In This Issue

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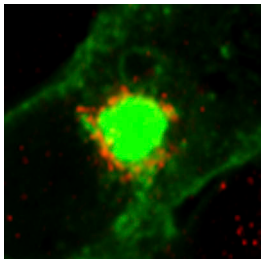
Ras makes a pit stop



Ras proteins (green) huddle inside the ring-shaped Golgi apparatus (red).

Ras proteins are restless, continually flitting from the cell membrane to the Golgi apparatus and back again. Misaki et al. reveal that the proteins enter recycling endosomes during the journey to the plasma membrane.

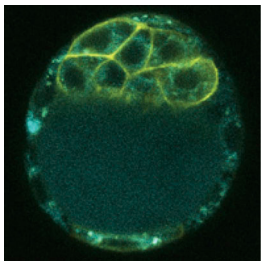
One mystery is why Ras proteins—which spur cell growth, differentiation, and survival—move so often. The cluttered cell interior has also made it difficult to discern how the proteins travel. Proteins heading for the Golgi might zip through the cytosol or hitchhike in endosomes. Some evidence suggests that they pass through recycling endosomes, whereas other studies indicate they shun the endocytic pathway altogether.

Misaki et al. used COS-1 cells in which recycling endosomes are easier to observe because they gather in the so-called Golgi ring near the organelle, separate from early and late endosomes. The researchers found that Ras proteins do spend time in recycling endosomes, but only on the outbound leg from the Golgi to the cell membrane. Addition of two palmitoyl groups directs Ras to recycling endosomes, the team discovered.

The researchers think that an unidentified vesicle ferries the proteins from the Golgi to the recycling endosomes. Whether recycling endosomes deliver Ras proteins to the cell membrane or hand off their cargo to other carriers is unclear. Receptors such as the epidermal growth factor receptor also slip into recycling endosomes and might activate Ras proteins there.

Misaki, R., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200911143.

CLIP catches enzymes in the act



Yellow and blue have separated in this mouse blastocyst, indicating that two PCs are active.

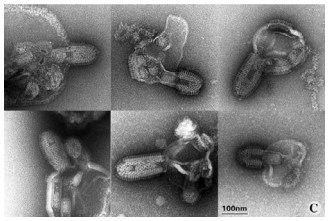
Proprotein convertases (PCs) are big shots in the body because they snip and turn on numerous hormones, receptors, adhesion molecules, and other crucial proteins. Mesnard and Constam describe a new technique to track the activity of some of these ubiquitous but hard-to-study enzymes.

The targets of the nine PCs range from insulin to the blood pressure regulator renin to several proteins implicated in Alzheimer's disease. Cancer cells and pathogens such as HIV often co-opt the enzymes for nefarious ends. For example, PCs turn on matrix metalloproteinases that clear away extracellular matrix and allow cancer cells to spread. But the enzymes' widespread distribution and overlapping functions have made it difficult to tease out what jobs individual enzymes perform.

To simplify the task, Mesnard and Constam devised a method to determine when and where PCs are working. They fused yellow and blue fluorescent proteins to create a biosensor they call CLIP. When PCs are absent, the two colors remain together. But active PCs cut CLIP and separate the colors. Researchers can thus track PC activity inside a cell and at its surface, or even in whole tissues. Mesnard and Constam used the approach to find out when two PCs, Pace4 and Furin, switch on in early mouse embryos. The enzymes were on the job before the blastocyst implanted, earlier than expected. The researchers say that CLIP could improve drug design, allowing scientists to pin down where certain PCs are functioning in diseases and monitor the effectiveness of inhibitors dispatched to those sites.

Mesnard, D., and D.B. Constam. 2010. *J. Cell Biol.* doi:10.1083/jcb.201005026.

How a virus enters without breaking



VSV particles in the process of fusing with liposomes.

Viral glycoproteins are the flexible keys to the cell. By changing shape, they open the cell so that the virus can enter. Libersou et al. show how one glycoprotein helps the vesicular stomatitis virus (VSV) gain access to mammalian cells.

Glycoprotein contortions reshape the viral and cellular membranes, allowing them to fuse. VSV carries a surface glycoprotein called G. Previous work indicated that G has at least three configurations—a pre-fusion state, an intermediate form that interacts with the target cell membrane, and a post-fusion conformation. Using electron microscopy and tomography, Libersou et al. tracked G to determine how its alterations spur fusion of VSV particles.

Instead of going in tip first, the virus, which is shaped like a bullet, backs in with its flat base. Low pH triggers the viruses to fuse and trips G molecules into the post-fusion arrangement. However, fusion requires more than gymnastics by G. The researchers found that if they reduced the pH just enough so that the glycoproteins distorted into the post-fusion shape, the viral particles remained locked out.

G undergoes another transformation—glycoproteins not located on the viral base interconnect to form helical arrays. The arrays can also reshape membranes, the researchers found. Libersou et al. conclude that fusion requires two rearrangements of G. First, glycoproteins on the viral base remodel and establish a connection with the cell membrane that initiates fusion. Then G molecules on the sides of the virus connect into helices that can deform the viral membrane to fully achieve fusion.

Libersou, S., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201006116.