Review TRENDS in Cell Biology Vol.16 No.10

Special issue: Membrane Dynamics

Online Article: Insights into COPI coat assembly and function in living cells

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Eukaryotic cells use an elaborate machinery involving the COPI coat complex to control protein trafficking in the secretory pathway. Although individual components of this complex are well known and their roles in deforming lipid membranes into coated carriers are well described, the precise sequence of molecular events by which these components assemble into and release from the COPI coat lattice remains unclear. Here, we present images and movies characterizing the dynamics of protein components of the COPI coat in living cells. We discuss the self-assembly of these coat components into a molecular machine for sorting and trafficking membranes.

Introduction

Secretory transport depends on membrane-bounded carriers to move protein and lipid between intracellular compartments. The COPI coat has a central role in this process, creating a sorting domain on the membrane into which cargo proteins, destined to return to the endoplasmic reticulum (ER), concentrate. The membrane domain deforms into a coated bud, pinches off the membrane as a coated carrier, and then uncoats. Successful operation of the COPI coat system is necessary for selective retrieval of protein and lipid components back to the ER, which enables secretory membranes to functionally and morphologically differentiate from the ER.

Much of our understanding of how the COPI coat assembles and functions has come from traditional genetic and biochemical studies [1,2]. These studies have provided a basic model for COPI coat assembly and disassembly. Coat assembly in this scheme begins with the membrane recruitment and activation of the small soluble GTPase Arf1. This is mediated by guanine nucleotide exchange factors (GEFs) that convert Arf1 to its active, GTP-bound form. Upon being activated at the membrane, Arf1 recruits a preformed coatomer complex (consisting of seven subunits) from the cytoplasm. Coatomer bound to Arf1 then binds membrane-associated ArfGAP, the GTPase activating protein for Arf1. The complex of coatomer–Arf–ArfGAP polymerizes into a coat lattice on the membrane, concentrating cargo proteins directly beneath it. The lattice deforms the membrane into a coated bud, which then pinches off the membrane as a coated vesicle. Disassembly of the lattice occurs when ArfGAP hydrolyzes the GTP on Arf1. This releases Arf1 into the cytoplasm, which in turn triggers the release of coatomer and ArfGAP from the membrane. The components are then free to be reused for coat reassembly.

This molecular description has proved invaluable for understanding many aspects of COPI coat control. Nevertheless, it leaves unanswered key questions related to the dynamics and operation of the COPI coat. These questions include: how is Arf1–GTP hydrolysis coupled to cargo sorting and coat disassembly; are coat components put on and taken off once or multiple times in a single cycle of coated vesicle formation and consumption; and does the coat have broader roles in membrane trafficking than just in the sorting and mechanical deformation of the membrane?

Recent quantitative live cell imaging approaches have provided a way to begin investigating these questions. By enabling individual components of the COPI coat to be marked with a fluorescent protein reporter and visualized directly, these approaches have made it possible for the dynamics of COPI coat components to be understood in relation to coat self-assembly in living cells. Here, we present video images characterizing the molecular events of COPI coat assembly and disassembly *in vivo* and discuss how these findings are expanding our insights into COPI coat maintenance and function.

Sites of COPI coat assembly on membrane

When and where the COPI coat acts in membrane sorting and deformation is dictated by the intracellular sites where COPI components bind and dissociate from membranes. The intracellular sites of coatomer binding have been visualized in *ldlF* cells expressing the coatomer subunit εCOP labeled with yellow fluorescent protein (εCOP–YFP) [3]. The construct readily assembles with other COPI subunits into the coatomer complex, and in *ldlF* cells – which contain an εCOP subunit that is inactive at high temperature – it functionally substitutes for endogenous εCOP. It thus provides a reliable readout of the intracellular distribution and dynamics of coatomer.

