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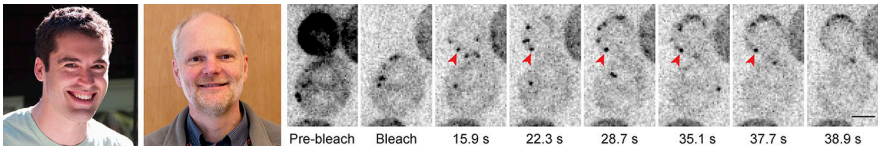
A timeline for exocytosis

Researchers track the tethering and fusion of individual secretory vesicles in budding yeast.

Focal Point



PHOTOS COURTESY OF THE AUTHORS

Kirk Donovan (left) and Tony Bretscher (right) track the behavior of individual secretory vesicles in budding yeast in order to provide a timeline of the events involved in exocytosis. As shown in this time-lapse series, vesicles labeled with the Rab GTPase Sec4p consistently tether to the bud cortex for 18 seconds (red arrowheads) before undergoing membrane fusion and disappearing (final frame). This tethering period, which may allow cells to ensure vesicles are targeted to the right location, is regulated by Sec4p and the myosin motor Myo2p. Myo2p dissociates from the vesicles about four seconds before fusion, in contrast to other components, such as the exocyst complex, that remain bound to vesicles until exocytosis is completed.

Exocytosis is essential not only for secreting proteins outside of the cell, but also for supporting cell growth (1). Secretory vesicles are delivered to the plasma membrane and tethered there for short periods before undergoing membrane fusion to release their contents. Researchers have identified and characterized many of the proteins involved in these processes, but precisely when, and in which order, they all act remains unclear. By tracking the tethering and fusion of individual secretory vesicles, Donovan and Bretscher sketch out a timeline for exocytosis in budding yeast (2).

Yeast secretory vesicles carry the Rab GTPase Sec4p, which, once activated by its guanine nucleotide exchange factor Sec2p, recruits a protein complex called the exocyst and binds and activates the myosin motor Myo2p, which transports the vesicle along actin cables into the bud. The exocyst then tethers the vesicle to the plasma membrane before Sec4p is inactivated and SNARE proteins and regulatory factors such as Sec1p stimulate membrane fusion.

Tony Bretscher, from Cornell University in Ithaca, New York, says that, despite their small size, yeast cells are an excellent system to track the progress of secretory vesicles. "They all go from the mother to the bud, so we know where they're going to end up and can therefore follow them," Bretscher explains. "That would be very difficult to do in most animal cells." Nevertheless, because yeast deliver so much secretory material into growing buds, tracking individual vesicles can be a challenge. To make things easier, postdoc Kirk Donovan labeled secretory vesicles with GFP-tagged Sec4p, and then photobleached vesicles already in the bud so that he could follow new vesicles as they arrived from the mother cell (2).

These vesicles moved around the bud rapidly before becoming immobilized at the cell cortex—a behavior Donovan and Bretscher ascribed to vesicle tethering. After a short time, the GFP-Sec4p signal would suddenly disappear, representing the vesicle's fusion with the plasma membrane. "What was really surprising was that the vesicles remained tethered for a well-defined time of 18 seconds," Bretscher says. "That strongly suggests that there's some sort of checking mechanism to make sure a vesicle is in the right place at the right time before it can undergo fusion with the plasma membrane."

> **"The vesicles remained tethered for a well-defined time of 18 seconds."**

This tethering period was extended—and became much more variable—in cells expressing a constitutively active version of Sec4p, indicating that the GTPase might regulate this checking mechanism. Furthermore, a mutant allele of *myo2* also prolonged vesicle tethering, indicating the motor contributes to a process that occurs during tethering.

Donovan and Bretscher previously found that Myo2p remains bound to secretory vesicles after they become tethered to the plasma membrane and only dissociates once Sec4p is inactivated (3). By simultaneously imaging Sec4p and Myo2p, the researchers found that Myo2p dissociated from vesicles about four seconds before fusion.

In contrast, the guanine nucleotide exchange factor Sec2p lingered on vesicles until the moment of fusion, perhaps suggesting that a portion of Sec4p molecules remain active during this final step of exocytosis. The exocyst, too, remained associated until the end. Given the complex's large dimensions, it may have to undergo some sort of rearrangement to allow the vesicle membrane to contact and fuse with the plasma membrane.

Donovan and Bretscher's findings provide an initial timeline for the events surrounding vesicle tethering and fusion. Vesicles attach to the plasma membrane for a defined period of time that appears to be regulated by Sec4p and Myo2p. Myo2p is then released and, after a short delay, this is followed by membrane fusion and the release of other vesicle components. A similar schedule of events is likely to occur in mammalian cells. The researchers now plan to fill in more of the timeline's details. "We want to follow the time courses of more components and see what happens when we make mutations or vary the concentrations of the different factors," Bretscher says.

1. Brennwald, P., and G. Rossi. 2007. *FEBS Lett.* 581:2119–2124.
2. Donovan, K.W., and A. Bretscher. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201501118
3. Donovan, K.W., and A. Bretscher. 2012. *Dev. Cell.* 23:769–781.