Membrane traffic: **Do cones mark sites of fission?**

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Membrane fission occurs in eukaryotic cells whenever a vesicle is produced or a larger subcellular compartment is divided into smaller discrete units. Recent evidence suggests this fission event is promoted by enzymes that generate phosphatidic acid and thereby cause a distortion of the lipid bilayer.

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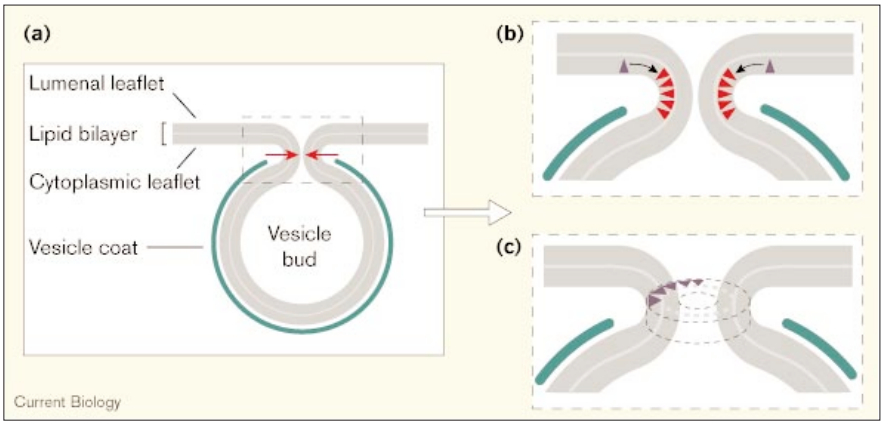
Vesicle-mediated transport in the secretory and endocytic pathways of eukaryotic cells involves the assembly of coat protein complexes onto the cytoplasmic face of the lipid bilayer [1]. These coat proteins are thought to be responsible for both the concentration of cargo proteins within the vesicle and the deformation of the lipid bilayer necessary to form the final, spherical, vesicle bud structure [1]. The final step in vesicle formation, termed fission or scission, is less well understood, but must involve some form of membrane fusion event at the restricted neck of the vesicle bud (Figure 1). Recent results [2,3] suggest that the fission event is promoted by enzymes that catalyse the transfer of fatty acids from coenzyme A to lysophosphatidic acid (LPA), generating phosphatidic acid that causes a destabilising distortion of the lipid bilayer.

For two classes of vesicle, those bearing COP I or COP II coat proteins, vesicle formation has been reconstituted *in vitro* with only purified proteins and synthetic lipid vesicles, suggesting that specific fission factors are not absolutely required [4,5]. This led to the hypothesis that the coat proteins serve as a mechanical device that drives, not only the vesicle budding process, but also the final fission event. Whether this is actually sufficient for vesicle fission *in vivo*, and how fission events that are not coupled to vesicle formation occur, are still matters for debate.

For other classes of vesicle, such as the clathrin-coated vesicles that form at the plasma membrane and trans-Golgi network [6,7], there is evidence that members of the dynamin family of proteins act as specific fission factors. The suggestion is that dynamin assembles at the site of fission and garrottes the membrane in a process driven by GTP hydrolysis. This notion is supported by the ability of purified dynamin to mechanically vesiculate liposomes *in vitro* in a GTP-dependent fashion [8]. There are, however, now suspicions that dynamin might not act as a fission factor *in vivo*, as mutations that compromise its ability to hydrolyse GTP actually promote receptor-mediated endocytosis. This observation led to the proposal that it is the downstream effectors of dynamin that physically mediate vesicle fission [9].

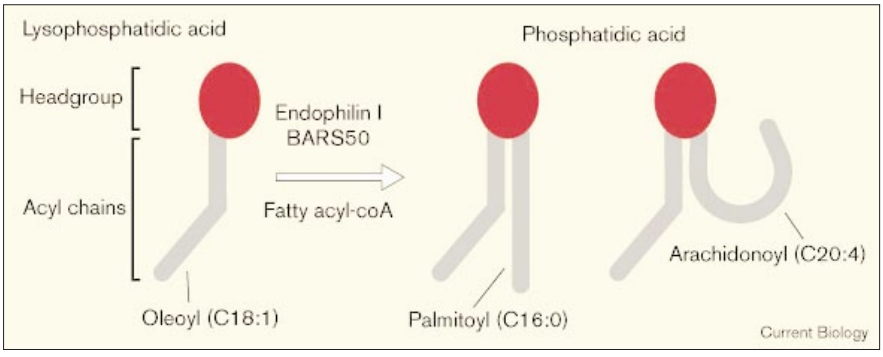
Membrane fission as envisaged in the examples discussed above is fundamentally a mechanical process, but recent evidence suggests this is not the whole story. Work carried out in two *in vitro* systems for either synaptic vesicle

