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Review

Increasing Diversity of Biological Membrane Fission Mechanisms

Henri-François Renard,[1,*] Ludger Johannes,[2] and Pierre Morsomme[1]

Membrane fission is essential to life. It is required for many fundamental cellular processes, as diverse as cyto- and karyokinesis, organelle division, membrane repair, and membrane trafficking and endocytosis. While membrane fission was originally seen as resulting from the action of mechanoenzymes such as dynamin, it is clear that the reality is more complex. In this review, we propose an updated overview of fission mechanisms, and try to extract essential requirements for each. We also present examples of cellular processes that involve these fission mechanisms. Finally, we list pending questions, whether they are specific to a peculiar fission mechanism or more general to the field.

Highlights

Several membrane fission mechanisms have been identified and can be qualified as active or passive, according to a direct requirement for cellular energy sources.

Passive membrane fission mechanisms can rely on amphipathic helix insertion, lipid domain formation and line tension, or protein crowding.

Active membrane fission mechanisms can rely on mechanoenzymatic machineries (dynamin and ESCRT-III complex), membrane-lipid-reorganizing proteins or complexes (actin cytoskeleton and CtBP/BARS), or the appearance of a lipid diffusion barrier (e.g., friction of a BAR domain protein scaffold on underlying lipids) combined with a pulling force induced by molecular motors (friction-driven fission).

Introductory Remarks on Membrane Fission

Biological membranes assume many different shapes, as illustrated by the elaborate and varied structures of cell-surface protrusions and membrane-enclosed organelles in eukaryotic cells. Membrane shape is controlled dynamically, as many essential cell processes (including vesicle budding/tubulation and fission, cell movement, and cell division) require transient membrane deformation. While mechanisms leading to the generation of membrane curvature are reasonably well understood, the situation is more complex for membrane fission, due to its highly dynamic and transient nature. Furthermore, many physicochemical parameters are involved in fission, such as membrane shape, composition, tension, rigidity, and presence of proteins, among others.

In this review, we focus on membrane fission. This process has been extensively studied, and is still an active research field with exciting recent discoveries showing that cells developed several mechanisms that likely operate in parallel. We have decided to classify fission mechanisms into two categories — passive or active — according to their energy requirement. Passive fission mechanisms result from spontaneous reorganization of lipids and/or proteins that lead to constriction without a direct use of energy. For instance, the asymmetric insertion of amphipathic helices into only one of the two membrane leaflets destabilizes the membrane and favors fission [1]. Active fission processes involve the direct consumption of cellular energy by nucleoside triphosphate hydrolysis. The best-studied example is the pinchase dynamin, which exploits the energy from GTP hydrolysis for membrane constriction [2].

Passive Membrane Fission Mechanisms

Asymmetric Transbilayer Stress by Amphipathic Helix Insertion

Insertion of amphipathic helices into one leaflet of a bilayer can induce membrane bending. Mechanistically, such helices behave as 'wedges' that create local membrane curvature. Upon clustering, they are able to generate larger curved membrane regions. For instance, the insertion of the N-terminal helix of the yeast Sar1p GTPase into membranes at the endoplasmic reticulum–Golgi interface generates membrane curvature required for the formation of coat protein complex (COP)II vesicles [3]. It has also been shown that amphipathic helix insertion can

[1]Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud 4-5, B-1348 Louvain-la-Neuve, Belgium
[2]Institut Curie, PSL Research University, Cellular and Chemical Biology unit, CNRS UMR3666, INSERM U1143, 26 rue d'Ulm, 75248 Paris Cedex 05, France

*Correspondence: henri-francois.renard@uclouvain.be (H.-F. Renard).

favor membrane fission [4,5] (Figure 1A, Table 1). Increasing the density of helix insertions directly promotes the transition from membrane tubulation to vesiculation *in vitro* and *in vivo* [1]. The action of amphipathic helices can be antagonized by scaffolding Bin/Amphiphysin/Rvs (BAR) domains (Box 1), which tend to stabilize curved membranes. Therefore, N-BAR domain proteins such as endophilins, which contain N-terminal amphipathic helices, possess both antagonistic properties. It should also be noted that the contribution of helices to scission was observed in liposomal model systems, where protein concentrations and lipid compositions are likely different from the cellular context [1]. Whether fission-compatible helix densities can be reached in biological membranes remains to be documented.

