In Brief

Rethinking Septin Function

Polymerization Is Dispensable

Septin deficiencies in budding yeast cause cytokinesis defects and the loss of filaments at the mother–bud neck. And mammalian septins are found at cleavage furrows. But three papers in this issue suggest that the septins may be more important as a docking site for proteins than as a cytoskeletal mediator of cytokinesis.

Frazier et al. (page 737) use antibodies to yeast Cdc3p to isolate a complex of four septins: Cdc3p, Cdc10p, Cdc11p, and Cdc12p. The yeast septins extend to form filaments of over 1,500 nm in length when dialyzed into physiological salt.

Yeast neck filaments were originally thought to be oriented around the bud neck. But the repeat length for the polymer in vitro is 32 nm—the same size as the gap between neck filaments. Thus the electron microscopy may be detecting a repeating structure in cells (perhaps a cross-linker) and not the filaments themselves. This led senior author Christine Field to propose in an earlier paper that the filaments run from mother to daughter cell, making it harder to envision a constricting role for the filaments.

Frazier et al. find that deletion of *CDC10* all but abolishes neck filaments (visible in 1/150 cells), but that it leaves cytokinesis and axial budding largely intact. Cdc3p and Bud4p still localize to the neck. The localization of Bud4p to the neck, and thus axial budding, is known to require septin function.

"It's looking like you can get some amount of septin function even if you don't form a filament," says Field. Polymerization could be useful for concentrating the septins in one place, and yeast cells may even use the polymer's orientation to differentiate mother from daughter in placing proteins on one side of the bud neck. But the *CDC10* results mean that septin's claim to be part of the cytoskeleton is extremely shaky.

Septins Are Needed for Depolarization

Yeast septin mutants were first defined in terms of cytokinesis defects, but they also have highly elongated buds. This looks suspiciously like the phenotype of *gin4* mutants in certain genetic backgrounds. When the cell is dependent on the cyclin Clb2p, Gin4p kinase directs the switch from polar growth (actin directed to the bud tip) to isotropic growth (actin directed all over the daughter cell). Without kinase activation, the cell cycle is delayed in early mitosis.

In a screen for mutants with the *gin4* phenotype, Carroll et al. turn up an allele of the *CDC11* septin (page 709). A Gin4p affinity column binds all four septins mentioned above, plus a new septin named Sep7.

Septins appear to be part of the Gin4p pathway: Cdc11p and Sep7p are necessary for activation of Gin4p kinase activity, and *cdc11* mutants mimic the delay in early mitosis seen in *gin4* mutants. Eventually the delay is overcome and the cytokinesis phenotype becomes evident.

Longtine et al. come across Gin4p in a synthetic lethal screen with temperature-sensitive *cdc12* (page 719). Gin4p and Cdc12p colocalize at the bud neck, Gin4p binds to Cdc3p in a two-hybrid assay, and Gin4p localization is dependent on septin function. In a *gin4* deletion strain the septins are still at the neck, but clumped into bars running through the neck. This is consistent with the orientation of the filaments proposed by Field and colleagues.

Rather than being a filament that pinches off a new cell, the septins (and Gin4p) may redirect actin from the bud tip to the rest of the daughter cell and, presumably, the neck region. A more direct interaction between septins and actin also remains likely, as septins are necessary for both establishment and maintenance of a contractile actomyosin ring in budding yeast (Bi, E., P. Maddox, D.J. Lew, E.D. Salmon, J.N. McMillan, E. Yeh, and J.R. Pringle. 1998. *J. Cell Biol.* 142:1301–1312), and septins are associated with actin-containing structures in interphase cells.

Making Space in the Extracellular Matrix

As cells cluster to form the islets of Langerhans in the developing pancreas, they need to make room for themselves in the extracellular matrix (ECM). It therefore makes sense that matrix metalloproteinase 2 (MMP-2) is necessary for islet morphogenesis, as reported by Miralles et al. on page 827.

