Published Online: 20 January, 2004 | Supp Info: http://doi.org/10.1083/jcb1642iti3

In This Issue

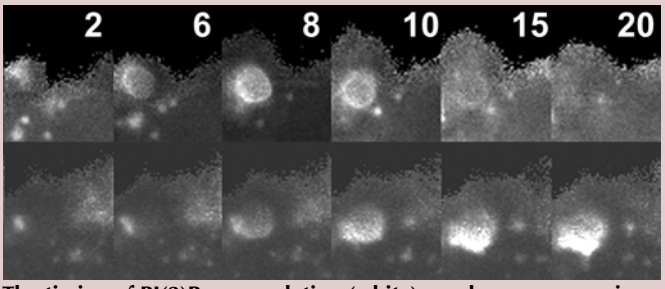
More than one way to skin a phagosome

Phagosome studies have rested on a major assumption: that in a given cell, all phagosomes with the same cargo mature alike. On page 185, Henry et al. test this assumption, and find it wanting.

Using ratiometric fluorescence microscopy, the authors followed the maturation of individual macrophage phagosomes containing IgG-opsonized erythrocytes. Most of the results were uniform and unsurprising. Actin is present briefly during phagosome formation, and then all of the phagosomes sequentially associate with the small GTPases Rab5a and Rab7. The lysosomal membrane protein LAMP-1 associates with the phagosomes after Rab7, consistent with lysosomal fusion.

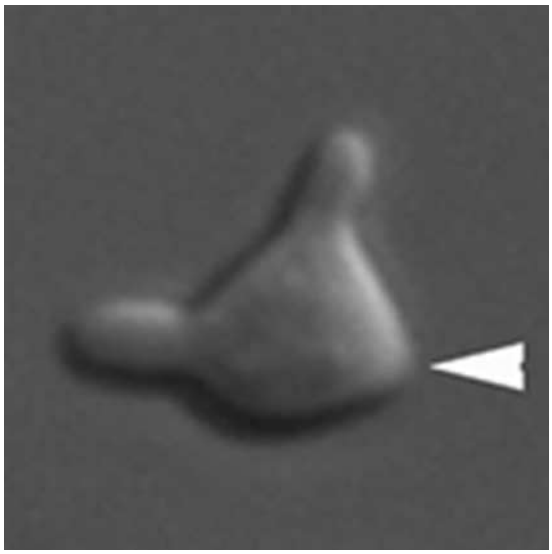
A marker for phosphatidylinositol 3-phosphate (PI[3]P), however, reveals two distinct populations of erythrocyte-carrying phagosomes. In one population, PI(3)P levels spike rapidly after phagosome formation, and then fall to undetectable levels within 20 min. In the other population, PI(3)P rises slowly and persists for several hours.

The differences may reflect stochastic variations in phagosome content that are amplified by kinase/phosphatase systems. The functional significance of the two states remains unclear, although different levels of PI(3)P may be used to recruit different levels of NADPH oxidase.



The timing of PI(3)P accumulation (white) on phagosomes varies.

If organelle maturation were driven solely by localized receptor action, then the levels of PI(3)P on a given phagosome should represent an average of all of the receptor signaling on the phagosome membrane. The complete conversion of each phagosome to one of two extreme states shows, however, that membrane chemistry is integrated over the entire organelle. This contrasts with the initial formation of the phagosome, which is driven by the zipper-like local activation of individual receptors along a pseudopod. The authors are now using FRET-based methods to examine the surface chemistries of phagosomes in more detail and hope to identify appropriate markers for the signals that integrate PI(3)P levels.


Cdc42 and the polarisome control the timing of multiple outgrowths in yeast.

How to terminate a shmoo

Many types of cells grow polarized structures of defined sizes, but virtually nothing is known about how a cell starts and stops this process. On page 207, Bidlingmaier and Snyder provide the first report of regulatory proteins involved in this timing control, and find that initiation and termination of polarized growth are controlled by distinct but overlapping sets of proteins.

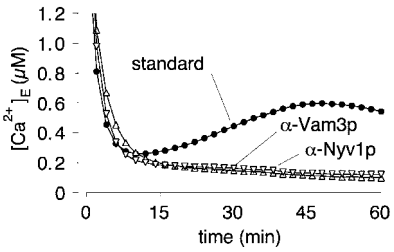
The authors investigated the growth of mating projections in *Saccharomyces cerevisiae*. This yeast responds to gradients of pheromone by assuming a shmoo-like shape pointing in the direction of the signal. Shmoos still form in the presence of uniformly high concentrations of pheromone (as may result when cells are closely packed together), but they poke out sequentially in random directions to sample the environment. Bidlingmaier and Snyder found that the time between the initiation of each of these new mating projections varied depending on the relative activity of both Cdc42 and its effectors in the multiprotein, actin-polymerizing polarisome complex. Timing of growth termination was independent of the upstream Cdc42 regulators, but dependent on polarisome components and two proteins implicated in the downstream process of cell fusion.