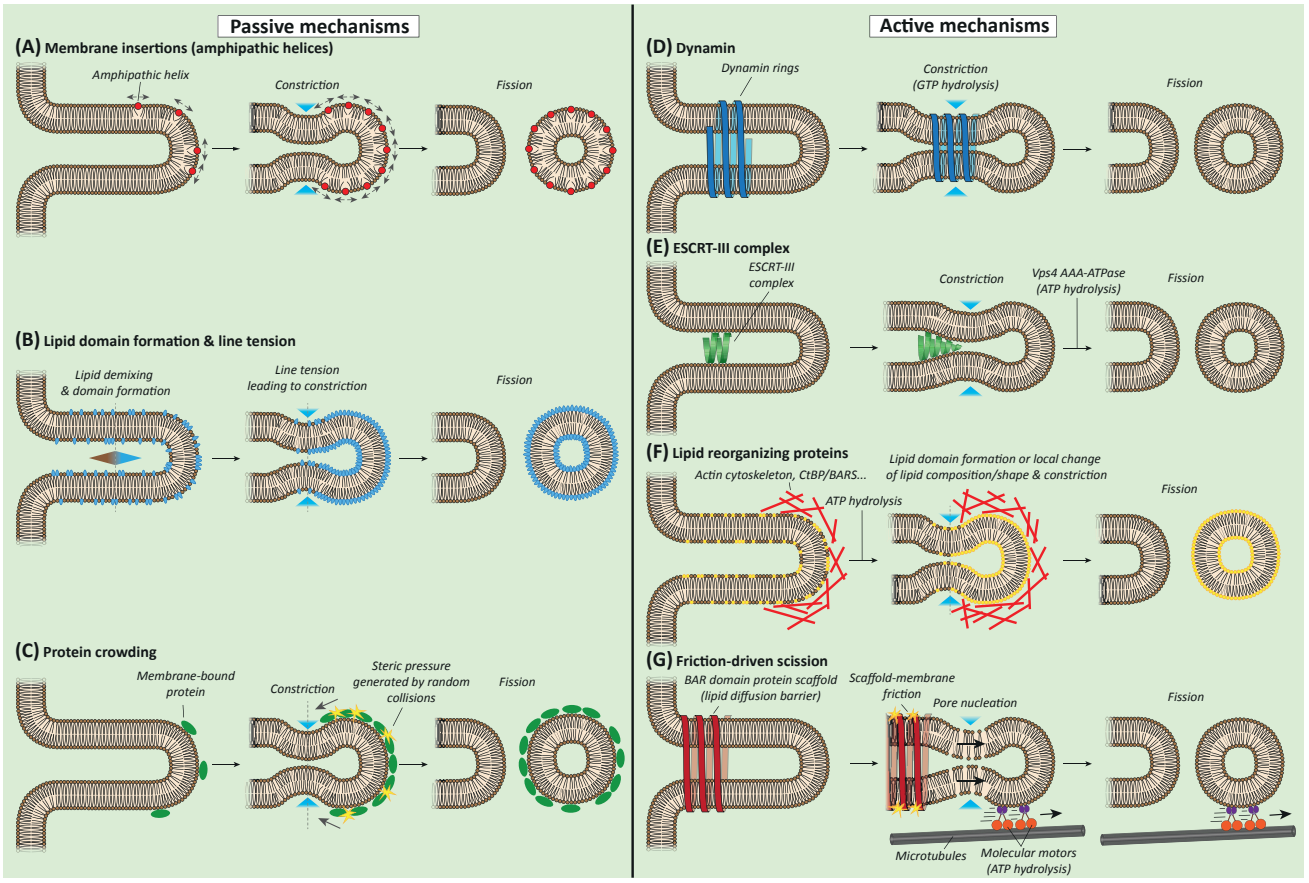

Figure 1. Membrane Fission Mechanisms at a Glance. Membrane fission mechanisms can be classified as passive (left panel) or active (right panel). Passive mechanisms do not require the direct consumption of cellular energy. They can be based on the insertion of amphipathic helices into the membrane (A), the formation of lipid domains leading to the generation of line tension (B), or the crowding of membrane surface by proteins (C). Active mechanisms require the direct use of cellular energy (i.e., hydrolysis of GTP or ATP) at some step of the process to complete membrane fission. Mechanoenzymatic machineries such as dynamin (D) or ESCRT-III complex (E) act directly as membrane constrictors. While dynamin is a GTPase, ESCRT-III complex requires the activity of the AAA-ATPase Vps4 to complete the fission process. Other proteins or complexes are thought to modify the lipid composition and/or organization in the membrane (F), leading to domain formation and line tension. For instance, actin polymerization at the membrane surface modifies lipid organization. CtBP/BARS complexes are associated with enzymatic activities that act directly on local membrane lipid composition (e.g., lipases and acyltransferase). Finally, friction-driven scission (G) requires the scaffolding of the membrane by BAR domain proteins in combination with pulling forces exerted by molecular motors or possibly also by the expanding actin cytoskeleton. Of note, the pictures of the various fission modalities in this figure do not represent the real scale, and the distances to which they can squeeze membranes are not quantitatively reproduced. In the physiological context, they might act on membrane structures that possess very different shapes and sizes. Abbreviations: BAR, Bin/Amphiphysin/Rvs; CtBP/BARS, C-terminal-binding protein/brefeldin A ADP-ribosylated substrate; ESCRT, endosomal sorting complex required for transport; Vps4, vacuolar protein sorting-associated protein 4.