MMP-2 is present at all stages of pancreatic development, but the activated form is present only from embryonic day 17–19 in rats—the 2-d period when the islets are forming. This holds true in the in vitro model used by Miralles et al., in which translucent structures emerge from pancreatic epithelium grown in a collagen gel.

An inhibitor of metalloproteases, BB-3103, prevents islet formation in vitro; other protease inhibitors have no effect. Adding TGF-β1 to the system accelerates MMP-2 activation and islet formation. TGF-β antibodies prevent MMP-2 activation and islet formation, while leaving cellular differentiation intact.

The next step will be to determine the link between TGF-β and MMP-2. As in heart valve morphogenesis or osteoclast migration, this may involve increased mobilization of the MMP-2 activating protein to the cell surface.

CFTR's Chloride Sensor, Not Conductance, Triggers ATP Release

The discovery that the cystic fibrosis transmembrane conductance regulator (CFTR) was a chloride channel was not, as it first appeared, the simple and complete solution to the mechanism of disease. Aberrant ion levels in airway fluid probably aid the bacterial colonization seen in cystic fibrosis. But as evidence for CFTR regulation of

other channels mounts, the relative importance of chloride conduction through CFTR has become increasingly unclear.

Another potential mechanism involves ATP release, which could occur either through CFTR or a channel regulated by CFTR. This field has been controversial, not least because of stretch-activated ATP channels on many cell types. "If you poke a cell it secretes ATP," explains John Engelhardt, senior author of a report starting on page 645. "It's very easy to see that and dismiss the whole phenomenon of CFTR-associated ATP release. Therefore we reconstituted the system in *Xenopus* oocytes to control for these non–CFTR-related pathways of ATP secretion."

Jiang et al. observe ATP release that is dependent on injection of CFTR cRNA, activation of CFTR by protein kinase A, and an increase of extracellular chloride levels from ~0 to >100 mM. Oocytes bathed in high chloride from the start do not release ATP. "To be physiologically significant in the airway, these changes in extracellular chloride would probably have to be on the order of 10 mM," says Engelhardt. Other ions or the altered ionic microenvironments around gland ducts may control the reactivity of the ATP release system in vivo.

Chloride conductance is not the key event for eliciting ATP release; chloride release by other channels, or even by a mutant CFTR, does not lead to ATP release. ATP is released, however, by activation of a CFTR mutant that can apparently sense but not conduct chloride.

Jiang et al. suggest that a chloride sensor in CFTR triggers a conformational change in the activated protein, and that this drives ATP release. ATP could bind to purinogenic receptors, which could open or close the chloride or sodium channels that are known to be regulated by CFTR.

Immunosuppression by Disrupting Rafts

The polyunsaturated fatty acids (PUFAs) found in sunflower and fish oils may suppress the immune system by displacing Src-like kinases from signaling rafts, according to Stulnig et al. (page 637).

Some costimulatory molecules necessary for T cell activation, such as CD59, are anchored to the membrane via a glycosyl phosphatidylinositol (GPI) anchor. With only a lipid tail in the outer leaflet of the membrane, how can these proteins transduce signals into the cell? One clue is that the proteins colocalize with Src family kinases such as Lck, which also have lipid tails. Both CD59 and Lck are found in detergent-resistant membrane domains (DRMs), or rafts.

Rafts are detergent-insoluble thanks to the sphingolipids and cholesterol in their outer leaflet. The saturated chains of the raft lipids seem to promote an ordered arrangement by self-association of lipid tails.

Stulnig et al. find that PUFAs inhibit calcium transients and tyrosine phosphorylation in response to CD59 stimulation. The same is true for signaling through CD3, a transmembrane protein that associates with the T cell receptor.

CD59 remains in rafts after PUFA treatment, but Lck becomes diffuse and can no longer be sedimented in rafts. The angled conformation of unsaturated fatty acid chains may selectively disrupt the inner leaflet of rafts. Alternatively, PUFAs may be added to Lck in place of palmitoyl groups, creating a molecule that does not associate preferentially with rafts.

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