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Review

Mechanical requirements for membrane fission: Common facts from various examples

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ABSTRACT

Membrane fission is the last step of membrane carrier formation. As fusion, it is a very common process in eukaryotic cells, and participates in the integrity and specificity of organelles. Although many proteins have been isolated to participate in the various membrane fission reactions, we are far from understanding how membrane fission is mechanically triggered. Here we aim at reviewing the well-described examples of dynamin and lipid phase separation, and try to extract the essential requirements for fission. Then, we survey the recent knowledge obtained on other fission reactions, analyzing the similarities and differences with previous examples.

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1. Introduction

The early hypothesis of membrane traffic, as it was conceived just after the discovery that proteins could be transported between organelles [1], involved the formation of small vesicles that were separated from the donor membrane by a process called *membrane fission*. As a consequence, the compartimentalization of eukaryotic cells ensuring the specialization and function of each organelle was regarded as strictly dependent on this process: without membrane fission, all the organelles would end up being connected, mixing their contents and losing their function/specialization.

One of the first proteins found to be implicated in fission was dynamin. It was genetically shown to be involved in the release of synaptic vesicle from the plasma membrane [2], and the helical polymer it forms in vitro [3] and in vivo at the neck of endocytic buds [4] immediately suggested that it could trigger fission by constricting the neck of buds. In this paper, we first review 15 years of work on dynamin in order to understand how well this hypothesis is verified. The fact that dynamin-like proteins only work in a subset of fission reactions then prompts us to ask what the common features and/or functions of proteins/lipids involved in membrane fission are, and which other proteins are involved in other reactions.

2. Constriction versus shearing: what really triggers membrane fission?

As often in cell biology, morphological analysis at the ultrastructural level trigger hypothesis on molecular mechanisms. This is best exemplified by seminal work on dynamin: dynamin could constrict and fuse the two sides of the neck. In this picture, fission would be similar to fusion, as it would involve the same metastable intermediates [5]. This view was supported by studies showing that dynamin has all the features to actually drive fission by constriction/fusion: GTP is needed for fission [4], and, as shown in a milestone paper [6] by the Jenny Hinshaw group, dynamin alone can deform membranes into tubules circled by the dynamin helix. It was moreover shown in this work that upon GTP treatment such tubules constrict and break into very small vesicles. Therefore, it seemed at this point that a large-scale dynamin conformational change could provide enough work to constrict the tubule and fuse it locally, which would result in membrane breaking.

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2.1. Dynamin: the paradigm of constriction

Several questions were brought about by the work of Hinshaw and co-workers: where does the constriction come from? Does the conformational change of the helical polymer induce torsion, similar to the wringing of linen fiber? Or does each individual monomer constrict, causing the helix to shrink in size, without really changing shape?

Early evidence of a linen-like conformational change came from a careful study of the biochemical interactions between different dynamin domains [7]. The strongest interactions were observed between hetero-domains and proposed to be in the continuity of the helical turn. They remained unchanged when the nucleotide load was modified, whereas the interactions between homo-domains were weaker in the presence of GTP and proposed to be between contiguous helical turns. This suggested that during the hydrolytic cycle of GTP, dynamin oligomers could undergo a cycle of binding/unbinding between adjacent helical rings. The authors of this study thus favored a mechanism by which sliding of adjacent helical turns would cause constriction.

The 3D structures [8] obtained by cryo-EM before and after constriction yielded a more detailed picture of this complex situation: as the dynamin polymer went from its non-constricted to its constricted state upon incubation with GMP–PCP, the number of dimers per turn went from 14 to 13, while constriction and bending of each dimer was also observed. This is a direct proof that some of the constriction actually occurs by torsion. The huge inward bending of each dimer however also has a dramatic influence on the membrane, and constricts it even more.

At the structural level, it thus seems that dynamin constriction comes from the combination of a global (torsion of the helical polymer) and a local (compaction of the monomers) conformational change. On the functional level, the structural studies of the Hinshaw group [8] have a remarkable feature: they show that long, continuous constricted tubules can be isolated, which is not at all expected in a situation where constriction alone induces tubule breaking. Pointing this out, the Hinshaw group also showed a striking difference between dynamin-coated tubules treated by GTP when observed by negative stain or by cryo-EM. When performing negative stain [6], which involves attaching the tubules to a substrate before GTP treatment, they observed a large amount of fission. On the other hand, when cryo-EM was used, which implies treating dynamin-coated tubules with GTP in bulk, no obvious fission occurred [9]. As a matter of fact, in the 3D constricted structure of Ref. [8], whole membrane tubules (as opposed to hemi-fission intermediates) are seen. It should however be noted that the tubules in this last reference were not treated with GTP, but with GMP–PCP, and that the comparison might therefore not stand as fission is observed with GTP only.

In a nutshell, there is compelling data supporting the early hypothesis that in addition to being required for fission, dynamin constricts membrane tubules upon GTP hydrolysis. Still, the essential question of whether this constriction is sufficient to induce membrane fission on its own was still open at this point.