Table 1. Summary of Various Membrane Fission Modalities and Features

Fission modality	Proteins involved	Cellular location	Mode of fission	Refs
Passive mechanisms				
Amphipathic helix insertion	e.g., Epsin, N-BAR domain proteins . . .	Plasma membrane, Golgi, ER, others	Wedging effect of amphipathic helix in one leaflet of the bilayer and generation of asymmetric stress	[1,4,5]
Line tension	Cholera toxin	Unknown (model membranes only)	Phase separation of lipids and constriction by line tension at the boundaries between phases	[6–9,94]
Protein crowding	Any bulky protein	Unknown (model membranes only)	Pressure generated by collisions between membrane-bound proteins	[13]
Active mechanisms				
Dynamin	Dynamins, BAR domain proteins	Plasma membrane, mitochondria, others	Oligomerization of dynamin into helical scaffolds and constriction upon GTP hydrolysis	[2]
ESCRT-III	ESCRT-III complex subunits (CHMP2A/B, CHMP6, CHMP3, CHMP4A/B/C, CHMP5, CHMP7, CHMP1A/B, IST1), Vps4 AAA-ATPase	Plasma membrane, endosomes (MVBs), mid-body, nuclear envelope, others	Oligomerization of ESCRT-III components in spiral filaments able to store elastic energy (like springs), likely used for membrane constriction and fission. ATP consumed by Vps4 for the disassembly and turnover of ESCRT-III components.	[47–50]
CtBP/BARS	CtBP1/2 + 14-3-3γ, PI4KIIIβ, ARF1, FAPP2, PLD, LPAATδ + ARF1, PLD2, LPAATγ	*trans*-Golgi (PGCs) *cis*-Golgi (COPI vesicles)	Membrane lipid reorganization through local *de novo* synthesis of lipids (phosphatidic acid) by associated energy-dependent enzymes? Role of actin cytoskeleton? Molecular motors?	[64–69]
Actin cytoskeleton	Actin, others	Plasma membrane, mitochondria, others	Membrane lipid reorganization, lipid domain formation and constriction by line tension at the boundaries between phases. Mechanical constriction by actin polymerization.	[77–80]
FDS	BAR domain protein (e.g., EndoA2), microtubule-dependent molecular motors (e.g., dyneins)	Plasma membrane, others?	Pulling forces exerted by molecular motors on a membrane tube covered with a BAR domain protein scaffold that reduces lipid diffusion. Fission through lysis.	[87–90]

Box 1. BAR Domain-Containing Proteins

BAR domains are crescent-shaped structures that are ~20 nm long, and formed by the dimerization of an elongated coiled-coil bundle. The BAR domain contains positively charged residues allowing the interaction with the negatively charged head groups of phospholipids. BAR domain dimers assemble to form highly organized oligomeric structures that are able to scaffold onto tubular membranes. Hence, BAR domain proteins are perfect sensors or inducers of membrane curvature.

One distinguishes between several subfamilies of BAR domain proteins according to the shape of the BAR domain and the presence of additional functional domains: classical BAR, N-BAR, BAR-PH, PX-BAR, F-BAR and I-BAR (Table I). Classical BAR domains have affinity for highly positively curved membranes. With an additional N-terminal amphipathic helix that inserts in a shallow manner into membranes, N-BAR domains prefer even higher positive curvatures. PH or PhoX (PX) domains confer affinity to different negatively charged phosphoinositides. The F-BAR (FCH-BAR) domains recognize shallow positive curvature, while I-BAR (Inverse-BAR) domains interact with shallow negatively curved membranes. Additional domains can be found adjacent to the BAR domain in these proteins: SH3 (binding to dynamin as well as actin regulatory WASP/WAVE proteins), RhoGAP, RhoGEF, tyrosine kinase, phosphotyrosine binding domain, PDZ domain, etc. Over the years, BAR domain proteins have been implicated in many cellular functions involving sensing or induction of membrane curvature, such as endocytosis and membrane trafficking, podosome and filopodia formation, or mitochondria and autophagosome shape. Many BAR domain proteins have been linked to various human pathologies, such as genetic disorders or cancers. For further information, the reader is referred to excellent reviews [95,96].

Table I. Subfamilies of Human BAR Domain Proteins, Structures and Associated Diseases (Adapted from [95])

Subfamily	Structure	Names of human BAR domain proteins	Associated diseases
Classical BAR		Arfaptins, ICA69, PICK1, DNMBP (Tuba)	Alzheimer's disease (DNMBP), diabetes mellitus (ICA69)
N-BAR		Amphiphysins/BINs, endophilins/BIFs, nadrins	Paraneoplastic stiff-person syndrome (amphiphysin-1), centronuclear myopathy (amphiphysin-2)
BAR-PH		Oligophrenins, APPLs, GRAFs, centaurin-βs	Mental retardation, epilepsy, and cerebellar hypoplasia syndrome (oligophrenin)
PX-BAR		SNX 1,2,5,6 and 9	
F-BAR		Toca-1, FBP17, CIP4 FCHo1, FCHo2, Fes/Fer kinases, syndapins, srGAPs, PSTPIP-1, PSTPIP-2	Pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PSTPIP), 3p-syndrome (srGAP3)
I-BAR		IRSp53, MIM, IRTKS	Bladder and prostate carcinoma (MIM), Tourette syndrome (IRSp53)

Abbreviations: BAR, Bin/Amphiphysin/Rvs; PH, Pleckstrin Homology; PX, PhoX.

