Membrane curvature and the control of GTP hydrolysis in Arf1 during COPI vesicle formation

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Abstract
The GTP switch of the small G-protein Arf1 (ADP-ribosylation factor 1) on lipid membranes promotes the polymerization of the COPI (coat protein complex I) coat, which acts as a membrane deforming shell to form transport vesicles. Real-time measurements for coat assembly on liposomes gives insights into how the GTPase cycle of Arf1 is coupled in time with the polymerization of the COPI coat and the resulting membrane deformation. One key parameter seems to be the membrane curvature. Arf-GAP1 (where GAP stands for GTPase-activating protein), which promotes GTP hydrolysis in the Arf1–COPI complex is highly sensitive to lipid packing. Its activity on Arf1-GTP increases by two orders of magnitude as the diameter of the liposomes approaches that of authentic transport vesicles (60 nm). This suggests that during membrane budding, Arf1-GTP molecules are progressively eliminated from the coated area where the membrane curvature is positive, but are protected from Arf-GAP1 at the bud neck due to the negative curvature of this region. As a result, the coat should be stable as long as the bud remains attached and should disassemble as soon as membrane budding occurs.

Introduction
Figure 1(A) shows the classical GTPase cycle of a G-protein as seen in many textbooks. The G-protein undergoes a binary switch between a GDP conformation and a GTP conformation through the catalytic activities of a GEF (guanine nucleotide-exchange factor) and a GAP (GTPase-activating protein). When bound to GTP, the G-protein interacts with an effector target. The consequence of this GTP-dependent interaction ranges from change in enzymatic activity to change in ionic conductance or change in localization depending on the nature of the effector target. However, regardless of the exact nature of the effector, the molecular response follows the binary switch of the G-protein because it is based on the formation of a simple 1:1 complex. In other words, the output is in phase with the input. Yet there are mechanisms for which the relationship between the binary switch of a G-protein and the molecular response is less trivial.

Protein coats are large polymeric structures that assemble at the cytoplasmic surface of cell membranes, from which they promote the budding of small (<100 nm in diameter) transport vesicles. For the COPI (coat protein complex I) and COPII coats, which drive the formation of transport vesicles between the endoplasmic reticulum and the Golgi, it has been clearly established that their assembly is controlled by small G-proteins from the Arf (ADP-ribosylation factor) family (Arf1 in the case of COPI and Sar1 in the case of COPII) [1,2]. When Arf switches to the GTP conformation, it becomes firmly anchored to the membrane surface through the exposure of its N-terminal amphipathic helix. Arf-GTP promotes the membrane recruitment of large COP complexes, which can collect the cytosolic tails of transmembrane proteins and self-assemble to form a polymeric two-dimensional structure. This structure has an intrinsic curvature and thereby promotes the formation of a coated vesicle by a budding/fission process.

Coat assembly and membrane budding can be observed when Arf is permanently activated by non-hydrolysable analogues of GTP (such as GTP[S]). Conversely, coat disassembly requires GTP hydrolysis in Arf1. Therefore it is tempting to conclude that the assembly–disassembly cycle of the coat is in phase with the GDP/GTP cycle of Arf. But this is probably an over-simplification. Compare the two coat-building units shown in Figure 1(B). The first is a recently formed 1:1 complex between Arf-GTP and a COP complex wandering at the membrane surface by lateral diffusion. The second is an older unit, which has been incorporated in the coat lattice. If GTP hydrolysis occurs in the ‘young’ unit, both Arf-GDP and the COP complex will obviously dissociate from the membrane. This is a futile cycle, as the coat has not performed its function yet. Now what happens if GTP hydrolysis occurs in the ‘old’ coat unit? Here, the answer depends on the lateral interactions between the polymerized COP units. If these lateral interactions are strong enough, they should keep the COP unit assembled in the lattice despite the loss of Arf. So one key question in protein coat dynamics is the temporal and spatial control of the GTP hydrolysis reaction as the coat lattice starts to grow and to curve the lipid membrane [3]. Here, we will depict two mechanisms that give such a temporal and spatial control of GTP hydrolysis: (i) coat polymerization and (ii) membrane curvature.

Key words: Arf1, coat protein complex, G-protein, GDP, GTP, liposome.
Abbreviations used: Arf, ADP-ribosylation factor; BAR, bin, amphiphysin and Rvs; COP, coat protein complex; 1,2-DOG, dioleoylglycerol; GAP, GTPase-activating protein; GEF, guanine nucleotide-exchange factor.

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Control of GTP hydrolysis by COP polymerization

Sar1 is a ‘cousin’ of Arf1 and controls the assembly of the protein coat COPII. This coat consists of three protein ‘layers’: Sar-GTP and two large complexes Sec23/Sec24 and Sec13/Sec31. These proteins assemble in a stepwise manner at the surface of the endoplasmic reticulum membrane [4]. First, Sar1 translocates to the lipid bilayer in a GTP-dependent manner. Then Sec23/Sec24 interacts with Sar-GTP. Lastly Sec13/Sec31 polymerizes the Sar1-GTP–Sec23/Sec24 complex in a coat structure. One puzzling characteristic of the COPII coat is that Sec23/Sec24 is a GAP for Sar1. Sec23/Sec24 has thus two seemingly opposite faces: it is a structural component of the coat but on the other hand its GAP activity may counteract COPII complete assembly.

