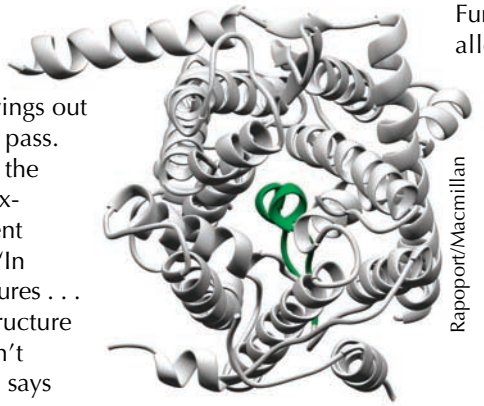
Protein translocation pulls the plug

Membrane-bound compartments present an immediate problem: proteins need to get into and across those membranes. Now, we have the first picture of how that process works, thanks to the structure determined by Bert van den Berg, William Clemons, Jr., Stephen Harrison, Tom Rapoport (Harvard Medical School, Boston, MA), and colleagues. They find that a single SecY complex from *Methanococcus jannaschii* forms an hourglass-shaped pore with a basal plug that probably swings out of the way to let nascent protein chains pass.

The complex was not an easy target: the structure came only after five years of experiments with proteins from ten different organisms. The effort was worthwhile. "In contrast to ion channels and other structures . . . where people had models before the structure was solved, in this case we really didn't have any ideas of how this would work," says Rapoport. "We were shocked because we didn't expect the pore to be in one complex."



Rapoport/Macmillan

A plug (green) stoppers the protein-conducting channel (seen from top).

Earlier EM experiments had suggested that a large pore formed in a gap between four associated complexes. But more recent EM data are consistent with this supposed pore being only an indentation, and the new structure clearly suggests a path for nascent proteins through a single complex.

Translocation begins, according to cross-linking data, when the signal sequence of the nascent protein inserts between transmembrane domains (TM) 2b and 7 of the main, channel-forming α-subunit. This probably has two effects. First, a plug formed by TM2a swings ~22 Å out of the way, revealing a narrow, central constriction delimited by a ring of six hydrophobic residues. Second, the two pseudosymmetric halves of the channel are pried open a little to widen the pore ring. Further separation of the two halves should allow release of membrane-spanning domains of the nascent protein.

The plug and pore ring are Rapoport's candidates for forming the tight seal that prevents passage of molecules other than the translocating protein. This sealing function had previously been ascribed to either the ribosome in the cytoplasm or (in eukaryotes) BiP in the lumen. Now, Rapoport needs to check that his presumed pore region is where the polypeptide really goes, and that the channel works in single copy and using the proposed plug movement. In the longer term he hopes to see the channel in action with a ribosome or even a translocating polypeptide; but for now, he says, seeing the current structure "has been a dream for me." ■

Reference: van den Berg, B., et al. 2003. *Nature.* 10.1038/nature02218.

A circular uncoating mechanism

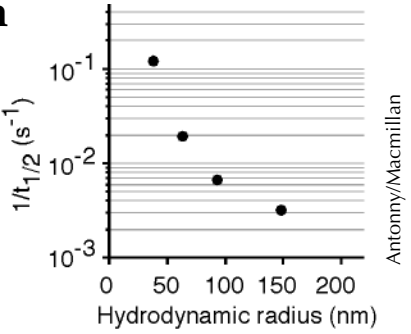
In retrospect it seems obvious. If you need to uncoat a vesicle only after the vesicle has formed, use the spherical shape of the vesicle as a trigger for uncoating. Now, Joëlle Bigay, Bruno Antonny (Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne-Sophia-Antipolis, France), and colleagues have evidence for this mechanism.

Morphology was not the first suspect as a trigger for uncoating Golgi transport vesicles. Lipid metabolism was a possibility, and Antonny and others experimented in vitro with the effect of different lipid mixtures on uncoating. But in vivo evidence for changes in lipid composition during vesicle budding was lacking.

The different in vitro lipid compositions did result in different uncoating dynamics, however. Antonny suspected that some of the in vitro mixtures were mimicking a distorted lipid arrangement seen during membrane curvature. Sure enough, vesicles with constant lipid composition but decreased diameter and thus increased curvature showed two changes: more active ArfGAP1, and faster dispersal of the COP1 coat. ArfGAP1 helps Arf1 to hydrolyze its bound GTP, thus releasing COP1 components that were originally recruited by Arf1-GTP.

That process of COP1 assembly begins before the budding of a vesicle. "If the membrane is flat, the coat must stay assembled because the job is not done," says Antonny. But then COP1 polymerization helps bend the membrane, thus increasing the spacing between lipids in the outer membrane. It may be this spacing that ArfGAP1 detects.

Arf1-GDP on the positively curved membrane will rapidly disperse, but the negative curvature at the edge of a vesicle bud should ensure that a ring of Arf1-GTP will remain as a protective rim to maintain the coat until the vesicle is fully formed. This concept of Arf1GAP1 action "remains a model, but I think it can explain many things," says Antonny. "The key point now is to find the region in ArfGAP that is sensitive to curvature." ■



Antonny/Macmillan

High curvature on smaller vesicles speeds hydrolysis of Arf1-GTP.

Reference: Bigay, J., et al. 2003. *Nature.* 426:563–566.