Pheromone signaling is thought to cause Cdc42 activation that is initially spread over the membrane and gradually localized via a positive feedback loop involving Cdc42 transport. The resultant actin polarization process may deplete a rate-limiting initiation factor such as Cdc42 or pheromone receptor from the membrane, but this factor gradually builds up again to initiate polarization at a new site. Termination of outgrowth at one site is not requisite for reinitiation at a new site, but may supply some factors that increase the rate of reinitiation. ■

TEXT BY ALAN W. DOVE
ALANWDOVE@EARTHLINK.NET

SNAREs drum up calcium

On page 195, Merz and Wickner show that when two intracellular vesicles fuse, the interaction of their SNARE proteins triggers a short-lived calcium release. The work provides the first clear connection between calcium release and the fusion cycle, and supports a model in which the pairing of SNAREs in trans coordinates the downstream signals that lead to vesicle fusion.



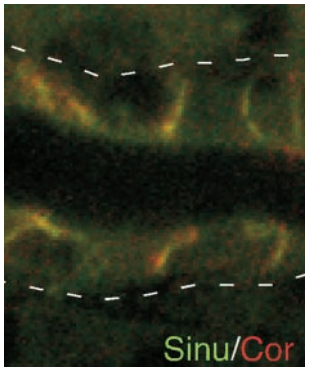
SNARE interactions release calcium for fusion.

Previous work had hinted at a requirement for calcium signaling during vesicle fusion, but it was unclear what triggered the calcium release. In the new work, the authors isolated vacuoles from mutant yeast strains and analyzed their fusion in vitro. For calcium release to occur in this system, four different vacuolar SNARE proteins from two different membranes must interact after the priming step of the fusion cycle. Staging experiments suggest that each of these trans-SNARE interaction events triggers a short-lived calcium release.

Merz and Wickner propose that, after priming, the formation of SNARE complexes between apposed membranes during the docking step could serve as a checkpoint in the fusion cycle. Once the trans-SNARE complexes form, the resulting calcium release signifies that the priming step is over, inducing downstream calcium-dependent signals that ultimately bring about fusion. ■

Conjunction of junctions goes beyond function

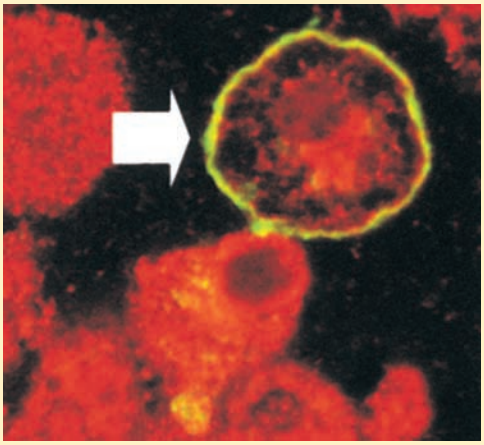
The growth of a network of branching tubes during organogenesis and the formation of a watertight epithelial barrier may seem like very different processes, but on page 313, Wu et al. uncover a surprising connection between the two. The work also reveals a common molecular basis for the formation of invertebrate septate junctions and vertebrate tight junctions.



Sinuous (green) localizes to septate junctions.

In the new work, the authors cloned the *sinuous* gene of *Drosophila*, which was previously found in a screen for mutations that affected tracheal tube formation. The product of *sinuous* shares homology with claudins, the family of proteins responsible for forming the seals of vertebrate tight junctions. *Sinuous* localizes to the fly septate junction, and is essential for the formation of normal barriers.

The molecular and functional similarities between tight junctions and septate junctions—structures that were previously considered analogous rather than homologous—suggest that different types of barrier junctions arose from a single, claudin-containing ancestral structure. The nonbarrier functions of junction components could have driven their divergence. For example, the activity of *sinuous* in tracheal development seems to be distinct from its function in septate junction barrrier formation. The authors are now trying to determine how this novel claudin works in both processes. ■



Active TC10 (green) drags PKCζ/λ (red) to the membrane.

How insulin signals globally and acts locally

Insulin stimulation in adipocytes sends signals through two pathways, one dependent on phosphatidylinositol-3-kinase (PI3K) and the other acting through the small membrane-associated GTPase TC10. On page 279, Kanzaki et al. reveal an unexpected convergence of the two pathways, and show that the localization of atypical protein kinase C (PKCζ/λ) to specific lipid raft microdomains confers specificity on the system.

Previous work had suggested that PI3K could activate PKCζ/λ or protein kinase B, but the relative importance of these downstream effectors was controversial. In the new study, the authors found that both the PI3K and TC10 pathways can activate PKCζ/λ in adipocytes, but only the TC10 pathway recruits PKCζ/λ to TC10-containing lipid raft microdomains. The localization is directed by Par6 and Par3, proteins that are known to link GTPases to PKCs in the worm *C. elegans*.

PI3K activation is a common result of many signaling pathways and thus has many readouts, some of them inapropriate for insulin signaling. Kanzaki et al. believe that insulin stimulates PI3K to levels that are by themselves insufficient to generate all downstream events, either desirable or undesirable. But the convergence of the TC10 stimulation on raft-localized PKCζ/λ pushes the stimulation to a level sufficient to turn on only the few desirable downstream events, such as the recruitment of glucose transporters, that function in raft microdomains. ■