Traffic COPs: rules of detection

Anne Spang

Growth & Development, Biozentrum, University of Basel, Basel, Switzerland. Correspondence to: anne.spang@unibas.ch

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How specific cargo recognition by coat proteins is achieved and how this recognition event may regulate vesicle formation are still under investigation. In two recent papers by the Owen and Goldberg labs, the binding mode of dilysine motifs to the coatomer of the COPI coat has been analysed. Collectively, their findings suggest that the dilysine motif containing cargo proteins may stabilize coat complexes on membranes and enhance the chance for coat polymerization and vesicle budding.

The balance of anterograde and retrograde transport between the endoplasmic reticulum (ER) and the Golgi apparatus and within the Golgi is essential for organelle identity and maintenance, and ultimately for cell survival. Communication between organelles along this secretory pathway is maintained by coated transport vesicles. While anterograde transport to the ER is dependent on COPI vesicles, retrograde transport within the Golgi and back to the ER requires COPI action. The main function of COPI vesicles is to retrieve proteins and lipids back to the previous compartment along the pathway. COPI-dependent cargoes display motifs on their cytoplasmic tail that are recognized by the coat complex to allow for their transport. Unfortunately, pathogens—or factors expressed by them—also explore the COPI traffic route to exert their detrimental function on host cells. For example, toxins from shigella and cholera use the COPI transport system to reach the ER, from which they escape to the cytoplasm to fulfill their harmful function. In addition, functional COPI traffic is essential for the replication of a number of viruses, presumably because they transport obligatory factors for replication initiation. Thus, understanding the molecular basis of cargo recognition by the COPI coat is not only exciting for intracellular transport aficionados, but will also guide the rationale to fight pathogens depending on COPI transport for infection.

Transmembrane proteins exposing a KKXX or a KXXX motif on their C-terminal tail have been shown to be COPI-dependent cargo molecules that are retrieved from the Golgi apparatus to the ER. These motifs are also found in either ER-resident proteins that may escape sometimes or proteins that facilitate export of other cargo from the ER in COPII vesicles, and which serve as some sort of export receptors. Once the cargo-transport receptor complex has reached the Golgi, the complex dissociates and while the cargo moves through the Golgi, the transport receptors are recycled back to the ER through the retrieval sequence.

Given the importance of the dilysine-based motifs, researchers mapped the interaction site of the cargo tails with COPI. The COPI coat consists mainly of the small GTPase Arf1 and the heptameric protein complex coatomer (α, β, β′, δ, ε, γ, ζ). The coatomer complex, although recruited en bloc to membranes, can be subdivided into two subcomplexes: a clathrin adaptor-like complex (β, δ, ε, γ, ζ) and an outer shell complex (α, β′, ε). In clathrin-dependent trafficking, cargo recognition occurs in the adaptor complex, which is also true for the COPI coat with respect to a di-arginine-based signal recognition motif. However, a number of experiments by different groups demonstrated that the dilysine-based signals are recognized by the outer shell complex (Cosson

Figure 1  Schematic depiction of how the KKXX motifs might stabilize the coatomer on Golgi membranes, even when Arf1-GTP hydrolysis has occurred.
and Letourneur, 1994; Schroder-Kohne et al., 1998; Eugster et al., 2004).

Interestingly, two different subunits, α and β′, have been implicated in binding of dilysine motifs through their β-propeller domain. Lee and Goldberg reported a structure of α–β′-COP, in which the ends of the β-propeller in β′-COP would converge into a trimer (Lee and Goldberg, 2010). Hence, the outer shell might form triskelions, consistent with recent EM data (Faini et al., 2012). Whether this would affect or obscure cargo binding to the same domain remained unclear. A few months ago, Jackson et al. (2012) reported that the structure of the β-propeller of β′-COP bound to both types of dilysine motifs in a similar manner, and they concluded that cargo binding might still occur in the trimeric assembly of the outer shell complex. In a new paper published in The EMBO Journal, Ma and Goldberg (2013) extend this knowledge by reporting structures of the α–β′ subunits with several dilysine-based retrieval motifs. Both subunits can bind with about the same specificity to KKKX and KXXXX motifs. Most surprisingly, however, KKXX and KXKXX bind with about the same specificity to KKXX and KXKXX motifs. Both subunits can discriminate between distinct, functional di-lysine signals in vitro (Figure 1). Initial recruitment of the coatomer to the membrane is driven by the interaction of the adaptor subcomplex with activated Arf1. Interaction of cargo with the outer shell complex might extend the residence time of the coatomer on the membrane, independent of GTP hydrolysis by Arf1, even more so when several dilysine motifs are present, like in oligomeric assemblies of p24-family proteins or the Mst27/28 complex (Bremser et al., 1999; Sandmann et al., 2003; Aguilera-Romero et al., 2008). Consistent with this idea, overexpression of dilysine motif-containing proteins rescues mutations in the adaptor complex subunit Sec21 (γ-COP), which interacts with Arf1-GTP (Zhao et al., 1999; Sandmann et al., 2003). Thus, coat complex residence time on membranes and probably also on vesicles may not be strictly coupled to GTP hydrolysis and the presence of GTPase on vesicles, and uncoating of vesicles may mostly rely on factors on the target membrane, such as tethers and Rab-GTPases. In fact, the DSL complex, which tethers COPI vesicles to the ER, recognizes first the coatomer and not a naked vesicle (Zink et al., 2009).

Given the conservation of vesicle formation along the secretory pathway, it will be interesting to see whether coat-omeral stabilization on membranes by dilysine motifs is a conserved feature as well.

Conflict of interest

The author declares that she has no conflict of interest.

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