2.2. What triggers membrane fission?

Recent studies have used live imaging and sensitive measurements to directly visualize dynamin-mediated membrane fission, and try to isolate the minimal requirements for dynamin-mediated membrane fission [10–12]. As membrane fission is a very transient event, the strength of these studies was the ability to follow a membrane template in real time while it is being broken by dynamin. Using dynamin-coated tubules grown from planar membrane sheets, a first study showed that rapid twisting of the dynamin helix occurred upon GTP treatment [10]. This twisting activity was further evidenced by the formation of supercoils, which also caused the long dynamin-coated tubules to retract. Surprisingly, tubules anchored only at one end never broke, while tubules anchored at both extremities ruptured after experiencing longitudinal tension, probably generated by the torsional activity of dynamin through the formation of supercoils. On top of confirming that part of the constriction comes from torsion, this study stresses the role of mechanical forces in dynamin-mediated membrane breaking. Such mechanical forces could be provided by the actin cytoskeleton, which would account for its known involvement in this process [13,14].

The fact that torsion occurs very rapidly led to the hypothesis that dynamin could break membranes by shearing/tearing (Bruno Antonny, private communication). Indeed, although a membrane sheared on long time scales will tend to flow, applying a torque very quickly to the membrane tubule could tear the membrane (see Fig. 1). An interesting feature of this mechanism is that its efficiency is crucially dependent on the velocity associated with torsion. If torsion is slower than membrane's viscoelastic time (defined as its viscosity over its stretching modulus and thus of order 10^{-8} s), dynamin will just crawl on the liquid membrane, which will be drained out by the squeezing action of the helix. If it is faster, shearing-induced breakage could occur. On those short time scales, the tube is expected to behave like a piece of rubber, and thus to collapse on itself under shear (see Fig. 1C). This collapse should occur in the early stages of the shearing (i.e. prior to tearing), meaning that membrane breakage will immediately be followed be self-sealing of the two resulting pieces into two separate tubules. Membrane breakage through shearing/tearing should thus be a non-leaky process. A recent theoretical description of dynamin's helical torsion/constriction [15] showed that the propagation of the helix' strain along the axis of very long helical polymers should follow a diffusive dynamics. It also predicts that on experimentally observable time scales, the rate of this

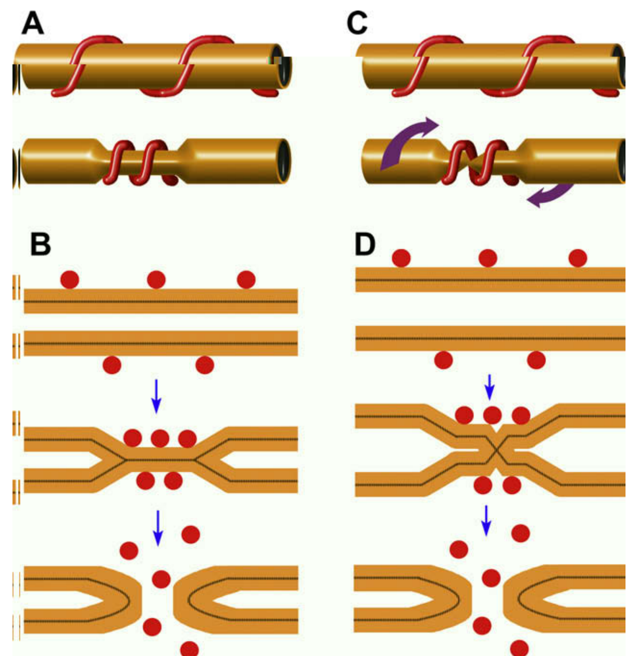

Fig. 1. Membrane tube fission by dynamin. (A and B) Dynamin breaks tubules by constriction. The conformational change (A) of the helix constricts the tubule until it hemi-fission is reached (B), and full fission is obtained when dynamin depolymerizes. (C and D) Dynamin breaks tubules by shearing. The conformational change (C) generates enough torsion to shear the membrane and either tear it (not shown) or fuse it. (D) Fission leads to dynamin depolymerization by removal of the membrane.

propagation and thus the torsion activity of dynamin are imposed by a friction of the helix onto the membrane. Strikingly, the longer the helix, the more difficult the membrane drainage and thus the slower the shearing of the membrane. Therefore, this study predicts that if dynamin breaks membrane by shearing, long dynamin helices break the membrane less efficiently than short ones.

This length-dependent efficiency of dynamin is indeed one of the important conclusions of a recent study [12]. Using supported bilayers on micron-size beads, Pucadyil and Schmid studied the fission efficiency when preformed dynamin-coated tubules were treated with GTP, or when a dynamin/GTP mix was directly applied to membranes. They showed that dynamin combined with GTP could create small vesicles out of the supported membranes. Using preformed dynamin-membrane tubes, they found that when dynamin was allowed to polymerize for a longer time on its own, less fission occured upon GTP addition. They concluded that fission was more efficient when dynamin formed short helices, a situation similar to the one encountered in vivo.