Figure 1



A diagrammatic representation of vesicle fission. **(a)** Vesicle coat proteins (green) assemble on the cytoplasmic face of the membrane, resulting in the formation of a vesicle bud with a constricted neck (red arrows). Membrane fission occurs when the lipid bilayers at this point fuse. **(b)** The localised production of a cone-shaped phospholipid such as phosphatidic acid (red triangles) from an inverted-cone-shaped phospholipid such as LPA (inverted blue triangles) in the cytoplasmic leaflet of the bilayer allows the bilayer at this point to adopt the extreme inward curvature along the bud axis suggested to favour fission [2,3]. **(c)** Note that the opposite curvature exists round the neck of the bud, something that inverted-cone-shaped phospholipids such as LPA (blue triangles) would appear to favour. If this model is correct, interconversion of LPA and phosphatidic acid would presumably destabilise the organisation of the bilayer at the bud neck.

Figure 2



Endophilin I and BARS50 catalyse the transfer of fatty acids from coenzyme A to lysophosphatidic acid (left), generating phosphatidic acid (right) at the site of membrane fission. Drawn approximately to scale, the red oval denotes the glycerol backbone and phosphate headgroup, while the fatty acid chains are shown in grey. The carbon chain length and number of double bonds of the various fatty acids are indicated in brackets. LPA is shown with an oleoyl fatty acid, as this was the form used by Weigert *et al.* [3].

formation [2] or the fragmentation of the Golgi apparatus [3] has provided surprising evidence for a common mechanism of membrane fission, involving the remodelling of membrane lipids rather than mechanical force generation. The groups concerned have identified proteins — endophilin I for synaptic vesicles and BARS50 for the Golgi apparatus — which mediate the transfer of fatty acids from coenzyme A to a LPA acceptor, generating phosphatidic acid (Figure 2). The two enzymes have similar properties, preferentially using LPA while showing little activity towards other lysophospholipids, and transferring a variety of saturated or unsaturated fatty acids.

Not surprisingly, a common model has been put forward to explain how endophilin I and BARS50 promote fission (Figure 1a,b). The model is based on the observation that the phospholipid LPA, with just a single fatty acid chain, has an inverted-cone shape, while phosphatidic acid, with two fatty acid chains, is thought to be cone shaped [10]. Production of a cone-shaped lipid in the cytoplasmic leaflet of the bilayer would induce an inward distortion of the membrane along the axis of the bud, promoting fusion of the lumenal leaflets and subsequently membrane fission [2,3]. Unfortunately, this simple model fails to explain how phosphatidic acid would be arranged round the constricted bud neck, which has the opposite curvature, something that would seem to be favoured by inverted cone phospholipids such as LPA (Figure 1c).

What criteria should be used to recognise an effector for membrane fission? Little is known about membrane fission or the proteins mediating it, but there are a number of criteria that such a protein might be expected to adhere to. Inhibition or depletion of the putative fission protein or its cofactors should prevent fission, and lead to the accumulation of a pre-fission intermediate, such as a completed vesicle bud. The protein, or in the case of an enzyme its reaction products, should localise to the site of fission — that is, the neck region of a vesicle bud or the constricted part of a tubule or tubular network. This association could occur at the cytoplasmic or lumenal surface of the membrane, or both in the case of a transmembrane protein. The protein should display a specific fission activity towards its target membrane when used at a physiological concentration. Finally, its presence should correlate with the membrane fission event under examination; for example, synaptic vesicle fission proteins would be expected only in the brain.

BARS50 was originally discovered by Luini and coworkers [11,12] as one of two proteins — the other is glyceraldehyde-3-phosphate dehydrogenase — that are ADP-ribosylated in cells treated with the drug brefeldin A (BFA). They reasoned that, as BFA is an agent that disrupts membrane transport and causes collapse of the Golgi apparatus back into the endoplasmic reticulum, these proteins might be important for normal Golgi apparatus function. Purification of BARS50 [13] showed that it is a member of a group of proteins thought to function in transcriptional repression [14], with a high degree of sequence similarity to glyceraldehyde-3-phosphate dehydrogenases. How these observations tied in with those on the function of BARS50 in Golgi membrane dynamics was unclear, but the recent study of Weigert *et al.* [3] has now provided evidence that BARS50 plays a direct role in Golgi membrane fission.

