dolphins, stingrays, yellowtail kingfishes, snappers and mulloway [5]. The latter two are reputed to be active nocturnal predators, but no careful studies have been performed to confirm this. The second implication is that the cuttlefish itself, in order to drape its body in the right disguise, must be able to reliably see the details of the substrate at night. Certainly, the eyes of this cuttlefish are large and probably very sensitive, but again, experimental data are lacking. Nonetheless, despite our fragmentary knowledge of Sepia apama's visual ecology, this new discovery of nocturnal camouflage suggests that night vision is better than previously imagined, a conclusion that has recently come from many corners of the animal kingdom [15,16].

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Department of Cell and Organism Biology, Zoology Building, University of Lund, Helgonavägen 3, S-22362 Lund, Sweden.
E-mail: Eric.Warrant@cob.lu

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Vesicle Tethering: TRAPPing Transport Carriers

Transport vesicles must dock with the appropriate acceptor membrane to ensure faithful protein delivery. Recent work identifies some of the molecular mechanisms that contribute to the specificity of this reaction.

Elizabeth A. Miller

Protein secretion from eukaryotic cells relies on the directional movement of vesicles that ferry proteins between the compartments of the secretory pathway. Once released from the donor organelle, vesicles must navigate the cell to find and fuse with the appropriate acceptor compartment. Given the large number of potential target membranes, delivery to the correct compartment is essential in maintaining the fidelity of intracellular protein transport. Specificity of vesicle delivery is mediated in part by the proteins that promote fusion between vesicle and acceptor membranes, such as SNAREs — coiled-coil proteins that interact in specific combinations to form a four-helix bundle that facilitates mixing of the opposing lipid bilayers [1]. However, SNAREs interact only over relatively short distances (~120 Å), so cells also employ additional tethering factors that act upstream of the SNAREs. These tethers are thought to act over long distances to bring vesicles into the vicinity of the appropriate acceptor membrane and confer additional compartmental specificity [2]. Two recent papers have advanced our understanding, at a molecular level, of how one of these tethering complexes, the transport protein particle TRAPP I, facilitates docking of transport vesicles derived from the endoplasmic reticulum (ER) [3,4]. Vesicles are sculpted from the ER membrane by the cytoplasmic COPII coat, comprising the small G protein Sar1, the cargo adaptor complex Sec23–Sec24 and the outer cage complex Sec13–Sec31 [5]. These COPII components act together to generate membrane curvature, populate nascent vesicles with appropriate cargo proteins and release small transport carriers from the ER membrane. COPII vesicles contain both newly synthesized cargo proteins that are destined for delivery to the Golgi apparatus as well as the SNARE machinery that facilitates the downstream fusion of vesicles. Before fusion can occur, however, vesicles must attain close proximity to the acceptor compartment. The intricate details of COPII vesicle formation and delivery to the Golgi have been defined largely through yeast genetics and in vitro reconstitution [6–8]. The components involved in these processes are likely to play similar roles in mammalian cells, although the immediate downstream fate
of a COPII vesicle in higher eukaryotes is likely to be homotypic fusion with another vesicle rather than direct delivery to the Golgi proper [9]. Recruitment of COPII vesicles to the Golgi in yeast requires two tethering complexes, the coiled-coil protein Sec23 (p115 in mammals), and TRAPP I [10]. TRAPP I is a Golgi-localized multi-component protein complex that functions as a guanine nucleotide exchange factor (GEF) for the early-acting Rab family G protein, Ypt1 (Rab1 in mammals). Significant insight into how TRAPP mediates vesicle tethering has been provided by two complementary papers that investigated the molecular architecture of TRAPP I [4] and defined a TRAPP-binding partner that forms the other half of the tethering equation [3].

One piece that has long been missing from the TRAPP-tethering puzzle is the identity of the vesicle-borne receptor that interacts with TRAPP I to bring the two opposing membranes within SNARE-able range. In new research from Susan Ferro-Novick’s lab [3], the COPII subunit Sec23 is defined as that elusive partner. Sec23 interacts directly with the Bet3 component of TRAPP I, and excess Sec23 added to an in vitro vesicle-tethering assay inhibits the ability of vesicles to become immobilized. Furthermore, stripping the COPII coat from vesicles also inhibits the ability of those stripped vesicles to tether to immobilized TRAPP. By definition, interaction between a tether and a component of the vesicle coat will result in capture of the correct flavor of vesicle, so this seems like a perfect solution to the vesicle-tethering specificity problem. In addition to defining the vesicle-specificity factor, this study also suggests that COPII vesicles retain at least part of their coat after release from the ER membrane. This is not surprising, given the affinity of Sec23–Sec24 for the cargo proteins contained within the vesicle, and has important implications for the exposure of the SNARE fusion machinery. Although the coiled-coil SNARE domains are extended flexible structures that may protrude up to 140 Å from the vesicle membrane, the position of the SNARE ER export motifs and the location of their binding site on Sec24 suggest that the vesicle-borne SNAREs, such as Bet1, are pinned close to the vesicle membrane and, therefore, restricted in their ability to interact with the SNAREs of the opposing membrane (Figure 1) [11]. Thus, release of the Sec23–Sec24 complex from the vesicle membrane, perhaps triggered by binding to TRAPP I, may be a prerequisite for the final stages of SNARE engagement.