Another conclusion of this paper is that fission is concomitant with the depolymerization of the dynamin coat. This is also the main conclusion of a study by Bashkirov and coworkers [11]. By using membrane tubules extracted with a patch-clamp micropipette from a black lipid membrane, Bashkirov and coworkers monitored the conductance through the tubule while dynamin interacts with it, with or without GTP. When dynamin is added to the tubule in the presence of GTP, the conductance abruptly fell to zero after a random time lag, which is a signature of fission. Conversely, when dynamin was added onto the tubule in the absence of GTP, a gradual decrease of the tubule's conductance was seen, stabilizing at very low values compatible with squeezing of the tubule by dynamin polymerization. When GTP was added to these tubules, a gradual increase of the conductance was first observed, which showed a release of dynamin squeezing by depolymerization. Then, after a time lag, a sudden decrease to zero was observed, which indicated fission. Based on their quantitative evaluation of the tubule radius when dynamin is polymerized, the authors propose that polymerization itself would drive sufficient constriction to bring the membrane into a metastable state. Then, hemi-fission and fission would spontaneously occur when dynamin comes off the membrane, as the dynamin coat maintains the continuity of the membrane until it is released through GTP-dependent depolymerization. It has been argued [16] that the radius measured (5–6 nm, including membrane) for dynamin-coated tubules in the absence of GTP is surprising in view of other studies [4,6,8,9], and might reflect a technical underestimation of the real radius. A tubule of 5 nm radius is certainly in a highly constrained state that makes it metastable, but 10 nm is compatible with stability.

The merit of these two studies is to put the focus on what really triggers membrane fission. Bashkirov et al. [11] clearly showed that fission occurs by hemi-fission, since no leakage is observed. These studies lead us to ask when sufficient constriction is reached to drive hemi-fission, and what triggers full fission. Clearly, these two papers agree on the fact that fission is triggered by dynamin depolymerization. But when is hemi-fission generated?

Putting all this data together, a two-step model for dynamin-mediated membrane fission can be proposed (see Fig. 1). After polymerization of a dynamin helix, full constriction is obtained by GTP-dependent constriction/twisting. This constriction could lead to hemi-fission of the membrane, and fission would occur subsequently to the depolymerization of the coat. This depolymerization could be induced either by conformational stresses appearing upon torsion, or directly from GTP hydrolysis that would weaken the polymer bonds. Alternatively, constriction/twisting could shear the membrane, leading to full membrane fission, promoting depolymerization by removing the substrate for dynamin continuity.

3. Role of membrane properties

In this section, we temporarily turn away from the role of proteins in membrane fission, and consider how the properties of the membrane itself might assist, or even drive its own fission. Lipid membranes are auto-sealable objects, a property that makes them very difficult to break. However, this is mostly true for membranes that are composed of one single lipid, as the non-miscibility of lipids makes lipid bilayers more fragile, and here we first review membrane fission driven by lipid separation. Consistent with our observation that mechanics is relevant for the action of dynamin, we then turn to two important mechanical properties of the membrane, namely its the bending rigidity and tension, which could affect the action of fission machineries, including dynamin.

3.1. Membrane fission by lipid phase separation

The first pieces of evidence for phase separation in lipid bilayers date back to the 70s [17,18]. The formation of domains with a certain lipid composition, floating in an ocean of a different composition, led to the "raft" hypothesis in the late 80s, revealing how membrane properties could affect membrane traffic.

Phase separation is usually associated with an energy cost proportional to the size of the interface. In a three-dimensional system, e.g. oil and water, interfaces are surfaces. The energy cost is thus proportional to the *surface area* of the interface between the two fluids, through a coefficient known as the interface's "surface tension". For two-dimensional lipid domains, interfaces are lines, and the energy cost is proportional to the *length* of the interface, thus defining a "line tension". Both surface and line tension measure how badly the different components want to separate. The requirement that the interfacial energy be minimal implies that fluid membrane domains have a circular shape, which minimized the interfacial length at constant domain surface area. For high line tensions, it was theoretically proposed [19] that another way of reducing the length of the interface would be to bud the domain out of the plane of the membrane, the connecting neck where the interface sits being narrower than the domain (see Fig. 2A). In this case, an extra energetic cost must however be paid to bend the membrane into a curved vesicle. In extreme cases where the

