The small GTPase forms an organized scaffold that can regulate the pinching of vesicles from the ER.

Vesicle formation involves curving membranes into buds that constrict at the neck and eventually pinch off completely. Coat proteins and their regulators induce these membrane deformations. In the case of ER to Golgi transport, vesicle formation is initiated by the small GTPase Sar1 and the COPII coat proteins that it recruits to ER exit sites. Long et al. now describe how Sar1 organizes itself on membranes to control the final stages of COPII vesicle release (1).

Sar1 bends membranes by wedging its amphipathic N terminus into the lipid bilayer, selectively expanding the outer leaflet (2, 3). Increasing curvature eventually leads to membrane constriction. However, although Sar1’s N terminus is required to fully constrict and pinch off vesicles (2, 3), the GTPase is too dispersed within the COPII coat for the amphipathic wedge to drive vesicle release (4, 5). Meir Aridor from the University of Pittsburgh therefore wondered whether Sar1 acts independently of other COPII proteins at the vesicle neck.

To investigate this possibility, Aridor and colleagues used a simplified system consisting solely of large liposomes and purified Sar1. The GTPase uses its N terminus to convert spherical liposomes into tubes (2, 3). “But amphipathic wedges are actually very dangerous—they can destroy liposomes,” says Aridor. “So Sar1 carefully controls how it inserts its N terminus into the membrane.” Membrane insertion is partly controlled by Sar1’s GTPase cycle, but Long et al. found that the protein’s arrangement on liposome membranes also regulates the amphipathic domain and membrane deformation.

Sar1 converted liposomes into two different kinds of tubes. Some were rigid and nonconstricted, while others looked more like beads on a string—electron microscopy revealed this second kind of tube to be a series of small vesicles separated by tightly constricted necks. Aridor and colleagues found that the GTPase was highly ordered on rigid, nonconstricted tubes, but more disorganized on the vesiculated membranes. The researchers think that when the protein is organized into an ordered scaffold, the amphipathic wedges drive membrane tubulation but are spaced too far apart to pinch the tubes into vesicles; when Sar1 is disorganized, the wedges become dense enough in places to tightly constrict the membrane.

Similar shifts in Sar1 organization at the bud neck in vivo could drive COPII vesicle release, just as the dynamin GTPase self-assembles to pinch off endocytic vesicles (6). “Sar1 seems to behave like dynamin and other proteins that tubulate and then constrict cell membranes,” Aridor says.

Aridor and his team identified several factors that regulate how Sar1 assembles on lipid membranes. Applying tension to liposomes through membrane attachment increased Sar1’s arrangement into a regularly packed scaffold and therefore boosted the formation of nonconstricted tubules. “In a cell,” explains Aridor, “this tension could come from the attachment of ER exit sites to the cytoskeleton.”

A C-terminal segment of Sar1 called the Ω loop is also critical for the protein’s regular arrangement and control of membrane constriction. Mutations in the loop prevented Sar1 from converting liposomes into rigid, nonconstricted tubes. Expressing the same mutant in cells blocked the ER to Golgi transport of procollagen molecules, although smaller cargo was still transported normally, presumably with the help of endogenous wild-type Sar1. Ω loop mutants still recruit COPII coat proteins but they may constrict nascent vesicles too quickly for large procollagen molecules to be efficiently packaged.

Sar1 therefore drives the regulated constriction of a vesicle neck independent of other COPII components. “We think that when more GTP-bound Sar1 is loaded onto the membrane than is removed through coat-induced GTP hydrolysis, Sar1 organizes itself and creates the vesicle neck,” says Aridor. “The neck starts off as an unconstricted structure, but at some point Sar1 loses its packed organization, resulting in membrane constriction. GTP hydrolysis then induces vesicle release.” Aridor now wants to investigate how the Sar1 scaffold at the neck is controlled by COPII, which coats the vesicle and induces Sar1’s GTPase activity.

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