A key question that remains to be addressed is how the interactions between tether and coat may influence the function of TRAPP I as a GEF for Ypt1/Rab1, which helps mediate vesicle fusion. In a recent study that represents a tour de force of biochemistry, X-ray crystallography and single-particle electron microscopy, Kim et al. [4] defined the architecture of the TRAPP I complex. The authors identified discrete subcomplexes formed by the TRAPP components and defined the minimal TRAPP subcomplex required for GEF activity. Yeast TRAPP I was reconstituted into a ~170 kDa particle that is composed of the six essential TRAPP subunits assembled from two heterotrimerers — Bet3–Trs33–Bet5 and Bet3–Trs31–Trs20 — linked together via Trs23. However, the minimal catalytic complex was a heterotetramer of Bet5–Trs23–Bet3–Trs31. Whether this particular arrangement of TRAPP I components exists in vivo remains to be seen, and the possibility remains that, in cells, only fully assembled TRAPP I is catalytically active. Consistent with the yeast TRAPP architecture, mammalian TRAPP I proteins also assembled into two subcomplexes comprising bet3–trs31–sedlin (equivalent to yeast Trs20) and...
bet3–trs33–bet5–trs23, which may be linked together in vivo by an unknown subunit. The X-ray crystal structures of the two mammalian subcomplexes were determined; in combination with single-particle EM analysis of the yeast TRAPP I holocomplex, the structural data suggest that the particle forms an elongated, flattened, bi-lobed platform. Both the mammalian and yeast TRAPP I particles contained two copies of Bet3, which is consistent with a role for bilateral interaction between Bet3 and Sec23 on opposing vesicles during homotypic fusion of COPII vesicles in mammalian cells. One appealing model is that Bet3 independently assembles on both the vesicle and acceptor membrane, forming two distinct complexes (Bet3–Trs33–Bet5 and Bet3–Trs31–Trs20) that are bridged by Trs23 to assemble the complete tether and simultaneously stimulate GEF activity. Thus the TRAPP I holocomplex could coordinate tethering and local activation of downstream fusion machinery. Together, these two important papers refine our model for COPII vesicle delivery to the Golgi and provide a strong foundation from which to explore the question of Rab-mediated regulation of vesicle tethering and fusion (Figure 1).

Direct interaction between Sec23 on the vesicle surface and TRAPP I on the Golgi membrane (or bound through the second copy of Bet3 to another COPII vesicle) would serve to anchor the vesicle to the target membrane (Figure 1, step 1). This tethering function may be necessary to unmask the SNARE proteins, which mediate the final, short-range interaction that results in the merging of the two compartments. Binding of TRAPP I to Sec23 may also trigger guanine nucleotide exchange activity on Ypt1/Rab1 (Figure 1, step 2) that would serve to recruit the coiled-coil tether, Uso1/p115. Uso1 may further anchor the two compartments and coordinate interactions between the opposing SNAREs to further ensure specificity and maximize the efficiency of vesicle coupling with the target organelle (Figure 1, step 3) [12]. Thus, direct interaction between the tether and coat may trigger a localized molecular handoff of the vesicle-borne SNARE, released from Sec24 following Sec23–TRAPP I interaction, to the target membrane SNARE complex, coordinated by recruitment of Uso1 by activated Rab.

Interaction between a tether and a coat protein not only defines a new function for the well-characterized COPII coat, but also represents a particularly appealing model for ensuring the specificity of vesicle delivery. Indeed, coat-dependent tethering may be such an integral part of vesicle consumption that it operates redundantly in COPII vesicle transport. The putative yeast tethering complex, Grh1–Bug1, also binds Sec23–Sec24, raising the possibility that multiple tethers attract COPII vesicles to the Golgi apparatus through interaction with the coat [13,14]. More detailed characterization of coat–tether interactions and their consequences with respect to coat release, SNARE pairing and Rab activation is clearly warranted and will be aided dramatically by the availability of the crystal structure of the remarkable vesicle TRAPP.

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Department of Biological Sciences, Columbia University, New York, New York 10027, USA.

E-mail: em2282@columbia.edu

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Plant Development: Three Steps for Stomata

Three basic helix-loop-helix proteins regulate sequential steps in the formation of stomata: SPEECHLESS initiates entry into the stomatal lineage; MUTE controls asymmetric divisions of stomatal precursor cells; and FAMA promotes guard cell differentiation.

Julie E. Gray

Stomata are microscopic pores in the aerial epidermis of plants. Each pore is surrounded by a pair of guard cells, which regulate the aperture of the pore and control gas exchange and water loss between the plant and the environment. Stomata are