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Sorting out endosome form and function

In the early 2000s, researchers redefined the organization and function of the endosomal system.

The system of tubules and vesicles that sorts and recycles molecules internalized from the plasma membrane is extremely complex. Newly endocytosed proteins are first delivered to early endosomes, from where they can be quickly returned to the cell surface, or directed to late endosomes and lysosomes for degradation. Alternatively, endocytic cargoes can take a slower route, passing from early endosomes into recycling endosomes where they are sorted for delivery to specific regions of the plasma membrane or the trans-Golgi network (TGN).

By the late 1990s, some of the molecular machinery controlling these transport pathways had been identified, but, given the constant exchange of membrane between endocytic organelles, researchers expected these proteins to be dispersed throughout the endosomal system, rather than confined to specific compartments.

Thus, it came as a surprise to Birte Sönnichsen, Marino Zerial, and colleagues at the European Molecular Biology Laboratory when they saw almost no overlap in the distribution of several Rab GTPases involved in endocytic recycling (1). "I'll never forget coming from the confocal microscope and telling [Coordinator of the Cell Biology Program] Kai Simons that I didn't see anything colocalizing," says Zerial, now at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. "Kai replied 'Are you crazy? That's too complicated!'"

Although they didn't colocalize, Rab4, -5, and -11 often labeled tubulovesicular structures in close proximity to each other, suggesting that the GTPases might occupy distinct subdomains on the same endosomal membrane. Sönnichsen et al. confirmed this by immunoelectron microscopy and found that the different domains didn't intermingle as recycling cargo made its way through the endosomal system. Instead, internalized transferrin molecules initially entered Rab5-positive vesicles and were then either quickly recycled through endosomes containing Rab4 and Rab5 subdomains, or passed on to slower recycling endosomes comprised of Rab4 and Rab11 compartments.

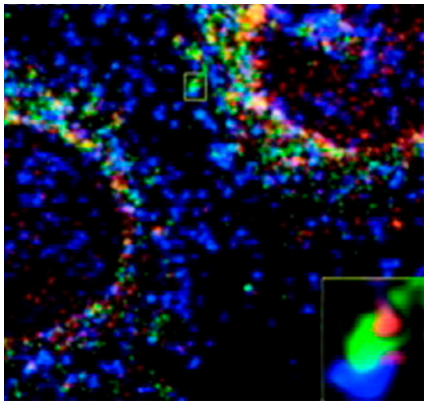
Thus, individual endosomes carried multiple Rabs that defined distinct membrane subdomains, which, by virtue of the effector proteins recruited by these GTPases, could have unique functions in endocytic trafficking. The researchers postulated that effector proteins capable of binding multiple Rabs could help neighboring subdomains communicate and coordinate their activities (2). Moreover, Zerial and colleagues later demonstrated that one Rab subdomain can convert into another over time, exemplified by the ability of Rab5 to initiate the recruitment of Rab7 as early endosomes mature into late endosomes (3). "We think that these subdomains are dynamic and are created by an upstream Rab domain to facilitate cargo transport," Zerial says.

> "The first place VSV-G went after leaving the TGN was recycling endosomes."

"Our paper made it clear that you had to look at the spatial organization of the transport machinery," he continues. "It isn't sufficient to say that a protein localizes to endosomes, you have to know where it is on the membrane and what its dynamics are."

A few years after Sönnichsen et al.'s paper, Ira Mellman and colleagues demonstrated that recycling endosomes are also intermediates in the secretory pathway (4). Several studies had hinted that some newly synthesized proteins passed through an endosomal compartment on their way from the TGN to the plasma membrane, but the secretory and endocytic pathways were generally considered to be completely separate.

Mellman, who was then working at Yale University before his later move to Genentech, was particularly interested in how proteins were targeted to the apical or basolateral surface of polarized epithelial cells. Endocytosed proteins were sorted to their correct destination in recycling endosomes, using the same sequence motifs that determined the localization of newly synthesized proteins (5). Moreover, many of the factors involved in delivering secretory proteins to the basolateral membrane, such as the clathrin adaptor AP-1B, localized to recycling endosomes. "We wondered if there was a common sorting mechanism taking place at a common site," Mellman says.



Rab4 (red), Rab5 (green), and Rab11 (blue) localize to distinct subdomains of endosomal membranes.

Mellman and colleagues, led by graduate student Agnes Lee Ang, therefore followed the secretion of GFP-tagged VSV-G, a model basolateral membrane protein, in live MDCK cells. To maximize their chances of catching the protein's passage through recycling endosomes, the researchers used a series of temperature shifts to accumulate newly synthesized VSV-G in the TGN, before releasing the protein for delivery to the cell surface. "The first place VSV-G went after leaving the TGN was recycling endosomes labeled with the endocytic cargo transferrin," Mellman explains. Importantly, when Ang et al. ablated recycling endosomes, VSV-G failed to make it to the basolateral membrane, indicating that the protein has to pass through the endosomal compartment on its way to the cell surface.

Though recycling endosomes sort both secretory and endocytic cargo, precisely how this process occurs remains unclear. Zerial and Mellman agree that improved microscopy techniques, including quantitative and superresolution imaging, should help contemporary researchers elucidate the organization and function of endosomes still further.

1. Sönnichsen, B., et al. 2000. *J. Cell Biol.* 149:901–914.
2. de Renzis, S., et al. 2002. *Nat. Cell Biol.* 4:124–133.
3. Rink, J., et al. 2005. *Cell.* 122:735–749.
4. Ang, A.L., et al. 2004. *J. Cell Biol.* 167:531–543.
5. Sheff, D.R., et al. 1999. *J. Cell Biol.* 145:123–139.