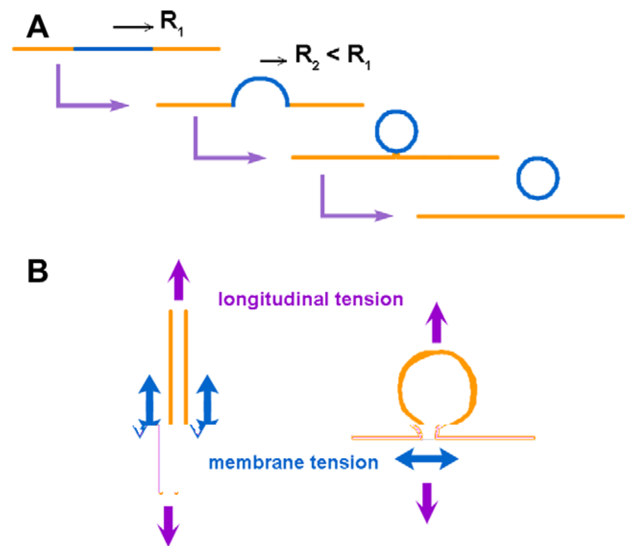

Fig. 2. (A) A lipid domain (blue) is budding and fissioning from the donor membrane (yellow) while line tension is increasing. (B) Combined effects of longitudinal tension and membrane tension depending on the geometry of the membrane (tubule vs. sphere).

line tension is high enough to override this bending cost [20], it was proposed that the constriction generated by phase separation at the neck of these buds would be sufficient to break the membrane. Deformation of membranes by lipid phase separation has been studied experimentally quite recently [21], and led to the first direct measurement of line tension between lipid domains, close to 1 piconewton (pN). In some case, the authors of this work observed the complete disappearance of the neck, suggesting fission, which was confirmed by other studies (see for example [22]). Similarly, lipid phase separation induced along membrane tubules formed by kinesins in vitro leads to fission [23] at the boundary between domains.

3.2. Role of membrane tension

In the instances of phase separation-driven membrane breaking, the membrane geometry and tension play an important role in crossing the energy barrier for fission. For instance, highly tense membrane tubules formed by kinesins (tension larger than 5×10^{-5} N/m) break less than 1s after induction of phase separation, whereas tubules formed at a low tension (smaller than 10^{-6} N/m) take up to 20 s to break ([24] and unpublished data). The role of membrane tension in facilitating tubule fission is twofold: first, as the radius of the tubule is dependent on membrane tension ($radius = \sqrt{\frac{\kappa}{2\sigma}}$, where κ is the membrane bending rigidity and σ is the membrane tension [25]), an increase in membrane tension drives the radius down, which takes the membrane closer to a fissioned state; second, *surface* tension (expressed in N/m) is concomitant with the tubule's *longitudinal* tension (in N), which is defined as the externally applied force required to prevent the tubule from retracting. This longitudinal tension could help fission by maintaining the structure during the operation of the fission machinery. As discussed previously, longitudinal tension facilitates dynamin-mediated fission. This is analogous to the case of a rubber band that needs to be slightly extended in order to be cut by scissors.

To further clarify the relationship between surface tension and longitudinal tension, we note that they may either act in the same direction or have antagonistic effects depending on geometry. As mentioned above, tubules are an example of a synergetic effect between membrane tension and longitudinal tension, as both are coaxial with the tubule (see Fig. 2B). In the case of a spherical bud, on the other hand, longitudinal tension (pulling on the bud) favors fission by facilitating the detachment of the bud, whereas membrane tension has an opposite effect (see Fig. 2B): it tends to flatten the membrane, and therefore to the collapse the bud into a flat membrane. By counteracting constriction, membrane tension could therefore hinder fission. In vivo, it was actually shown that an increased membrane tension can block endocytosis [26], whereas a decreased membrane tension tends to increase the endocytosis rate [27].

3.3. Conclusion

Dynamin and lipid phase separation are two examples of how tubular membrane structures can be broken. Beyond the specifics of these two examples, we are interested in extracting some basic principles of how membrane fission is mediated, which could help understand other fission machineries. In the case of lipid phase separation, fission occurs by constriction, as the domains are fluid and no torsion occurs [23,24]. Membrane parameters can crucially up- or down-regulate the energy barrier to be crossed for fission to occur. A theoretical model [28] shows how the combined actions of actin pulling on an endocytic bud and constriction generated by lipid phase separation could promote fission in systems lacking the active role of dynamin. The main lessons from studies on pure lipid membranes are: (1) fission by a pure constriction mechanism can occur; (2) membrane tension, depending on the geometry of the membrane (a neck between two vesicles or a tubule) can either reduce or enhance the energy barrier to fission and (3) applying external stresses on the constriction neck can help overcome the energy barrier.

However, it is not clear yet how small the radius has to be made in order to lead to fission. The structure of the dynamin helix provides some information about this threshold constriction radius. Dynamin does drive constriction on two occasions: (1) when it polymerizes, and (2) when it undergoes a conformational change while hydrolyzing GTP. Most probably, polymerization does not provide enough constriction to reach fission, as the internal radius is larger than 10 nm after polymerization, a tubule size compatible with membrane stability, as tubules of this size are experimentally observed. After GTP hydrolysis, internal radius was measured to be in the range of 4–5 nm by cryo-EM [9]. This is larger than the thickness of a bilayer (3 nm). However, it is smaller than the thickness of a bilayer plus the threshold radius (3 nm) that was proposed to spontaneously lead to membrane hemi-fission intermediates [29]. It is thus difficult to conclude on the state of the membrane inside the coat after GTP treatment. These data however indicate that the threshold radius for fission must be smaller than 5 nm.

