GAPs in the context of COPI

Enzymes, coat components or both?

Rainer Beck,^{1,*} Britta Brügger² and Felix Wieland^{2,*}

¹Department of Cell Biology; Yale University School of Medicine; New Haven, CT USA; ²Biochemistry Center; Heidelberg University; Heidelberg, Germany

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Trafficking in the early secretory pathway at first glance is well understood according to textbook knowledge: To achieve secretion and to maintain organelle homeostasis, protein and lipid cargo need to be transported constitutively from their origins of biosynthesis to their respective destinations. Thus, secretory cargo exits the ER and is shuttled to the Golgi via vesicular COPII carriers. Lipid and protein cargo is enzymatically modified in the Golgi, transported from cis- to trans- (by mechanisms that are still debated today), and from there travel to their final destinations. The best established roles for COPI vesicles, simply spoken, is to mediate retrograde trafficking of cargo molecules that were transported forward, but need to be transported back.

The discovery and characterization of COPI vesicles dates back to the early 1980s, when Rothman and coworkers described vesicular transport intermediates in trafficking events between isolated compartments in cell-free systems.^{1,2} Inter-organellar transport could be blocked by either treatment with N-ethyl maleimide (NEM), a sulfhydryl alkylating agent,³ which resulted in a fusion block and the accumulation of uncoated vesicles,⁴ or by treatment with the non-hydrolyzable GTP analog GTP γ S, which accumulated coated vesicles instead.⁵ The most straightforward explanation of these results leads to the paradigm that GTP-hydrolysis mediates uncoating. Following these early landmark studies was the purification and isolation of COPI vesicles in vitro,^{6,7} and the discovery of the major COPI coat constituents coatamer⁸ and Arf1.⁹ Arf1 itself lacks GTP hydrolysis activity¹⁰ and requires GTPase activating proteins. ArfGAP1 today is one of the best characterized Arf1 effectors on the Golgi.¹¹

Based on these pioneering studies, whether Arf GAP-mediated GTP hydrolysis in Arf1 directly triggers coat disassembly remains debated today, but several key findings, and this list is most likely far from complete, show a role for GTP hydrolysis at least as a prerequisite for uncoating:

(a) detailed electron microscopical characterization of Golgi-derived COPI vesicles, using GTP γ S or a mutant ArfQ71L

that is deficient in GTP hydrolysis^{5,12} prevented uncoating and blocked transport between organelles,

(b) live cell imaging results established that mutant Arf1Q71L stabilizes coatamer on the Golgi,^{13,14}

(c) overexpression of ArfGAP1 in living cells leads to the release of Arf1¹⁵ and coatamer¹⁶ from the Golgi,

(d) experiments from the Wieland lab demonstrating that in a minimal reconstituted system Arf GAP catalytic domain uncoats COPI vesicles,¹⁷ and that Arf GAP activity inhibits the accumulation of coated vesicles from the Golgi,^{18,19}

(e) real-time measurements of coat assembly and disassembly on liposomes.²⁰

While it is only fair to conclude that GTP hydrolysis is certainly a prerequisite for uncoating, Dr. Kahn in his recent review²¹ discussed the possibility that in the context of a living cell this need not be the immediate trigger.

By measuring fluorescence recovery after photobleaching, Arf1-GFP recovery kinetics were faster (by a factor of two) than coatamer on Golgi membranes of living cells, suggesting that additional interactions hold coatamer in place, even after the small GTPase had left the membrane.²² No significant fluorescence recovery could be observed for either Arf1 or coatamer when GTP hydrolysis was blocked. The different release times of Arf1 and coatamer could be interpreted in the following ways: Coatamer with its ~30-fold mass as compared to Arf1 has an accordingly slower diffusion rate²³ and/or whereas Arf1 dissociates as a small monomeric protein, the coat represents a polymerized network that has to be dissociated before it can be released by diffusion.

Recently published data imply a role for the tether complex Dsl1 to mediate uncoating at the ER by direct interaction with coatamer.^{24,25} Intriguingly, the loss of Dsl1 in yeast lead to a phenotype similar to the loss of the Glo3 Arf GAP: the accumulation of coated vesicles in vivo.²⁵

While the question remains to be answered if also in a living cell Arf GAP-mediated GTP hydrolysis in Arf1 directly triggers uncoating, there can be no doubt that Arf1 activation and deactivation, or more precisely, GTP loading and GTP hydrolysis, dictate Arf1 membrane association and dissociation, and that these processes are tightly regulated.