Text By William A. Wells
wellsw@rockefeller.edu

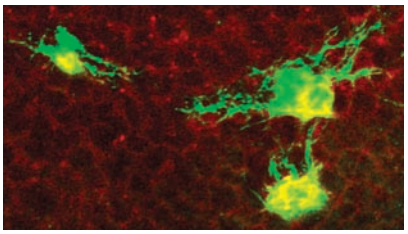
Giving neural precursors the finger

Epithelial cells communicate amongst themselves using finger-like projections, according to Cyrille de Joussineau, Daniel Alexandre, and colleagues (Université Montpellier II, Montpellier, France). The filopodia help individual fly neural precursors to create an island of nonneural cells around themselves, thus allowing the formation of discrete structures such as bristles at regular intervals.

The filopodia only become visible when single cells are labeled. Their inhibitory action is mediated by Delta attached to the filopodia, which contacts membrane-bound Notch on the surrounding, inhibited cells. The extent of visible filopodia roughly matched the range of inhibition. And overexpression of Delta increased the range of the filopodia, so Delta actually promotes formation of its own means of transport.

Inhibition of filopodial outgrowth did not prevent local Delta–Notch signaling, but did shut down longer range inhibition. The result was an increased density of neural cells and more of the associated structures such as microchaetes. Thus, epithelial cells can communicate at long distances without resorting to either diffusible mediators or cell relay mechanisms. ■

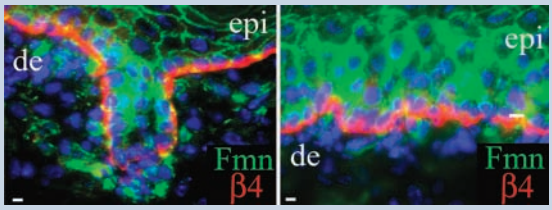
Reference: de Joussineau, C., et al. 2003. *Nature.* 426:555–559.



Alexandre/Macmillan

Filopodia allow epithelia to communicate over long distances.

Formin' adherens junctions



Fuchs/Macmillan

Formin-1 (green) localization at cell junctions (left) is lost in an α-catenin knockout (right).

Epithelial cells zipper together thanks to linear actin cables—cables that assemble at newly formed adherens junctions to stabilize them, thereby counteracting retractive forces at sites of cell–cell contact. Now, Agnieszka Kobielak, Amalia Pasolli, and Elaine Fuchs (Rockefeller University, New York, NY) have found that formin-1 drives the actin polymerization that creates the cables.

Formin-1 entered the story as a binding partner of α-catenin, a component of cadherin adhesion complexes. Cells lacking α-catenin fail to form actin cables at adherens junctions, and the Rockefeller group found that the same was true when the formin-1–α-catenin interaction was disrupted in vivo. In vitro, formin-1 was shown to polymerize actin into linear filaments. Finally, fusion of a β-catenin-binding domain to the actin-polymerization domains of formin-1 restored adhesion ability to cells lacking α-catenin.

Actin polymerization is important in two steps of adhesion. First, branched polymerization of actin by Arp2/3 pushes out both filopodia and broad areas of membrane as lamellipodia. Many of the resultant contacts are not productive, and the processes retract. But any surviving contact prompts the formation of an actin cable, which stabilizes the contact. It also pushes on a specific area of membrane so that more adherens junctions form nearby, thus zippering cells together. Just how formin-1 is regulated during this process remains to be determined. ■

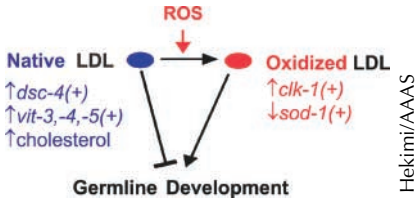
Reference: Kobielak, A., et al., 2003. *Nat. Cell Bio.* 10.1038/ncb1075.

Oxidation makes a germline

Food and sex go hand in hand, according to Yukimasa Shibata, Siegfried Hekimi, and colleagues (McGill University, Montreal, Canada). They find that worms that have less oxidation of certain lipoprotein particles—possibly an indicator of a slowed metabolism—have slowed development of their germline. Only when food is abundant and metabolism active would the germline get the stimulus to develop to maturity.

The proteins in question are vitellogenins: analogues of vertebrate apoB, a component of low-density lipoprotein (LDL). In the *clk-1* worm mutant, an increase in the levels of an antioxidant results in less oxidation of lipoprotein. The result is a slowing in germline development.

The slowed development is reversed by blocking the production of lipoproteins, or by reestablishing a more normal level of oxidation by reactive oxygen species (ROS). "The degree of oxidation is a measure of general metabolism," says Hekimi. "The germline may want to know that the worm is running fast. It could be sensing the general quality of metabolism." ROS effects on signaling have been seen before in vitro, but the new results are the most dramatic to be detected in vivo.



Hekimi/AAAS

Oxidized LDL may signal that metabolism is active enough to support germline development.

The effects of lipoproteins on germline development go through a receptor-associated kinase called ARK-1. Although ARK-1 is known to work downstream of an EGF-like receptor, it is not clear whether this or another type of receptor is a mediator for the lipoprotein signal, or which lipoprotein species (oxidized or nonoxidized) is doing the signaling. ■

Reference: Shibata, Y., et al. 2003. *Science.* 302:1779–1782.