BIOLOGY REPORTS

Published: 11 May 2010

Membrane deformation and separation

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F1000 Biology Reports 2010, **2**:35 (doi:10.3410/B2-35)

Abstract

Biological membranes are highly dynamic (e.g., during cell division, organelle biosynthesis, vesicular transport, and neurotransmitter release). They can be shaped into protein-coated transport vesicles or tubules and undergo regulated fusion. The life of transport vesicles depends on highly specific and tightly regulated protein machineries, which not only shape the donor membrane into nascent budding structures but also help to overcome the energy barrier to break the bilayers apart in order to pinch off nascent vesicles. Ultimately, vesicular membranes have to fuse with a target lipid bilayer, a process that again requires remodeling. Here, we highlight recent insights into mechanisms that lead to membrane deformation in the process of vesicular budding.

Introduction and context

Mechanisms underlying membrane deformation are highlighted in numerous recent reviews [1-16]. Various modes of phospholipid bilayer deformation were classified by McMahon and Gallop [5]. These include stereochemical properties of lipid building blocks or conformations of transmembrane proteins. In addition, the organization of the cytoskeleton can cause membrane deformation. In general, sculpting of membranes is assumed to be achieved by membrane scaffolding proteins, such as vesicular coats (coatomer for coat protein [COP]I vesicles and Sec 23/24 and Sec 13/31 for COPII vesicles) or clathrin and adaptor complexes for clathrin-coated vesicles (CCVs), or by amphipathic helices of proteins that insert into and increase the area of one leaflet of the bilayer (or by both).

The molecular mechanisms underlying budding of carrier vesicles in endocytosis or biosynthetic transport pathways are the focus of molecular cell biological research at present. These pathways employ GTPases that subsequently can recruit cytosolic coat protein complexes. The GTPase dynamin serves the generation of endocytic vesicles in combination with the adapter protein complex AP2 and clathrin [17]. In contrast, biosynthetic transport vesicles employ small GTPases – Sar1p and the coat complexes Sec23/24 and Sec13/31 for COPII vesicles and Arf for COPI vesicles and various CCVs – in combination with coatomer (for COPI) or adapter complexes AP1, 3, and 4 (for CCV).

Major recent advances

Dynamin-mediated fission was thought to require a power stroke generated by a concerted conformational change in assembled dynamin and triggered by rapid GTP hydrolysis [18,19]. However, more recent studies demonstrated that dynamin assemblies stabilize highly curved templates [20] and that fission requires cycles of GTP hydrolysis [21]. During these cycles, the underlying membrane undergoes squeezing and relaxation, resulting in the stochastic generation of a hemifission intermediate assumed to cause fission. Thus, cycles of membrane binding and GTP hydrolysis-dependent dissociation were shown to be necessary for dynamin-catalyzed membrane fission (reviewed in [22]). Another recent study showed that dynamin nucleation, and hence membrane deformation, occurs preferably at sites of high local curvature [23].

In analogy to dynamin, Sar1 was found to induce membrane curvature on liposomal membranes [24], and in more recent work, such a role has been reported for Arf1 [25-27]. This surface activity, however, does not require GTP hydrolysis. A minimal machinery consisting of liposomes, Arf1, and coatomer has been described to be sufficient for COPI reconstitution *in vitro* [28,29] in the presence of non-hydrolyzable GTP analogs. While additional factors, such as Arf-GTPase-activating protein 1, were reported to be required for the release of vesicles [30], this finding was recently challenged [31].

Which mechanism then would apply for the release of a budded COPI vesicle? As vesicle separation is observed in minimal reconstituted liposomal systems [29,32,33], it is basically the coat protein or the small GTPase or both that catalyze the scission reaction. Indeed, Lee *et al.* [24] have observed that, although a truncated form of Sar1p supports bud formation in the COPII system, the GTPase, when lacking its amphiphatic helix, lost the ability to deform the membrane. As a consequence, separation of the nascent vesicle was inhibited [24]. This opens a possibility that in the early secretory pathway, the small GTPases, in addition to recruiting coat proteins, have a role in membrane fission. Along these lines, free Arf1-GTP has been recently reported to preferentially localize to areas of low membrane curvature [34] when GTP-hydrolysis is stimulated by the curvature-sensitive ArfGAP1 enzyme [35]. Thus, Arf1 is likely to reside at sites where fission finally occurs, at the neck of the nascent bud.

Future directions

Models for the molecular mechanism of membrane separation have been forwarded on the basis of the assumption that the protein involved in a scission reaction would stabilize a transition state. Conceptually, it may be useful to consider models in which an unstable, energetically unfavorable transition state that can be relaxed by membrane separation is generated. For the small GTPases, this would imply that they must have affinity to membranes strong enough to remain in a membrane at sites of increasing negative curvature, as represented by the growing neck of a maturing bud. To avoid escape from energetically unfavorable sites, the GTPase would need to be stably anchored to the bud's coat. In the case of COPI vesicles, Arf1 is tightly bound by multiple specific interaction sites with its covering sheet of polymerized coatomer [36], and thus firmly kept in place even at a location of negative curvature, which forms in the bud neck and is energetically unfavorable to accommodate the small GTPase. Scission of a bud would then occur in case the energy barrier for spontaneous fusion of adjacent membranes in the neck was lower than that for an escape of Arf1 from high-energy sites. Arf1 was recently described to dimerize upon activation with GTP, and an Arf1 mutant unable to dimerize did not support COPI vesicle formation [25]. Thus, it seems attractive to speculate that the avidity to bind to membranes gained by dimerization of small GTPases adds to the mechanisms of membrane separation. It will be exciting in the future to experimentally challenge this hypothesis.

Abbreviations

CCV, clathrin-coated vesicle; COP, coat protein.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We would like to thank the German Research Council for funding our research (SFB 638, A10).

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