We have devised real-time assays to follow COP coat dynamics[5]. One is based on a large tryptophan fluorescence change that accompanies the GDP/GTP switch of Arf or Sar. By measuring the level of tryptophan fluorescence in a solution of Arf/Sar with liposomes, one can easily follow Arf activation by GEFs or Arf inactivation through GTP hydrolysis by GAP. The second assay is based on the scattering of light. When the various layers of the coat cover liposomes, a light scattering increase is observed due to the increase in the refractive index of the membrane. One can thus follow a cycle of COP assembly and disassembly on liposomes.

With these two assays, we have observed that the GAP activity of Sec23/Sec24 is regulated by COPII polymerization [5]. GTP hydrolysis in an isolated Sar1-GTP–Sec23/Sec24 complex is slow (halftime = 1 min). In contrast, when such a complex is polymerized through the action of Sec13/Sec31, the rate of GTP hydrolysis is accelerated 10-fold (Figure 2). With such a two-gear mechanism, new Sar-GTP–Sec23/Sec24 complexes would have the time to diffuse and collect cargo molecules before GTP hydrolysis. GTP hydrolysis would occur only after incorporation of the complex in the polymerized coat by Sec13/Sec31. Therefore the probability of having Sar1-GTP in the lattice should decrease from the periphery to the centre of the coat, since the lateral distribution of the COP complexes reflects their sequence of assembly. At the molecular level, the mechanism by which Sec13/Sec31 accelerates the GAP activity of Sec23/Sec24 is not known.
Control of GTP hydrolysis by COP-induced membrane curvature

Whereas the GAP for Sar1 is a structural element of the COPII coat, the GAP for Arf1 in the COPI coat is an accessory protein, which is not required for COPI polymerization [6]. Arf-GAP1 displays puzzling properties regarding its interaction with the lipid bilayer [7]. Incorporating a few mol% of 1,2-D0G (dioleoylglycerol), a diacyl glycerol with two mono-unsaturated C18:1 acyl chains, promotes the recruitment Arf-GAP1 to liposomes and facilitates its action on liposome-bound Arf-GTP. Surprisingly, the effect of 1,2-D0G does not depend on the position of the ester bonds that connect the acyl chains to the glycerol group but was reduced when the oleoyl chains were replaced by saturated acyl chains. These observations rule out a PKC (protein kinase C)-like model, in which Arf-GAP1 would recognize specifically the diacyl glycerol molecule. So what explains the DOG effect? Compared with classical lipid species, DOG has conical shape due to the lack of polar head and the presence of a kink right in the middle of the acyl chains. As a result, DOG does not pack well against more cylindrical lipids. We postulated that Arf-GAP1 senses these packing defects by intercalating bulky hydrophobic residues [7]. Whether the effect of DOG is physiologically relevant is unknown, however the physical basis for the DOG effect leads to an intriguing hypothesis.

When a membrane is curved through the mechanical stress imposed by a protein coat, lipid packing in the inner (lumenal) leaflet must increase, whereas lipid packing in the outer leaflet (cytosolic) must decrease. On a short-time scale (minutes), lipid flip–flop cannot compensate for this stress as it takes hours for most lipids to translocate between two lipid leaflets. We therefore postulated that Arf-GAP1 could sense these lipid-packing changes and act more readily on Arf-GTP as the membrane becomes curved [8]. As shown in Figure 3, the rate of GTP hydrolysis on Arf1 initiated by the addition of Arf-GAP1 indeed increases with the curvature of the liposomes. Reducing the liposome radius from 150 to 35 nm (the approximate size of a transport vesicle) increases the rate 30-fold. Other experiments suggest that the remarkable sensitivity of Arf-GAP1 to liposome radius also applies when Arf-GTP is engaged in the COPI coat [8].

If we now extrapolate these results to a COPI-coated bud, Arf-GAP1 should eliminate most Arf1 molecules from the coat except at the edge of the coat where membrane curvature is negative. This is exactly what you need to maintain a polymer in a metastable state: remove some links when they become dispensable in the centre, but protect the edges, where the lateral interactions are not complete.

How does Arf-GAP1 recognize membrane curvature?

There are probably not very many ways to recognize a curved membrane. One is to recognize the curvature itself, and this is exactly what BAR (bin, amphiphysin and Rvs) domains do [9]. These domains have a banana shape with a concave basic face, ideally adapted to interact with highly curved and negatively charged membranes. Depending on the strength of the BAR–membrane interaction, a BAR domain can either sense or induce membrane curvature. The structure of the BAR domain is a dimer made by a coiled-coil of six helices, three from each monomer.

Except for the presence of an N-terminal 120 amino acid zinc-finger module, which is responsible for its GAP activity, Arf-GAP1 bears no structurally identified domain. Notably, the lack of predicted coiled-coil regions strongly suggests that Arf-GAP1 contains no BAR domain. However, this is nothing but surprising. Indeed and as mentioned above, Arf-GAP1 is not sensitive to membrane curvature by itself, but to the lipid packing defects induced by membrane curvature, and sensing lipid-packing changes should require a deeper protein–membrane interaction as compared with sensing the geometric curvature of the lipid bilayer.

To identify the putative lipid-packing sensing region in Arf-GAP1, we have taken advantage of the homology between Arf-GAP1 and Gcs1p. Gcs1p is the closest homologue of Arf-GAP1 in yeast. In contrast with other Arf-GAPs, the homology between Gcs1p and Arf-GAP1 extends three from each monomer.

Concluding remark

The dynamics of protein coat is a challenging issue because, as for other polymers, there is no linear correlation between the behaviour of each unit (e.g. GDP or GTP) and the behaviour of the polymer (assembled or disassembled) [11]. One key parameter is the position of a COP unit within the lattice. The experiments presented here suggest two mechanisms that permit the control of COP units according to their position (central of peripheral) in the lattice.
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References

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