

this cell, named IG for 'inhibitor of GGN', may play a role in memory recall: by regulating the firing threshold of Kenyon cells, it could create a sliding scale for the resolution of object recognition. This feedback mechanism could additionally help stabilize GGN's membrane potential.

The discovery of GGN's powerful effect on Kenyon cells will reshape our understanding of olfactory coding in higher brain regions. How it works in the context of other sparsening mechanisms, such as the feed-forward inhibition pathway mediated by the lateral horn, will be interesting to determine [13]. Combinations of feed-forward and feed-back inhibition have been observed in the vertebrate olfactory system: Stokes and Isaacson [14] recently showed that a feed-forward inhibition mechanism acts immediately upon stimulus onset, and a feed-back inhibition mechanism contributes more slowly, in slices of the piriform cortex, a brain region in many ways analogous to the invertebrate mushroom bodies. And, in *Drosophila*, Papadopoulou *et al.* [4] recorded from the APL, a neuron similar in structure to GGN, and

found that the two neurons are functionally equivalent. Thus, global normalization mechanisms for maintaining sparse olfactory codes appear to be common. The relatively simple nervous systems of insects will no doubt continue to pave the way for unraveling the evolutionarily conserved mysteries of olfaction.

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## Vesicle Transport: Springing the TRAPP

When a coated transport vesicle docks with its target membrane, the coat proteins and docking machinery must be released before the membranes can fuse. A recent paper shows how this disassembly is triggered at precisely the right time.

Elizabeth Conibear

Transport vesicles are created when coat proteins assemble on a flat membrane, select cargo, and deform the membrane into a bud. The budded vesicle is then carried to its target organelle, where it docks by means of 'tethers' before undergoing membrane fusion. The vesicle coat was once thought to fall off as soon as budding was complete, but we now know the coat is important for binding the tethering factors that help the vesicle identify the correct organelle. Coat proteins and tethers must be removed before fusion can take place, but what triggers their disassembly has always

been a mystery. A paper recently published in *Nature* [1] now shows that, when one kind of transport vesicle docks with its target membrane, it encounters a kinase that breaks the bond between the coat proteins and the tethers, kick-starting the disassembly process.

To learn more about tethering, Lord *et al.* [1] focused on the coat protein complex II (COPII) coated vesicles that transport proteins from the endoplasmic reticulum (ER) to the Golgi. Studies over the past 20 years have given us a detailed picture of how this process works [2]. Formation of a COPII-coated vesicle begins with the activation of the small GTP-binding

protein Sar1, which associates with ER membranes and recruits the Sec23/24 complex to form the inner layer of the coat. Whereas Sar1-GTP interacts with Sec23, Sec24 selects the cargo. Subsequent assembly of the outer subunits, Sec13/31, completes the budding process. Once the vesicle is released, Sar1 hydrolyses its bound GTP and dissociates from the membrane. However, thanks in part to stabilizing interactions with membrane-associated cargo proteins, the rest of the coat does not fall off right away.

Once the COPII vesicle reaches the Golgi, it is recognized by two different tethers—Uso1 and TRAPPI [3]. TRAPPI is a multi-tasking, multisubunit complex that acts not only as a tether, but also as a guanine nucleotide exchange factor (GEF) for the Rab GTPase Ypt1 [4], whereas Uso1 (the ortholog of mammalian p115) is a long coiled-coil tether that binds Ypt1-GTP. A few years ago, the Ferro-Novick group discovered that the COPII coat protein Sec23 binds directly to the Bet3