Antonny and co-workers reported that ArfGAP1 responds to lipid-packaging, acting as a membrane curvature sensor,²⁶ and therefore conferring Arf1 localization to membranes of defined (low) curvature.²⁷ Likewise, such hydrophobic patches mediated binding of ArfGAP1 to Golgi membranes in mammalian

*Correspondence to: Rainer Beck and Felix Wieland;
Email: rainer.beck@yale.edu and felix.wieland@bzh.uni-heidelberg.de
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cells.^{28,29} In contrast, the two Golgi resident Arf GAPs of the Glo3 family, ArfGAP2 and ArfGAP3, while sharing with ArfGAP1 striking homology in their catalytic GAP-domains, do not display this curvature sensitivity, and were shown to depend on coatamer interactions for membrane binding.^{19,30} Note that while interactions of all three Arf GAPs with coatamer have been reported, these interactions differ between the Gsc1 and Glo3 GAPs, and may represent distinct non-redundant layers of COPI regulation.³¹

In a cellular context, individual or pair-wise knockdowns of any of the three Arf GAPs did not give rise to any significant phenotypes, neither in terms of coatamer localization to the Golgi, nor transport of selected anterograde and retrograde cargo markers.³² However, the simultaneous downregulation of ArfGAPs1,2,3 lead to a robust increase of Arf1 GTP levels *in vivo*, resulting in severe phenotypes in Golgi morphology, block of transport,³² and cell death,³³ consistent with previous studies in yeast.

Our understanding of Arf GAP function(s) in COPI vesicle biogenesis becomes even more complex, as we and others^{13,34-37} have found that Arf GTP must be hydrolyzed in order to allow efficient uptake of cargo into COPI vesicles.

One of the most controversial questions in the ongoing debate is whether Arf GAPs represent structural stoichiometric coat components for COPI vesicle biogenesis, as suggested by the Hsu lab.³⁸ There can be no doubt that Arf GAPs are required for COPI function. As to whether they are essential mainly because they catalytically confer GTP hydrolysis to the system, as we see it, or because they are, apart from being catalytically active, also stoichiometric essential building blocks of the COPI coat is the point where our interpretations of experimental data diverge.

The answer must be found in the design of the experiments, so I will try to summarize both sides without going too much into detail. What we observed,^{18,19} for reconstitution and subsequent purification of COPI-coated vesicles from Golgi membranes in the presence of GTP, is that Arf GAP-mediated GTP hydrolysis (by adding ArfGAPs in ranges of substoichiometric concentrations to our one step incubations) antagonizes the accumulation of coated COPI vesicles in those fractions of sucrose gradients that represent the buoyant density of coated vesicles. This of course does not answer the question whether GTP hydrolysis in this case prevents sufficient binding of Arf GTP to the donor membrane for vesicle formation to occur in the first place, or whether reconstituted COPI vesicles in the presence of Arf GAP activity rapidly shed their coat prior to purification.

In contrast, the Hsu lab observed a requirement of ArfGAP1 for the reconstitution of COPI vesicles.³⁸ They use a two-step incubation system: Arf1 GTP and coatamer are recruited to Golgi membranes first, in the absence of Arf GAP activity. These

primed membranes are isolated by centrifugation and re-incubated in a second stage with stoichiometric amounts of ArfGAP1. The Hsu group observed that this second stage incubation is essential to release coatamer from the donor membranes, in the form of COPI vesicles. We have repeated these experiments and published our interpretations^{18,19} and we are looking forward to a vivid discussion in the blog.

Another experimental detail that is worth mentioning here is the fact that, consistent with Lelio Orci's and James Rothman's pioneering work on COPI vesicle biogenesis, and their thorough characterization by electron microscopy,³⁹ we also observed that reconstituted COPI vesicles derived from Golgi remain associated with the donor membranes, but could quantitatively be released by salt treatment with 250 mM KCl. Rothman and Orci described this as 'vesicles on strings' and postulated a tight interaction of membrane tethers to vesicles in order to mediate a spatially regulated trafficking. In this context it also seems worthwhile to mention that we could rule out any mechanical force needed to release COPI vesicles,¹⁸ as had been suggested in various publications.

We speculated that part of the observed discrepancies between the Hsu and Wieland labs might be explained by a role for Arf GAP activity in releasing vesicles from their tethers. Bruno Antonny's group elegantly demonstrated that the golgin GMAP-210 is able to tether flat to curved membranes by interacting with the curved phospholipid bilayer on one end, and by binding to Arf1 GTP on the other.⁴⁰ This finding might open the door for additional roles for Arf GAP activity that we did not yet consider experimentally in our *in vitro* budding assays.

In general, the data from both our and other labs combined suggest that Arf GAP-mediated hydrolysis needs to be regulated and modulated for productive vesicle formation to occur, be it by (1) combinations of membrane curvature/lipid interactions, (2) regulation through machinery/cargo proteins, as the data from various other groups suggest, or (3) in the case of the *in vitro* experiments from both our labs, potentially by lacking regulatory factors that would be present in a cell, by a spatial/temporal restriction of Arf GAP activity.

What counts now is to look ahead and try to understand why the time point of Arf GAP addition here, or any other experimental detail, might lead to different results and/or interpretations.

What we share is our enthusiasm in trying to understand the fascinating and elegant molecular mechanisms behind vesicle biogenesis and function. There are many pages that could be written to discuss the pros or contras of experimental design and interpretation on either side, and we would like to leave this open for the commenting on the CellLogBlog (www.landesbioscience.com/journals/cellularlogistics/blog/5).

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