Lipid Domain Formation and Line Tension

Depending on their composition, membranes undergo lipid segregation, leading to the coexistence of phases that have different physical properties (i.e., thickness or stiffness). At phase boundaries, perturbations arise that are energetically unfavorable, leading to line tension. The membrane system tries to reduce these perturbations by reducing the total length of the phase

boundaries (i.e., the line energy of the system). If this occurs in a tubular membrane such as the neck of an invaginated vesicle, the tube/neck diameter is reduced (spontaneous squeezing), which — when strong enough — leads to fission (Figure 1B, Table 1). Line tension-driven fission of tubular membranes has been demonstrated in theoretical studies and model membrane experiments [6–9]. In this experimental context where lipid mixtures are far away from phase transition, line tension can be strong enough to lead to spontaneous squeezing and fission of membranes.

Cellular membranes are more complex than the purely lipidic model membranes that were used in the above-mentioned studies, owing to the presence of large varieties of lipids, and of peripheral or transmembrane proteins. Cellular membranes are maintained close to phase transition, which allows cells to fine tune lipid domain formation required for biological processes. In this context, line tension is weak and might not be sufficient to efficiently drive membrane fission alone. In addition, lipid segregation leading to domain formation in cellular membranes most likely occurs in a triggered fashion. Most *in vivo* examples indicate that in cells, domain formation and line tension-driven scission are tightly controlled by energy-dependent machineries, notably the cortical actin cytoskeleton (see the 'Lipid Reorganization-Driven Fission: Actin Cytoskeleton' section). Moreover, actin polymerization could favor further fission trough mechanical forces applied directly on the membrane.

Protein Crowding

Experimental studies on model membranes [10,11] and theoretical studies [12] have shown that protein crowding can cause membrane bending. Briefly, the lateral pressure generated by collisions between membrane-bound proteins can drive spontaneous membrane budding and tubulation. This phenomenon is independent of the way proteins are attached to the membrane, be it via helix insertion or binding to head groups of phospholipids. A protein coverage >20% is sufficient to induce membrane bending [11]. This mechanism can be reconstituted with bulky proteins whose natural functions are unrelated to membrane bending, such as GFP. Domain size and membrane tension are two global parameters that influence the size of deformations induced by protein crowding [10]. Indeed, tubular deformations generated by protein crowding frequently consume the entire protein-coated domains such that the domain sizes tightly define the tubule surface area. In addition, the size of tubular deformations is constrained by the maximum membrane tension that can be reached by the system.

More recently, it has been proposed [13] that protein crowding can also lead to membrane fission, if pressure generated by collisions between proteins is not counterbalanced on the opposite membrane surface (Figure 1C, Table 1). Similar to previous studies about the role of protein crowding in membrane bending, membrane fission efficiency depends on protein coverage, but not on the way by which proteins are attached to the membrane. Moreover, proteins with large hydrodynamic radii drive fission more efficiently than small proteins.

While protein-crowding-driven fission seems to be effective *in vitro*, it is still unclear to what extent this mechanism contributes to membrane fission in cells.

Active Membrane Fission Mechanisms

Membrane-Constricting Mechanoenzymatic Machineries: Dynamin

Dynamin is the first mechanoenzyme shown to catalyze membrane fission [14] (Figure 1D, Table 1). Dynamin is a 100-kDa GTPase that has three isoforms in mammals: dynamin 1 and 3 expression is restricted to neurons, while dynamin 2 is ubiquitously expressed. Based on its remarkable property to form contractile helical polymers around membrane tubes, dynamin

isoforms and related proteins are involved in cell functions as diverse as organelle division and fission of endocytic membrane invaginations. While dynamin was first thought to be associated only with clathrin-dependent endocytosis, it is now clear that the protein plays a role in some, but not all clathrin-independent endocytic events [15,16].

Dynamin proteins have three well-established properties (detailed review in [2]): (i) their ability to oligomerize into helical scaffolds around membrane tubes; (ii) their ability to constrict the membrane in a nucleotide-dependent manner by conformational changes; and (iii) their ability to induce membrane fission in a GTP-hydrolysis-dependent manner. Despite having been studied for more than two decades, key aspects of the molecular mechanism of dynamin function still remain debated.

The ability of dynamin to oligomerize around membrane tubes has been known for ~30 years (for a complete bibliographic overview about this property, see review [2]). In the absence of GTP, dynamin assembles into a helical scaffold with an outer diameter of 50 nm, and a pitch of 10–20 nm between helix turns. The membrane tubule is thereby constricted to 20 nm in diameter. While the GTPase domains face outside of the polymer, dynamin possesses plekstrin homology (PH) domains on the inside that mediate binding to the membrane [17].

