and germline development.

Amini et al. found that germ cells became progressively more interconnected during the development of wild-type gonads, but the intercellular bridges were largely absent in worms lacking ANI-2. In contrast, depleting ANI-1 increased the number and width of intercellular bridges and rescued bridge stability in *ani-2* mutant gonads, suggesting that the two Anillin proteins counteract each other to regulate the gonad's syncytial architecture.

Late in larval development, germ cells lacking ANI-2 became multinucleate as the membranes separating individual nuclei collapsed. This multinucleation coincided with the onset of cytoplasmic streaming, in which maturing oocytes at one end of the gonad grow by sucking in cytoplasm from the rest of the organ. Blocking cytoplasmic streaming reduced the multinucleation of *ani-2*–deficient gonads, suggesting that intercellular bridges help germ cells resist the mechanical stresses associated with this process. Author Nicolas Chartier now wants to see if shorter Anillin isoforms have a similar function in higher organisms, including humans, whose germ cells are also connected via stable intercellular bridges.

Amini, R., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201310117.