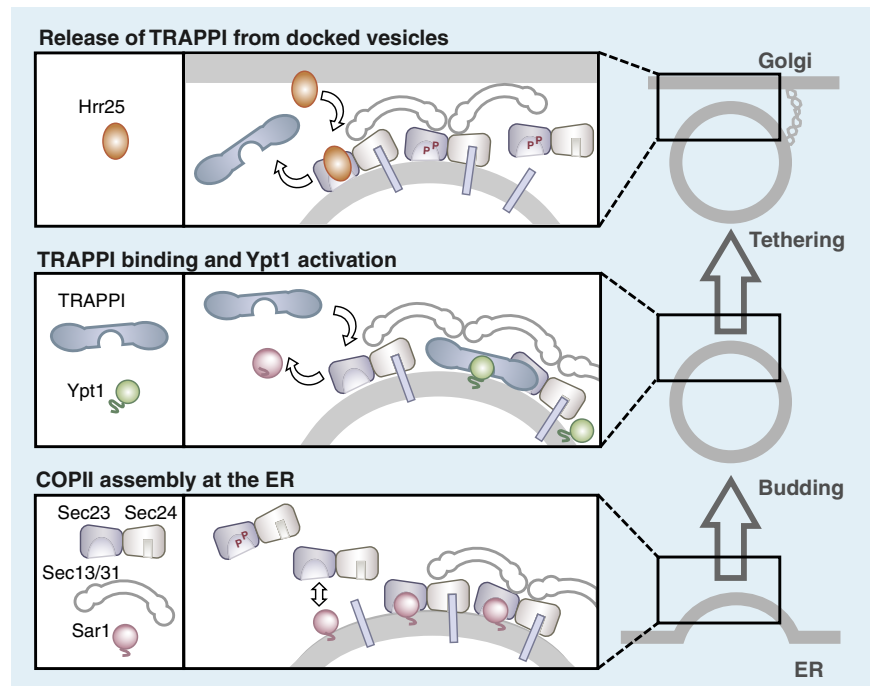
subunit of the TRAPPI complex [5]. At the time, the idea that vesicles keep their coats until they tether seemed heretical, but it now appears to be true for vesicles involved in other transport steps as well [6]. This presents a dilemma: how can a vesicle hang onto its coat long enough to reach its target, but shed it once it arrives?

An important clue came from the observation that, when COPII vesicles reach the Golgi, most retain their coats, yet quickly lose their TRAPPI-binding ability. From this finding, Lord *et al.* [1] reasoned that Sec23 could be modified by a Golgi-associated factor and searched for a kinase that was essential, Golgi-localized, and highly conserved. Their top candidate, Hrr25 (the ortholog of casein kinase I  $\delta$ ), fit the bill nicely. What's more, this kinase had previously been implicated in ER-to-Golgi transport, and the authors showed it could phosphorylate both Sec23 and Sec24 *in vivo* and *in vitro*.

Further investigation showed that Hrr25 competes with TRAPPI for binding to Sec23 and phosphorylates Sec23 at three residues, two of which are conserved. Mutations that mimic phosphorylation at these conserved sites reduced TRAPPI binding, blocked vesicle budding and fusion *in vitro*, and impaired cell growth. All this fits nicely with the idea that a Golgi-localized pool of Hrr25 modifies the COPII coat to displace TRAPPI. Shedding of TRAPPI also appears necessary for subsequent fusion, since an inhibitor of the Hrr25 kinase activity did not affect tethering but did block the final fusion step.

Interestingly, the two conserved Hrr25 phosphorylation sites are at or near the Sec23–Sar1 binding interface, and competition experiments confirmed that Sar1, TRAPPI, and Hrr25 all bind at the same or overlapping sites on Sec23, suggesting that Sec23 phosphorylation could affect vesicle budding. Indeed, it seems the non-phosphorylated form of Sec23 carries out the early stages of COPII assembly: the phosphomimetic form of Sec23 did not bind Sar1, and, whereas excess Hrr25 reduced vesicle budding *in vitro*, inhibition of Hrr25 kinase activity stimulated budding.

These results paint a picture of Sec23 as a master regulator of budding and fusion, participating in successive interactions that are regulated by phosphorylation (Figure 1). First, non-phosphorylated Sec23 binds



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Figure 1. Sec23 phosphorylation regulates sequential steps in COPII budding and fusion. Activated Sar1–GTP preferentially recruits the non-phosphorylated form of Sec23 to the ER during the formation of the COPII vesicle (lower panel). After vesicle budding, Sar1 dissociates and the TRAPPI tethering complex binds Sec23 in its place. TRAPPI then promotes GTP exchange on the Rab protein Ypt1 (middle panel). After the vesicle tethers at the Golgi, the Golgi-localized kinase Hrr25 phosphorylates Sec23, releasing TRAPPI (upper panel). After the coat is shed, Sec23 must be dephosphorylated to participate in further rounds of transport.

Sar1–GTP during coat assembly. After budding is complete, Sar1 hydrolyses its bound GTP and dissociates, allowing TRAPPI to bind Sec23 in its place. The COPII-coated vesicle then tethers to the Golgi, where it encounters Hrr25, which phosphorylates Sec23, releasing TRAPPI from the coat and allowing fusion to proceed. This not only explains how coat shedding is initiated once tethering is complete, but also suggests that the phosphorylation–dephosphorylation cycle could impart directionality to the vesicle transport process.