Membrane tubes are more constricted upon dynamin binding to, and hydrolysis of, GTP. Indeed, a superconstricted state of dynamin helices could be trapped around a membrane tube either by using the K44A mutant, whose GTPase and fission activities are impaired, or by observing wild-type dynamin after short incubation time [18]. To achieve constriction, dynamin undergoes a conformational change by twisting or torsion, and generates a rotational force or torque [19,20].

The GTP-hydrolysis-dependent fission property of dynamin has been established for many years, based on experiments on cells and in model membrane reconstitution systems (reviewed in [2], most recent publications [19,21,22]). As expected for any kind of membrane fission mechanism, dynamin-mediated fission proceeds through a hemifission state that has been documented both *in vitro* (reviewed in [2]), and *in vivo* [23]. Moreover, membrane tension and rigidity are two crucial parameters that modulate the rate of dynamin-driven membrane fission. Indeed, dynamin-mediated fission is accelerated either by increased membrane tension [19], or by reduced membrane rigidity; the latter of which can be achieved in the presence of polyunsaturated lipids [19,24].

The final step leading from hemifission to total fission probably remains highly contentious. Several models have been proposed (reviewed in [25]); two of which appear as the most likely ones, according to the latest developments in the field: the disassembly model, and the constriction/ratchet model. The disassembly model proposes that dynamin assembles to constrict the membrane, and then disassembles upon GTP hydrolysis to free the highly constricted membrane, which now can complete fission. The constriction/ratchet model proposes that dynamin acts as a molecular motor, where energy from GTP hydrolysis serves to generate a torque that twists the dynamin helix leading to the constriction of membrane tubule underneath. Both models can account for some experimental datasets, but not for others (see review [2] for more details). Yet, using high-speed atomic force microscopy, a recent study by Colom *et al.* [26] provided clear evidence for dynamic remodeling of the dynamin helix upon GTP hydrolysis leading to constriction, favoring the constriction/ratchet model. This model is further supported by another recent study showing that the disassembly of dynamin is not coupled to GTPase activity [27].

Another important aspect under debate is the precise role of dynamin binding partners in the fission process. In particular, dynamin interacts through its proline-rich sequences with SH3 domains of BAR domain proteins (Box 1). These BAR domain proteins may help to recruit dynamin and facilitate its polymerization. However, a direct role of BAR domain proteins in dynamin-dependent fission is still under debate, as both inhibitory and stimulatory effects on GTPase activity and fission have been observed (see review [2]). Recently, endophilin was shown to block dynamin-dependent membrane fission when present in excess by intercalating between turns of the dynamin helix [28]. To what extent this *in vitro* observation also applies in the cellular context remains to be established. Other BAR domain proteins, without interacting directly with dynamin, might prepare the foundation for dynamin polymerization at the membrane. For instance, using computational modeling and super-resolution imaging, Schöneberg *et al.* [29] recently showed how the lipid-mediated recruitment of the PX-BAR domain protein sorting nexin (SNX)9 at the neck of clathrin-coated pits regulates constriction, thereby providing a template for dynamin-mediated fission.

Membrane-Constricting Mechanoenzymatic Machineries: ESCRT Complex

The endosomal sorting complex required for transport (ESCRT) is a conserved membrane remodeling machinery that is composed of four multisubunit protein complexes, ESCRT-0, I, II, and III. ESCRT was initially discovered in the vacuolar pathway of yeast for its function in the sorting of ubiquitinated cargoes into intraluminal vesicles of multivesicular bodies (MVBs) [30–33]. Among the four subcomplexes, ESCRT-III proved to be a transplantable membrane fission machinery able to catalyze membrane separation at various locations in cells (Figure 1E, Table 1). Recent studies suggest that ESCRT-III complex is involved in cellular egress of enveloped retroviruses such as HIV [34–37], extracellular vesicle release, plasma membrane repair [38,39], sealing holes in the nuclear envelope [40–42], abscission during cytokinesis [43], neuronal pruning, and quality control of nuclear pore complex in yeast [44–46] (see also reviews [47,48]). All of these processes rely on the same basic mechanism, that is, the fission of membranes that were previously connected by a channel filled with cytoplasm. ESCRT-III localization to the various sites in cells is mediated through its interaction with specific adaptors (see reviews [49,50]).

From a mechanistic perspective, many unanswered questions persist on how ESCRT-III achieves membrane fission. ESCRT-III components organize in spiral filaments able to store elastic energy (like springs), which most likely is used for membrane deformation and fission [51–55]. ESCRT-III components also interact with vacuolar protein sorting-associated protein (Vps)4, an AAA-ATPase that drives the disassembly of complex components [56–58]. A recent study showed that Vps4 not only induces disassembly of ESCRT-III polymers, but also turnover of its subunits, leading to dynamic growth and shrinkage of ESCRT-III spirals [59]. Whether ATP hydrolysis on Vps4 actually occurs before or after fission is still subject to ongoing investigations. For more details on the various proposed models, the reader is referred to other recent reviews [50,60]. Little is known regarding the physical parameters required for ESCRT-III-dependent membrane fission. It was shown that membrane tension release is required for ESCRT-III assembly and efficient fission during cytokinesis [61]. Further studies, in particular biophysical approaches are necessary to understand how membrane fission occurs.