4. Other examples in the light of these principles

As far as we know, most of the fission events happening within a cell are dynamin-independent. Although the fission mechanisms underlying most of these events are still largely unknown, the insight gained from the examples of dynamin and lipid phase separation may help understand the mechanisms at work in other systems involving fission.

In this section, we consider other fission machineries in the light of the systems described above. This discussion is not intended as an exhaustive review, but rather as an attempt to extract similarities and divergences between various biological solutions to the membrane fission problem.

4.1. Other dynamin-like proteins

Many homologues of dynamin have been identified and most of them are involved in membrane remodeling [30]. Several examples come from the fission machinery of mitochondria and chloroplasts. The protein Dnm1/Drp1 (yeast and mammalian, respectively) is the most characterized member of the dynamin superfamily, other than dynamin [31]. It is the main player of mitochondria division, which is mediated by a single fission machinery to break the two mitochondrial membranes in a single event. In chloroplast division, ARC5, another dynamin-like protein, forms the ring necessary for constriction. ARC5 is a cytosolic protein that binds to the outer membrane of the plast, and ARC6 is involved in the alignment of this ring with the matricial collar of FtsZ [32]. Chloroplasts have kept the prokaryotic division machinery through evolution (the FtsZ ring) and its positioning system (the Min proteins). ARC5 was shown to participate in a GTP-dependent constriction of purified chloroplast rings [33], and the amazing supercoiling of these rings could be reminiscent of a twisting activity as described for dynamin constriction [10].

Dnm1 forms helices much wider than those formed by dynamin (55 nm compared to 25 nm, outer radii) that fit the thickness of a double membrane [34]. By analogy, one could expect that ARC5 is structurally similar to Dnm1, and that Dnm1 is able to mediate constriction in a similar GTP-dependent way than ARC5. They also both bind to the outer membrane of the organelle through binding to trans-membrane proteins (PDV1 for ARC5 and Fis1 for Dnm1 through a cytosolic linker called Mdv1, [31,35]).

All these similarities suggest that the mitochondrial and plastid fission machineries work in a very similar way to dynamin itself. However, as we discussed above, if dynamin fission occurs by constriction only, it requires a very tight constriction (radius < 5 nm). Although two membranes are present in mitochondria, Dnm1 has to reach the same final constriction to break the last membrane. Thus, the larger size of these rings that fits the fission intermediates observed in vivo is required for assembly on a larger structure, but would need to constrict to the same radius to complete fission. Increasing the starting size of the helix requires a bigger conformational change to complete fission: dynamin outer radius goes from 25 nm to 20 nm during constriction, an already considerable conformational change. For a similar constriction mechanism to occur in the case of Dnm1/drp1, the radius of the tubules it forms would need to go from 55 nm to 20 nm, and to break two membranes: this would be a formidable constriction, and would probably cause breaks in the polymer. Disruption of the Dnm1 spiral was actually seen when treated with GTP [34]. If dynamin-mediated fission is conducted through a shearing mechanism, the break in the membrane does not depend on the final radius of the tube, but rather on how fast the torsion is applied. Thus, shearing can in principle break thick membrane necks, but probably with leaks. The larger size of Dnm1/drp1 spirals may be the indirect indication that membrane is broken by shearing. Another explanation could account for this larger size. Indeed, the large radius of Dnm1/drp1 might just be required to accommodate the cytosolic domain of the transmembrane receptor (Fis1 for Dnm1) and the cytosolic linker (Mdv1). In this case, after assembly of the three components, the space left in the helical coat for the membrane must be much smaller than when Dnm1/drp1 is alone. In other words, the thickness of the coat containing Dnm1/drp1, the cytosolic linker and the transmembrane receptor would be much bigger than for Dnm1/drp1 alone. Thus, the membrane would already be more constricted by assembled coats and thus a smaller conformational constriction would be required to complete fission. Indeed, a recent study showed that Mdv1 enhances the ability of Dnm1 to self-assemble on liposomes in the presence of nucleotides [36]. The thickness of the coat is enhanced in the presence of Mdv1, even though the size of the helix is unchanged. However, the human equivalent of Dnm1, Dlp1, induces the formation of tubules both in vitro and in vivo [37], but their size is very similar to that of classical dynamin-coated tubules.

Surprisingly, almost all other dynamin-like proteins have been implicated in fusion instead of fission, and have either a transmembrane domain or a highly hydrophobic region that suggest a deep insertion in the membrane: whether there is a connection between these properties, which diverge from the classical dynamins, is still unknown.