Coatomer-containing membranes – assessed by εCOP–YFP labelling – include juxtanuclear Golgi structures and anterograde transport intermediates (pre-Golgi structures) [3]. The ArfGAP that binds coatomer, ArfGAP1, when

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Available online 7 September 2006.

tagged with cyan fluorescent protein (ArfGAP1–CFP), also co-distributes with εCOP–YFP on pre-Golgi and Golgi membranes [4]. Very little εCOP–YFP labeling is seen on structures budding off the Golgi as retrograde carriers [3]. These results suggest that the main sites for COPI assembly on membranes are pre-Golgi and Golgi structures, with the coat rapidly dissociating from retrograde transport vesicles.

In cells expressing εCOP–YFP, pre-Golgi structures containing εCOP–YFP move as globular or tubular shapes towards the Golgi apparatus (AVI movie 1 in supplementary material online). The amount of εCOP–YFP on the anterograde carriers remains constant as they migrate, often over long distances, through the cytoplasm. This raises the possibility that the coat lattice on these structures has other roles in addition to the budding of retrograde coated vesicles. These might include the recruitment of accessory factors for mediating interactions with cytoskeleton or tethering to an acceptor organelle, or the differentiation of membrane domains.

Regulating the temporal and spatial activation of Arf1

The formation of the COPI coat requires membrane recruitment and activation of Arf1. This has been demonstrated in cells treated with the drug brefeldin A (BFA), which binds Arf1–GDP, preventing its activation [5]. BFA treatment inhibits the assembly of the COPI coat [6]. This results in a dramatic cellular phenotype, in which membrane trafficking from the ER and Golgi apparatus stops and the Golgi apparatus disassembles [6,7].

Arf1 activation is normally mediated by guanine nucleotide exchange factors (GEFs) that convert Arf1 to its active GTP-bound state on the membrane [8]. One such GEF is GBF1, which when overexpressed in cells confers resistance to BFA [9]. The distribution and dynamics of GBF1 in living cells have recently been visualized using a construct of GBF1 tagged with YFP (YFP–GBF1) [10].

Confocal imaging and photobleaching results of cells expressing YFP–GBF1 are shown in Quick time movies 1 and 2 in the supplementary material online. At steady state, YFP–GBF1 resides on Golgi membranes [10]. Upon photobleaching of the Golgi pool of fluorescence in these cells, rapid replenishment from fluorescent pools in the cytoplasm occurs (Quick time movie 1 in the supplementary material online). This indicates that GBF1 molecules undergo rapid cycling on and off membranes rather than being stably associated with the membranes. The entire cellular population of YFP–GBF1 is engaged in this type of rapid exchange, because repeated photobleaching of a cytoplasmic area results in all cellular fluorescence being quickly diminished (Quick time movie 2 in the supplementary material online). The transient nature of the interactions of GBF1 with membrane shown in this movie could be important for enabling GBF1 to activate Arf1 at the right place and at the right time within cells.

The dynamics of membrane binding and release of GBF1 within cells is noticeably affected during BFA treatment (Quick time movie 3 in the supplementary material online). Within minutes of adding the drug, the amount of YFP–GBF1 associated with Golgi and pre-Golgi membranes increases dramatically. GBF1 remains associated with these membranes even after they undergo absorption into the ER. This is consistent with GBF1 being a direct target of BFA, and with the interaction between GBF1 and BFA occurring on Golgi and pre-Golgi membranes. Because nearly all GBF1 is recruited to membranes during BFA treatment, whereas only a small fraction of total Arf1 in BFA-treated cells is similarly recruited, the data additionally suggest that GBF1 acts catalytically in exchanging GTP for GDP on Arf1.

Cycling of Arf1, coatomer and ArfGAP1 on and off membranes

In models of COPI coat assembly and disassembly, after GBF1 activates Arf1 on membranes, the active form of Arf1 recruits coatomer and then ArfGAP from the cytoplasm. Together, the complex of coatomer–Arf–ArfGAP assembles into a lattice that concentrates cargo proteins and deforms the membrane into a bud that pinches off the membrane. Disassembly of the lattice is controlled by ArfGAP, which hydrolyzes GTP on Arf1. Exactly when coat components release from membranes relative to GTP hydrolysis in this scenario, and how these processes are spatially and temporally regulated for efficient vesicle formation and budding, remain unclear.