Lipid Reorganization-Driven Fission: CtBP/BARS

CtBP/BARS (C-terminal-binding protein/brefeldin A ADP-ribosylated substrate) should not be confused with BAR domain proteins. CtBP/BARS are ubiquitously expressed and encoded by two genes in invertebrates: *CtBP1* and *CtBP2* [62–64]. CtBP1 and CtBP2 proteins both have dual functions as transcriptional co-repressors in the nucleus, and as regulators of membrane fission in

the cytoplasm [62–64]. As transcriptional co-repressors, they regulate cellular activities, such as cell growth and differentiation [62–64]. In the cytosol, CtBP/BARS are involved in various membrane fission processes (Figure 1F, Table 1): the formation of Post-Golgi carriers (PGCs) at the *trans*-Golgi network (TGN) [65,66], release of COPI-coated vesicles at the *cis*-Golgi [67], and fluid-phase endocytosis [65,66] and micropinocytosis [68,69] at the plasma membrane.

From a regulatory perspective, the current model suggests that CtBP/BARS proteins shuttle between nucleoplasm and cytosol. The switch that determines the localization and the function of CtBP/BARS would be controlled by the binding of cofactors, the oligomeric status, and post-translational modifications [63,64]. CtBP/BARS dimerizes upon NAD(H) binding, which seems to inhibit its fission activity, while binding of acyl-coenzyme A to the same NAD(H)-binding site favors the monomeric conformation and increases membrane fission activity [63,64,67]. In addition, phosphorylation and SUMOylation of CtBP/BARS also intervene in their shuttling between the nucleus and the cytoplasm [64].

At the TGN, CtBP1/BARS is part of a larger complex together with 14-3-3γ proteins and phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ), which catalyzes the production of phosphatidylinositol 4-phosphate (PI4P) [64–66]. This complex appears to be crucial for the fission of PGCs from *trans*-Golgi membranes. PGCs are pleomorphic tubular shaped carriers from 100 nm to several micrometers in length, which elongate along microtubules. Other proteins interact with this complex, such as ADP ribosylation factor (ARF)1, which recruits and activates PI4KIIIβ on the Golgi, or the glycolipid-transfer protein FAPP2, which binds to ARF and PI4P and has been proposed to induce membrane bending (for review, see [64]). Furthermore, CtBP1/BARS might be an adaptor that recruits phospholipase (PL)D to Golgi membranes [70]. PLD catalyzes the production of phosphatidic acid (PA). In addition, CtBP1/BARS binds to and activates *trans*-Golgi localized lysophosphatidic acid (LPA) acyltransferase type δ (LPAATδ) that converts LPA into PA [71]. The local change in lipid shape by the transformation of LPA to PA might favor membrane bending and potentially facilitate fission.

At the *cis*-Golgi, CtBP1/BARS is involved in the fission of COPI-coated vesicles [67]. Similarly, CtBP1/BARS interacts with other proteins, such as ARF1, phospholipase PLD2, or lysophosphatidic acid acyl-tranferase type γ (LPAATγ) [64,66,70,72]. The LPAATγ-catalyzed transformation of LPA to PA is again expected to favor membrane constriction and fission. The fact that CtBP/BARS is found to interact with many enzymes that locally modify lipid composition, thereby favoring membrane constriction suggests that these activities are directly involved in the fission step. At this stage, such a lipid-mediated fission mechanism remains to be fully established. Some authors [64] have brought up the question of whether other cellular factors such as the actin cytoskeleton or molecular motors are also involved in CtBP/BARS-driven fission reactions. Indeed, fission is never completely arrested when CtBP/BARS is absent, which is also observed for other fission factors. Additional investigations thereby need to be performed to fully characterize the mechanistic details of CtBP/BARS-mediated fission.

Lipid Reorganization-Driven Fission: Actin Cytoskeleton

In mammalian cells, the actin cytoskeleton is considered as a major player in membrane fission during clathrin- and dynamin-independent endocytic processes [15]. Actin also functions in clathrin-mediated endocytosis when uptake needs to overcome resistance, such as that imposed by elevated membrane tension [73] or that experienced by integrins that are still in contact with extracellular matrix [74]. In yeast, the actin cytoskeleton and myosin motors play an essential role in membrane invagination and fission during clathrin-mediated endocytosis [75]. More recently, it was shown that the interaction between the yeast dynamin-like protein

Vps1 and actin is required to transduce the force of actin polymerization onto the membrane to drive successful scission [76]. In all of these actin-dependent processes, the energy barrier that needs to be overcome for effective fission of membrane invaginations is lowered due to the pulling forces exerted by the polymerization of actin, and/or the activity of molecular motors [77]. In addition, it has been proposed that actin polymerization on membranes promotes lipid domain formation [78], leading to the appearance of line tension, which might contribute to scission [79] (Figure 1F, Table 1; see the 'Lipid Domain Formation and Line Tension' section). In a study combining model membranes and cell experiments, it was shown that such a mechanism contributes to the scission of Shiga-toxin-induced endocytic plasma membrane invaginations in mammalian cells [80]. Further studies are required to identify adaptors that recruit the actin cytoskeleton onto membranes.