A recent study of Atlastin, a GTPase located at the ER, shows that it is critical for homotypic fusion of the ER, maintaining a dense, highly connected network [38]. Atlastin, besides having sequence and structural homology with dynamin [30], was recently shown to form tubules in vitro [39]. Also, the fusion of mitochondria is a two-steps mechanism as it involves two membrane fusions and therefore two fusion machineries, one for each membrane. Both of these machineries have dynamin-like proteins, the mitofusins 1 and 2 and Fzo1 for the outer membrane, and Mgm1/OPA1 for the inner membrane.

The fact that dynamin-like proteins (DLPs) are involved in fusion reactions supports the idea that fission is mediated through a fusion-like mechanism. In this interpretation, fusion would be mediated by the generation of high curvature, as in the case of synaptotagmin [40]. The tip of DLP-coated tubules would be a highly fusogenic point if sufficient curvature is reached. Even though nothing is known about oligomers formed by these specific proteins, one can expect that highly curved tubules and destabilization of the bilayer due to the deep insertion of their hydrophobic parts in the lipids would drive fusion. A consistent biochemical fact with this fusion activity is that they share low GTP hydrolysis rates compared to DLPs involved in fission [41]. It means that fusogenic DLPs would live longer in a GTP bound state, more favorable for polymerization and tubule formation. Long-life tubules would be then more favorable for fusion, having time to connect and fuse with the acceptor membrane.

Based on this assumption, one would predict that the recently described Epsin-Homology Domain (EHD) family of proteins [42] would belong to the fusogenic class of dynamin-like proteins. Proteins of this family are able to polymerize and form tubules coated by a helix. They hydrolyze ATP instead of GTP, but are otherwise both structurally and functionally very similar to other dynamin-like proteins. These proteins are implicated in membrane remodeling, but have no clear fission activity, at least in in vitro assays used for dynamin. They might thus just constitute another type of fusogenic dynamin-like protein.

4.2. Caveolae fission

Caveolae were shown to fission the plasma membrane by a dynamin-mediated process [43]. However, the lipid composition enriched in sphingolipids and cholesterol of caveolae led to the hypothesis that caveolin, the main component of caveolae, could drive lipid phase separation by locally increasing the cholesterol concentration in the membrane [44], as it binds cholesterol. This lipid phase separation could help/drive both the budding and the fission reaction of caveolae. A theoretical study [45] also proposed that not only the lipid phase separation, but the specific ordering of chiral and tilted lipids in the caveolin-coated phase could drive budding, fission and the formation of the surprising proteic patterns observed in vivo [46]. This lipid chirality-induced phenomenon was first proposed to explain the formation of endocytic tubular carriers driven by the binding of a toxin cargo to specific lipids [47].

It thus seems that caveolae are an endocytic route where all the known factors to drive fission are present, but nevertheless their time lapse at the plasma membrane is very long, arguing for a low fission rate. Experimental evidence suggests that caveolae could be endocytic structures blocked at the fission step [48]. It has been proposed that caveolin actually stabilizes raft endocytosis, probably by blocking fission [49]. One can speculate that the role of caveolin, if inhibiting fission, would be to reduce line tension at the boundary between caveolae and the plasma membrane, thus preventing their fission. This function is analogous to that of detergents, which can stabilize oil/water emulsions (i.e. very small droplets of oil in water) by reducing the surface tension of the oil–water interface. This would ensure good sorting of lipids and proteins by lipid phase separation without promoting fission. Fission would still be tightly regulated by the recruitment of dynamin, or by direct removal of the caveolin coat, which would enhance line tension and thus drive fission.

4.3. Golgi COPs

COPs I and II are coat proteins forming spherical carriers involved in trafficking between the ER and the Golgi. It is one of the most studied systems in membrane trafficking, and their sorting, assembly process and regulation are very well characterized. There is however only little knowledge about the fission reaction in these trafficking pathways. It has been proposed that the polymerization of the coat could drive fission by closing on itself as a sphere. This hypothesis is however in conflict with the fact that non-fissioned buds can be isolated in semi-reconstituted systems [50], and with the one that no obvious fission is seen in an assay