While this is an attractive model, it may not be the whole story. If Sec23 is the only relevant Hrr25 target, loss of Sec23 phosphorylation should block ER-to-Golgi transport. Surprisingly, the authors found that mutation of the two conserved Hrr25 phosphorylation sites in Sec23 had no effect on cell growth and only mildly impaired vesicle budding and fusion. Since Hrr25 also modified Sec24, it will be interesting to determine whether Sec24

phosphorylation influences the formation of pre-budding complexes or the release of Sec24–cargo interactions during uncoating. In fact, a previous study indicated that phosphorylated Sec24 does not bind to membranes in mammalian cells [7].

Just as Sec23 may not be the only target of Hrr25, Hrr25 may not be the only kinase involved in COPII formation and fusion. Previous studies have implicated a variety of kinases in ER-to-Golgi trafficking that could regulate transport under basal conditions or modulate secretion capacity in response to cargo load or the cell cycle (reviewed in [8]). Phosphatases must also play key roles because the Sec23 that is released when the vesicle uncoats must be dephosphorylated before it can undergo new rounds of coat formation. Further studies will be needed to identify the relevant enzymes and determine whether dephosphorylation is coupled to the uncoating process.

Although the activity of Hrr25 may be important for uncoating *in vivo*, it is

clearly not sufficient because purified Hrr25 was unable to uncoat COPII vesicles *in vitro*. Full uncoating may be triggered by additional, as yet-to-be-determined signals. However, the degree to which vesicles uncoat before fusion takes place is currently unclear. Although more than 64% of the COPII vesicles were reported to retain their outer coats during tethering [1], these data could also mean that each vesicle loses approximately one-third of its coat. In principle, a partially uncoated COPII vesicle could retain enough Sec23 to allow TRAPPI binding and tethering, while leaving enough exposed membrane to allow pairing of SNARE complexes between the two membranes and fusion of the vesicle with the Golgi.

Partial uncoating would make it easier for TRAPPI to interact with Sec23 on opposing membranes during the homotypic fusion of mammalian COPII vesicles [5]. With both COPII vesicles fully coated, the distance between Sec23/24 complexes on each vesicle is 140 Å [9]. TRAPPI is a rod-shaped particle that measures 180 Å from end-to-end [10]. However, for TRAPPI to interact with Sec23 on different membranes via its two copies of Bet3 and still be able to bind and activate Ypt1, it would have to lie flat on the membrane [4], requiring that the two vesicles be less than 75 Å apart.

Now that many different types of vesicle coat are known to interact with tethers [6], it will be possible to test whether regulation by Hrr25 or other kinases is a general feature of coat–tether interactions. Interestingly, in the case of vesicles bearing the AP-3 adaptor complex, which are linked to the vacuole by the HOPS tethering complex, coat–tether interactions are regulated by the Hrr25-related kinase Yck3. However, instead of phosphorylating the coat to release the tether, Yck3 does the opposite, modifying the HOPS subunit Vps41 to expose the binding site for the AP-3 coat and promote tethering [11]. Although the details may differ, the work of Lord *et al.* [1] provides a new paradigm for the regulation of vesicle tethering and fusion that may apply to all transport steps: namely, that each organelle harbours a kinase that lies in wait for incoming vesicles, ready to cut them free of their tethers and release them from their coats.

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## Animal Cooperation: Keeping a Clean(ing) Reputation

Cleaner wrasses are a model for the study of animal cooperation. Prospective clients can observe whether the cleaner works faithfully, and cleaners being watched remove just parasites while those that are not, nip the client for a tastier snack.

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Why do unrelated individuals help one another? Numerous studies have found answers to this question in direct reciprocity — ‘help me now and I’ll help you later’ — or mutualism — ‘if you don’t cooperate now, there will be a cost greater than if you helped’. Both of these mechanisms implicitly require some preparation for the future. But, can animals prepare for the future

without a representation of it? Possibly such future ‘preparation’ resides in an improved self image. For example, helpers can increase the chance that bystanders will assist them later by increasing their image score (e.g., how that individual is viewed by a group). Among non-human animals, eavesdropping bystanders offer an opportunity for helpers to improve how they are viewed but this has been tough to document.

Cleaner fish have long been studied as a model system for animal cooperation. The marine cleaner wrasse, *Labroides dimidiatus*, removes ectoparasites from visiting reef fish clients. This is a mutualistic relationship because the client gets cleaned of ectoparasites and the cleaner gets a meal, the parasite. But there’s a rub: cleaner fish prefer the client’s tasty layer of mucus to its ectoparasites [1]. This means that there’s a conflict of interest between cleaners and clients — clients want to be cleaned and cleaners prefer to eat mucus. These conflicting goals mean that clients want cleaners to do something they would rather not. However, cleaner fish service up to 2000 clients every day [2] and many of those encounters happen in the presence of observing bystanders, including future clients.