The role of actin and actin-related proteins in fission at the level of intracellular organelles has also been documented. It was proposed [81] that together with dynamin, actin and actin-related proteins are involved in the fission of carriers from the TGN. Indeed, the small GTPase Rab6 together with myosin II were observed to play a role in the biogenesis of tubular carriers at the Golgi [82]. However, the exact underlying biophysical mechanism remains to be described. Actomyosin has also been proposed to contribute to mitochondrial fission, together with the dynamin protein Drp1 [83,84], and to endosomal fission, together with dynamin and microtubule-dependent molecular motors [85].

Friction-Driven Scission (FDS)

Proteins from the BAR domain superfamily (Box 1) are specialized in membrane curvature recognition [86] and are able to form organized scaffolds around membrane tubes [87]. By screening a library of BAR domain proteins, endophilin-A2 (endoA2) was found to be localized on Shiga-toxin-induced plasma-membrane invaginations, and to play a role in the scission reaction [88]. In addition, microtubule-dependent motor activity of dynein exerts pulling forces on these invaginations [88,89]. Dynein-mediated pulling leads to membrane fission only when tubular invaginations are scaffolded by endoA2 [88]. Indeed, the endoA2 scaffold creates a frictional barrier that limits the diffusion of underlying lipids [90]. As a consequence, upon pulling with speeds that exceed 50 nm/s, local membrane tension builds up, and the lack in lipid supply leads to tube thinning, until the tube undergoes fission through lysis at the extremity of the scaffolded area. This newly discovered membrane fission mechanism was termed FDS (Figure 1G, Table 1) [90]. This FDS mechanism may operate more generally in processes of clathrin- and caveolin-independent endocytosis that are often poorly reliant on dynamin [15]. FDS might also operate in clathrin-mediated endocytosis in yeast, where both actin and the BAR domain proteins Rvs161/Rvs167 regulate fission [91–93]. Of note, it was shown that the N-terminal amphipathic helix of endoA2 is dispensable for scaffold formation and subsequent FDS, as the fission process is still efficient with endoA2 mutants lacking this helix [88], or with other BAR domain proteins that naturally are devoid of amphipathic helices [90]. Rather, the amphipathic helix is required for efficient binding of endoA2 to the membrane.

Concluding Remarks

The mechanisms that lead to the tight separation of a membrane-bounded organelle have fascinated an increasing number of researchers over the last 30 years. Much progress has been made since the discovery of dynamin as the first mechanoenzyme with pinchase activity. It is clear today that many other mechanisms contribute to fission. A striking fact is that the greatest advances in the field are made thanks to multidisciplinary approaches that combine cell biology and biophysics. Key predictions from cellular experiments can be stringently tested *in vitro*, which in turn informs new cell-based explorations. In addition, valuable data can also be

Outstanding Questions

Why did cells conserve so many different membrane fission modalities? Can these different modalities work together to achieve better control and higher efficiency of membrane fission? Such a cocktail hypothesis calls for further investigation.

In lipid reorganization-driven fission, how is the actin cytoskeleton bound onto membranes?

How does dynamin bring membranes to fission-compatible distance *in vivo*? What is the contribution of dynamin interactors (e.g., BAR domain proteins)?

How to observe the dynamics of ESCRT-III spirals *in vivo*?

What is the exact mechanism by which CtBP/BARS drive membrane fission? Are the CtBP/BARS-associated enzymes that catalyze changes in lipid shape and composition the only fission drivers in this mechanism?

How to assess the function of protein crowding-dependent fission and FDS *in vivo*? These exciting new fission modalities call for further exploration in the cellular context.

How are molecular motors recruited to membranes for FDS?

Does FDS also function on other organelles (e.g., endosomes with SNX proteins)? What are the molecular motors that could be involved (speed and directionality)?

In FDS, how is the scaffold attached to the plasma membrane such as to avoid that it is pulled in with the tubule?

Are there additional membrane fission modalities remaining to be discovered?

obtained from experiments in whole organisms. This back and forth between *in vivo* and *in vitro* approaches has already produced ground-breaking findings in the field and will be essential for future work.