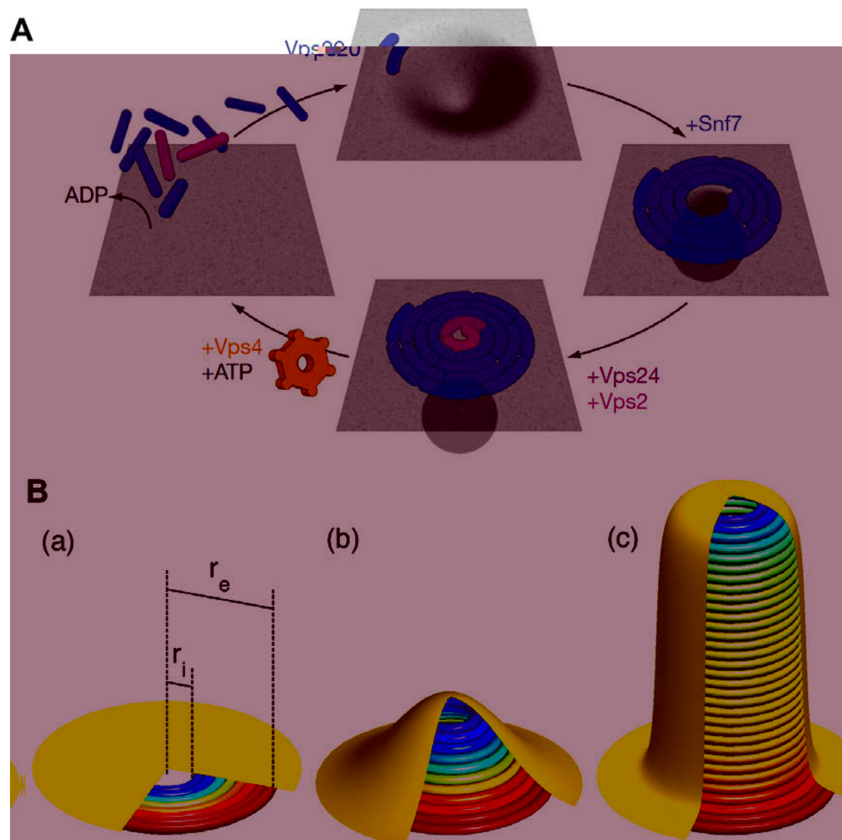

Fig. 3. (A) Budding and fission of membrane generated by the ESCRT-III complex. Polymerization of Snf7 into a spiral drive the deformation into a sphere, and then Vps24 stops the polymerization and finalizes the fission of the bud. Adapted from Ref. [59]. (B) Membrane buckling induced by Snf7 polymerization. As Snf7 polymerizes into rings (a), the smaller (blue) and the larger (red) rings accumulate potential energy as they are not at their preferred radius (yellow). A relaxation can occur by buckling the membrane (b), most of the rings being able to adjust to their preferred radius (c). Adapted from Ref. [58].

reconstituting purified COP I coats on Giant Liposomes [51]. In the COP I system, it was discovered early that Palmitoyl-CoA, a lipid intermediate in acyl chains metabolism, is necessary for the fission of buds [50]. Acyl-CoAs are strong detergents as they associate acyl chains with a large hydrophilic group (the Coenzyme A) which is required for their interactions with enzymes. This may drive fission per se, by destabilizing membranes, and by stabilizing pores and fusion intermediates. However, it is important to notice that non-hydrolysable analogs of Palmitoyl-CoA can block the fission reaction of these buds [50]. This strongly favors a role of Acyl-CoA in the acylation of fission proteins rather than a direct role in membrane fission.

Recent progress has been made on the fission reaction of COP II buds: Sar1p, the small GTPase controlling the recruitment of the coat to the membrane, was shown to participate both in the generation of curvature and in the fission reaction. It was shown that the amphipathic helix used by Sar1p to bind to the membrane could create curvature by insertion, thus tubulating membranes [52]. The same mechanism would help the squeezing of the necks of COP II buds. This could allow for direct fission [52] or fission upon release of Sar1p from the membrane (which itself occurs upon GTP hydrolysis) and the subsequent membrane destabilization [53]. Unexpectedly, the COP coat itself came back in the play recently. A mutation causing Cranio-lenticulo-sutural dysplasia was isolated in SEC23A, a component of the first block of COP II (Sec23/24), recruited to the membrane by Sar1p. Surprisingly, this mutation led to a defect of COP II traffic, where buds and pearled tubules accumulate in vivo [54]. Also, they showed evidences for a defect in recruiting the second level of COP II (Sec13/31 complex), and a synergy with Sar1p, as the Sar1A isoform partially compensate the SEC23A mutant phenotype, as the Sar1B does not. This is probably due to the higher affinity of Sar1A for Sec23/24, recruiting more Sec23/24 to the membrane. Taken together, these observations show that a defect of polymerization is associated with a defect of fission. All other steps of budding (membrane deformation, sorting) seemed unaffected. Thus, it suggests that closing of the bud by polymerization of the coat may cause fission. In other words, the forces needed to break the membrane by constriction could be in part provided by polymerization of the COP II coat. Nevertheless, as expected from previous studies, the coat alone is not able to perform fission and is probably assisted by co-factors, Sar1p in the case of COP II. The role of these co-factors is probably to reduce the energy barrier for fission by facilitating membrane bending, reducing the cost of constriction. However, it is still difficult to picture exactly how mechanically membrane fission occurs in the COPs systems, and hopefully future work will reveal interesting mechanical properties involved in this specific reaction.