Weigert *et al.* [3] analysed the function of BARS50 using a cell-free assay in which morphological changes in purified Golgi membranes were quantified following incubation with cytosolic extracts and partially purified, recombinant BARS50. They found that, when BARS50 was added to Golgi membranes in the presence of cytosol, it promoted fission of the tubules and tubular networks associated with the stacked Golgi cisternae, but not the central areas of the cisternae themselves [3]. Closer analysis of the membranes after 15 minutes of incubation revealed that constrictions, interpreted to be sites of fission, had appeared at regular intervals along the tubules, indicating some degree of organisation of the fission process. Perhaps inspired by the observations that transfer of palmitate from palmitoyl coenzyme A to an unidentified acceptor

molecule is required for the fission of COP I vesicles from Golgi membranes [15], Weigert *et al.* [3] then showed that the requirement for cytosol in membrane fission could be replaced by palmitoyl coenzyme A and BARS50 alone.

The ability of BARS50 to promote membrane fission was found to be closely correlated with its acyl transferase activity for all the fatty acyl coenzyme As that were tested [3], indicating that Golgi membranes have no specific requirement for a particular type of phosphatidic acid in fission. Considering the criteria for membrane fission defined above, there are still some key pieces of evidence missing before we can be certain that BARS50 is a fission factor. BARS50 does not appear to be localized to the site of membrane fission, and it has not been demonstrated that its depletion from cytosol results in an accumulation of pre-fission intermediates, such as COP I coated vesicle buds. Whether BARS50 really is the *in vivo* effector for the palmitoyl-coenzyme A-mediated fission of COP I vesicles remains an open question.

So, is endophilin I a fission enzyme? It is certainly in the right place, as it binds to dynamin, which is known to localise to the site of membrane fission during clathrin coated vesicle biogenesis [6]. Schmidt *et al.* [2] clearly demonstrated that endophilin I is required for synaptic vesicle formation in their *in vitro* assay. They found that depletion of endophilin I abolished synaptic vesicle formation, an effect that was rescued by readdition of the recombinant protein. In an *in vitro* assay for clathrin-coated vesicle formation, the Src homology 3 (SH3) domain of endophilin I blocked vesicle formation at a late stage, just prior to fission [16]. Somewhat contradicting this, antibodies to endophilin I blocked the formation of clathrin-coated vesicles in lamprey nerve terminals at an early stage, where the coated pit has just started to invaginate [17]. One possible explanation for the discrepancy is that the antibody acts by blocking an early stage of endophilin I action that involves functions associated with its SH3 domain, whereas the recombinant SH3 domain acts by blocking the recruitment of the full-length protein at a late stage where its fatty acyl transferase activity is needed.

Endophilin I is able to transfer both palmitic and arachidonic acid to LPA, but Schmidt *et al.* [2] found that only arachidonic acid supported the formation of synaptic vesicles *in vitro*, demonstrating there is some specific requirement for the type of phosphatidic acid produced [2]. They suggest that phosphatidic acid, with one arachidonyl fatty acid chain, allows greater curvature of the bilayer, which may be important given the small size of synaptic vesicles. Not only is the type of phosphatidic acid produced important in this assay, the method of production also appears to be critical. Phospholipase D, which generates phosphatidic acid by cleaving the headgroups from membrane lipids, was unable to bypass the requirement for endophilin I in this system [2]. Assuming that lipids with an arachidonyl fatty acid chain were present in the bilayer, and that the appropriate bioactive form of phosphatidic acid was generated, this observation supports a model in which localised production of phosphatidic acid by endophilin I is critical for synaptic vesicle fission. The restricted distribution of endophilin I, which is present mainly in the brain [18], is consistent with it having a role in synaptic vesicle formation.

Are we any closer to understanding the mechanism of membrane fission? Many issues need to be addressed before we can be certain that endophilin I and BARS50 really are fission factors. One of the major issues concerns the synthesis and turnover of phosphatidic acid and LPA, and how these might be regulated so that microdomains of phosphatidic acid are generated at the sites of fission. At this point it should be noted that the catalytic activities of BARS50 and endophilin I are extremely low, generating respectively 0.1 and 1 molecules of phosphatidic acid per minute per molecule of protein. There are many reasons why the proteins might not have displayed their full activity *in vitro*, but it may indicate that the true *in vivo* substrates have not been identified. The identification of two enzymes catalysing the transfer of fatty acids to LPA as potential key players in membrane fission are discoveries that clearly raise as many questions as they answer.

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