To illustrate the latter point, we direct readers to the two most recently identified membrane fission modalities – FDS [88,90] and protein crowding [13]. These new hypotheses have mainly been dissected on model membranes, and now need to be transposed in further detail in the cellular context, which will be challenging, owing to the dynamic and transient nature of membrane fission intermediates. Moreover, the exciting possibility that FDS can function in combination with other fission modalities increases the complexity of the situation. Further studies are needed to single out the individual contributions of each of them. Experimental setups in which the various fission modalities can be switched on and off (ideally with small molecule inhibitors that can be added acutely) would be ideal to address this goal. Exploration of protein crowding-driven scission will also be challenging. While this mechanism was shown to work on model membranes with low tension, it will be important to see how it operates in the cellular context, where membranes can be tensed locally, and where membrane-bound cytoskeleton and other proteins may increase membrane rigidity. Owing to the high number of proteins interacting with membranes, one would intuitively guess that protein-crowding-driven scission in cells must be highly controlled locally to avoid anarchic and deleterious membrane deformation and fission.

Another salient example of this necessity for back and forth between *in vivo* and *in vitro* studies is the controversy about the role of amphipathic helix insertion in membrane fission [1]. Recent studies indicate that amphipathic helices are dispensable for FDS to operate [88,90]: (i) despite the presence of amphipathic helices, a scaffold composed of the N-BAR domain protein endoA2 does not induce spontaneous fission if it is assembled on preexisting membrane tubes; and (ii) FDS still works with BAR domain protein scaffolds lacking hydrophobic insertions in the membrane. It is likely that the depth of helix insertion is critical for the fission outcome, and depends on whether the initial membrane substrate is vesicular [1] or tubular [88,90].

FDS is generic and may operate with any protein forming a scaffold around a membrane tube. In FDS, amphipathic helices mainly contribute to efficient membrane recruitment of BAR domain proteins and scaffold stability, rather than directly promoting fission. The recently described protein-crowding-driven fission also challenges this view according to which hydrophobic insertions are strictly required for membrane fission, suggesting instead that the role of insertions is to anchor proteins strongly to membrane surfaces, thereby amplifying steric pressure [13].

While dynamin-mediated membrane fission is the most studied and best understood mechanism so far, many questions are still unresolved [2]. Further discrimination between the various models proposed to explain how dynamin accomplishes fission is required. The most prominent question remains to understand how dynamin can bring membranes to fission-compatible distances in cells. Cryo-electron microscopy (cryo-EM) has already allowed to image superconstricted states of dynamin *in vitro* [18]. It should become possible to perform *in vivo* cryo-EM with high resolution, so that superconstricted states are then observable in living cells. A combination of *in vitro* and *in vivo* studies would be expected to address to what extent and how dynamin interactors such as BAR domain proteins collaborate with dynamin in the finals steps of membrane fission.

With the increasing number of membrane fission mechanisms, a fundamental question arises: how do these intertwine (see Outstanding Questions)? It appears likely that these various membrane fission modalities or modules act in a combined manner. This point is particularly important, as some fission modalities – such as protein crowding or amphipathic helix insertion – are expected to be weak within the context of crowded biological membranes. As a consequence, they would need to be combined together with other fission modalities to achieve proper membrane separation in the cellular context. A salient example in favor of this 'cocktail hypothesis' of fission factors has been recently proposed for the detachment of Shiga-toxin-induced tubular endocytic pits from the plasma membrane [88]. The fission of these invaginations requires the combined action of several modules: actin cytoskeleton, dynamin, and FDS (with endoA2 and dynein). In general terms, combining fission modalities may increase the robustness of membrane scission and might add several layers of regulation. Mechanistically, different fission modalities might act in a synergistic manner. FDS might exploit dynamin for scaffolding. In turn, dynamin function might be facilitated on membranes that are tensed by motor action. Furthermore, line-tension-driven squeezing might bring membrane tubules to radii that are favorable for dynamin binding. The dynamin scaffold might immobilize phophatidylinositides that in turn contribute to line tension. Examples of possible interplay between fission modalities can be extended in profusion, and should be a fruitful ground for future investigation.

To conclude, one might wonder why several membrane fission mechanisms coexist (see Outstanding Questions). It has already been mentioned that multiple entry points for regulation might thereby be generated, and that fission might become more robust. Requirements of the membrane substrate that are specific to given biological processes might also matter. For synaptic vesicles, tight coupling of dynamin onto the isotrophic clathrin coat appears optimal to assure the generation of even-sized carriers. In contrast, cargoes of clathrin-independent endocytosis such as Shiga and cholera toxins need microtubule tracks to reach the perinuclear area for intracellular sorting. In this case, coupling with dynein motors might occur early at the plasma membrane, which would favor FDS. The ESCRT machinery is yet another example where a specific membrane configuration (here: negative curvature) requires dedicated machinery. Due to their highly dynamic and transient nature, fission mechanisms are difficult to observe. The creativity of researchers will be required to overcome this obstacle. In particular, we believe that the combination of *in vitro* and *in vivo* approaches is a key for further exploration of these fascinating mechanisms and the interplay between them. This should help to understand how cells are able to coordinate fission events with specific biological functions in a spatial and temporal manner.

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