Results from live-cell imaging and photobleaching studies are helping to address these questions [3,4]. In cells expressing ArfGAP1–YFP or εCOPI–CFP, photobleaching of the Golgi pool of fluorescence reveals rapid recovery of fluorescence from the cytoplasmic pool of these proteins (Quick time movies 4 and 5 in the supplementary material online). The recovery kinetics reflected an exponential process, showing no delay for the exchange between the Golgi and cytoplasmic pools of these proteins [3,4]. This suggests that coatomer and ArfGAP1 are put on and taken off the coat lattice multiple times in a single cycle of coated vesicle formation and consumption (rather than remaining tightly bound to the lattice until it buds off as a coated vesicle).

Consistent with this interpretation, in cells expressing ArfGAP1–YFP or εCOPI–CFP that are incubated at 4 °C, a treatment that blocks the budding of coated vesicles, photobleaching Golgi fluorescence results in rapid and full recovery (Quick time movies 6 and 7 in the supplementary material online). Release of coatomer and ArfGAP1 from the coat lattice is therefore not coupled to or dependent on vesicle budding.

When the recovery kinetics for GFP-tagged versions of Arf1, ArfGAP1 and εCOPI into a photobleached Golgi region are compared side-by-side, the recovery kinetics of Arf1 are fastest [3,4]. This suggests that Arf1–GTP hydrolysis and release from membranes precedes the membrane dissociation of coatomer and ArfGAP1.

The effect of BFA on the dynamics of Arf1, coatomer and ArfGAP1

In BFA-treated cells, Arf1 is prevented from becoming active. Because activated forms of Arf1 that are already on the membrane are not affected by BFA, examination of the lifetime of activated Arf1 and its effectors on the membrane now becomes possible. In addition, an estimation of the lifetime of the coat lattice on the membrane can be made.

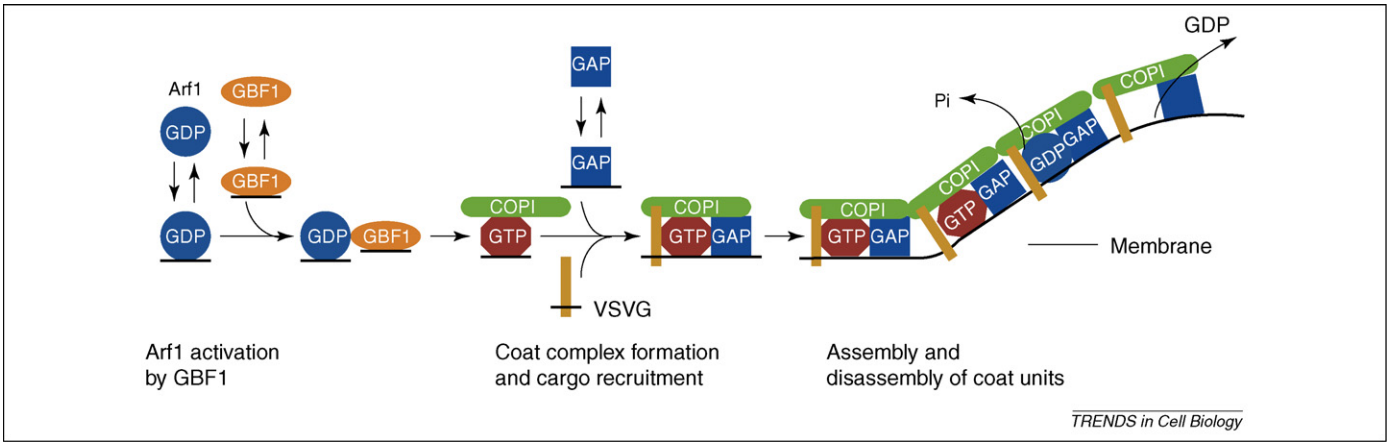

Figure 1. A model of COPI coat assembly. GBF1 (orange oval) promotes exchange of GTP for GDP by Arf1 and activation of Arf1 (blue circle, Arf1–GDP; red, Arf1-GTP). Active Arf1 then recruits COPI (green). The Arf–COPI complex then attracts ArfGAP (blue square), and the three components assemble into a lattice, which deforms the membrane. Hydrolysis of Arf1-bound GTP is induced by the recruited ArfGAP. Inactive Arf1–GDP then diffuses away from the membrane, initiating disassembly of the lattice.