4.4. ESCRT-III: deforming and severing the membrane from the inside

ESCRT complexes (Endosomal Sorting Complex Required for Transport) were first identified for their role in endosomal traffic [55]. Among the four known ESCRT complexes, ESCRT-III is the only one involved in the generation of intralumenal vesicles during the maturation of late endosomes to Multi-Vesicular Bodies (MVBs). These membrane-remodeling properties have recently been linked to two importants molecular features. First, one of the proteins of the ESCRT-III complex called Snf7 (CHMP4A,B in mammals) was shown to polymerize once nucleated by Vps20 (CHMP6), another protein of the complex. More precisely, when

overexpressed in cells, Snf7/CHMP4 binds at the plasma membrane, polymerizing into spirals that can form elongated tubules pointing out of the cell [56]. Two different explanations have been proposed for this membrane deforming activity: It was first proposed that the oligomerization of Snf7/CHMP4 would form a lasso-like structure, and that its depolymerization would cause the loop of the lasso to shrink in size, forcing the membrane enclosed by the polymer to curve in order to adjust the reducing size of the loop [57]. Based on morphological images obtained in Snf7/CHMP4 overexpressing cells, a recent theoretical study [58] proposed another, more intuitive explanation: it assumes that Snf7/CHMP4 filaments have a preferred radius of curvature and bind to each other as well as to the membrane, which accounts for the formation of tubular structures. Also, Snf7/CHMP4 has a strong affinity for the membrane. Therefore, in the presence of a membrane, the protein forms planar spirals covering the membrane, as observed experimentally [56]. In this configuration, the spiral rings smaller than the preferred radius of polymerization (the radius of helical polymers in the absence of membrane) are compressed, and spiral rings larger than the preferred radius are extended (see Fig. 3). This frustration of the polymer can be released by buckling the membrane in the center of the spiral, forming a tubule that allows most of the rings to adjust to their desired radius, as well as the binding of a larger number of filaments (see Fig. 3). This buckling mechanism resembles the spiral spring of a watch that pushes the frame out when overloaded.

Further discussions are required to understand how fission is mediated in this system. In order to tackle this question, we first note that the vesicles in the MVBs are budding inside the endosome, and that proteins or lipids involved in fission are *inside* the neck of the bud, which is the exact opposite of dynamin-mediated fission. This geometry seems incompatible with external forces applied to the membrane to squeeze it as in the case of dynamin. Thus, it was proposed that fission could be caused by depolymerization of Snf7/CHMP4, as it required ATP and Vps4 for completion. In the "lasso" hypothesis, fission would be triggered when the loop closes on itself. However, a recent study [59] showed that fission occurs when polymerization is stopped by Vps24. Vps4 and ATP, which are required for the disassembly of the complex, are actually required to resolubilize the proteins and to allow for several cycles of membrane deformation/fission, but not for fission itself. The authors of this last reference propose that the Snf7 spirals could curve the membrane in a similar way than in the "lasso" hypothesis, but with the difference that the reduction of the radius enclosed by the first ring of Snf7 is made by polymerization inside the first ring, forming a spiral (see Fig. 3). At the end, the spiral is closed by addition of Vps24, which completes fission.

Surprisingly, in this case, fission occurs without the need of an energy source. ATP and Vps4 are only required for depolymerizing both Snf7/CHMP4 and Vps24, Vps2 (CHMP3 and CHMP3 resp.) polymers. This means that the energy needed for fission comes from another source. Clearly, ESCRT-III generates membrane deformation and fission in a coupled manner. Vps24, which is the protein completing fission, when combined to its partner Vps2 is able to deform membranes into tubules and make a special dome-like structure that could participate in this fission event [60]. It also participates in the recruitment of Vps4 and Vps2 to the ESCRT-III complex and could be localized at the inner tip of the spirals generated by Snf7/CHMP4. One can speculate that the constriction needed for fission may arise from the tight association of the membrane on this dome-like structure. Then, depolymerization of ESCRT-III structures could occur at the tip/dome-like structure after fission has occurred. Because it challenges our views on membrane fission, ESCRT-III is obviously a system of choice to study membrane fission.

5. General conclusion

Membrane fission is an important topological change in the organization of cellular membranes. Here we have briefly described general principles of membrane fission mediated by lipid phase separation and by dynamin. The common principle of these two fission reactions seems to be a mechanism by which constriction brings the two sides of the membrane into close contact until they fuse, which is an energetically costly step. The differences in the origin of this energy in the examples presented here illustrates the diversity and richness of the field of membrane fission: at one end of the spectrum, the energy required for lipid phase separation originates in the physical interactions between different lipids, which manifest themselves as a line tension; on the other, dynamin-mediated fission is protein-driven and draws its energy from an active mechanism: nucleotide hydrolysis. Although the molecular ingredients involved in membrane fission are very diverse, emerging quantitative approaches taking into account physical parameters might provide a unified framework to study it. A first step in that direction could be a more precise determination of the energies it requires.

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