The effects of BFA on the membrane-association of GFP-tagged versions Arf1, εCOP or ArfGAP1 expressed individually within cells has been studied [3,4]. Most of the Arf1 redistributes from Golgi membranes into the cytosol within seconds [3]. The redistribution depends on GTP hydrolysis on Arf1, because a mutant form of Arf1 that fails to hydrolyze its GTP remains bound to membranes during BFA treatment [3]. BFA treatment releases εCOPI from Golgi membranes, but the release occurs more slowly than for Arf1 [3]. BFA treatment decreases the association of ArfGAP1 with Golgi membranes only slightly [4].

These findings offer several new insights into the respective dynamics and roles of Arf1, coatomer and ArfGAP1 on membranes. First, the fact that the majority of Arf1 is lost from the membrane during BFA treatment implies that the pre-existing membrane pool of Arf1 is primarily in an active, GTP-bound state (otherwise it would have become stabilized on the membrane as a complex with BFA and the Arf1-exchange factor GBF1). Second, because the half-time for Arf1 release into the cytoplasm was faster than for coatomer release (assessed using εCOP–YFP) during BFA treatment, GTP hydrolysis and cytoplasmic release of Arf1 during coat assembly must precede the membrane dissociation of coatomer and ArfGAP1. Third, the fact that nearly all coatomer is lost from membranes over the course of ~2 minutes of BFA treatment suggests that when input into the lattice of new coat components stops, the lattice has only a short, finite existence. Finally, because ArfGAP1 levels on the membrane drop only partially during BFA treatment, ArfGAP1 can bind to and release from membranes in the absence of activated forms of Arf1.

The response of cargo proteins to increased secretory traffic

The association of COPI coat components with Golgi membranes is affected by changes in levels in secretory traffic. This can be seen in ArfGAP1–YFP expressing cells upon release of a bolus of the temperature-sensitive vesicular stomatitis virus G protein tagged with CFP (VSVG–CFP) into the secretory pathway by temperature shift from 40 °C to 32 °C (Quick time movie 8 in the supplementary material online). A dramatic increase in the level of Golgi associated ArfGAP1–YFP occurs at the time when passage of VSVG molecules through the Golgi is at its peak. This increase depends on increased cargo transport and on the ability of ArfGAP to interact with Arf1 and coatomer, because in cells that are either not expressing VSVG–CFP or expressing an ArfGAP1 mutant that cannot bind Arf1, no change in the size of the Golgi pool occurs [4]. A similar increase in Golgi-bound βCOP in response to VSVG flow through the Golgi can be seen [4]. ArfGAP1 and coatomer levels on Golgi membranes are therefore both modulated in response to changes in secretory cargo transport through the Golgi.

Conclusion: a model of coat lattice dynamics

The live cell imaging data presented here, together with other results [11,12], suggest a simple model of COPI coat assembly (Figure 1). In this model, all of the key components and regulators of the COPI coat (including GBF1, Arf1, ArfGAP1 and coatomer) cycle on and off membranes in a manner that is uncoupled from vesicle formation, and in which feedback from productive vesicle budding is not necessary for their dissociation. Continuous membrane binding and release of these molecules enables the COPI lattice to be dynamically modulated. The COPI coat thus gains the capacity to self-assemble, enabling it to act in membrane sorting and transport processes as a molecular machine.

Appendix A. Supplementary material

Supplementary videos associated with this article can be found online at doi:10.1016/j.tcb.2006.08.008.

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