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Geometry of membrane fission

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ABSTRACT

Cellular membranes define the functional geometry of intracellular space. Formation of new membrane compartments and maintenance of complex organelles require division and disconnection of cellular membranes, a process termed membrane fission. Peripheral membrane proteins generally control membrane remodeling during fission. Local membrane stresses, reflecting molecular geometry of membrane-interacting parts of these proteins, sum up to produce the key membrane geometries of fission: the saddle-shaped neck and hour-glass hemifission intermediate. Here, we review the fundamental principles behind the translation of molecular geometry into membrane shape and topology during fission. We emphasize the central role the membrane insertion of specialized protein domains plays in orchestrating fission in vitro and in cells. We further compare individual to synergistic action of the membrane insertion during fission mediated by individual protein species, proteins complexes or membrane domains. Finally, we describe how local geometry of fission intermediates defines the functional design of the protein complexes catalyzing fission of cellular membranes.

1. Introduction

Geometry of membrane fission describes a sequence of membrane deformations leading to creation of two separate membranes from a single one. Fission begins from bulk rearrangement of the initial membrane. At this stage the dividing parts acquire their shapes and the division zone becomes apparent (Fig. 1A). The direction of these membrane transformations (inward or outward of the cytoplasm) will define the topology of proteo–lipid arrangements during fission.

The second stage in membrane fission consists in progressive constriction of a thin membrane neck forming between the dividing membranes (Fig. 1B). The constriction inevitably creates elastic stresses in the neck's membrane (Frolov and Zimmerberg, 2010; Kozlovsky and Kozlov, 2003). To minimize these stresses, the neck optimizes its geometry and molecular composition, soon becoming an isolated domain of the membrane system (Shnyrova et al., 2009). Acute accumulation of distinct protein species on the neck correlates with the severing of the neck (Taylor et al., 2011). Consequently, fission is often defined as the breakup of the neck, the definition ignoring the rest of the membrane system and emphasizing the local character of the forces operating at the neck. The geometry of the neck near the breakup point determines the energetics of fission and spatial organization of protein machinery responsible for fission in cells (Kozlovsky and Kozlov, 2003; Shnyrova et al., 2013).

The succession of non-bilayer intermediates underlying the neck breakup constitutes the third stage of fission (Fig. 1C). During this stage the actual membrane breaching localizes to as tiny neck as possible in order to avoid physiologically harmful leakage of contents from membrane compartments. Sure enough the breakup pathway remains the most speculative part of fission reaction. Experimental observations and computer simulations of fission

Abbreviations: COPI, coat protein I; COPII, coat protein II; ENTH domain, epsin N-terminal homology domain; BAR domain, Bin–Amphiphysin–Rvs domain; N-BAR domain, BAR domain with N-terminal amphipatic helix; F-BAR, Fer-CIP4 homology BAR domain; AH, amphipathic helix; PHD, plekstrin homology domain; GTP, guanosine triphosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; FAPP, four-phosphate-adaptor protein; ESCRTIII, endosomal sorting complexes required for transport-III; M2 protein, matrix protein 2; Arf1, ADP ribosylation factor 1; GUV, giant unilamellar vesicle; ArfGAP1, ADP ribosylation factor GTPase activating protein 1; CHMP2, charged multivesicular body protein 2; CHMP3, charged multivesicular body protein 3; Vps4, vacuolar protein sorting-associated protein 4; NT, nanotube; PLA2, phospholipase 2; k_B, Boltzmann constant; CLEM, correlative light and electron microscopy; SNARE, soluble NSF attachment protein receptor; DOPE, 1,2-dyoleoyl-*n*-glycero-3-phosphoethanolamine; PE, phosphatidylethanolamine; Rh-DOPE, rhodamine DOPE.

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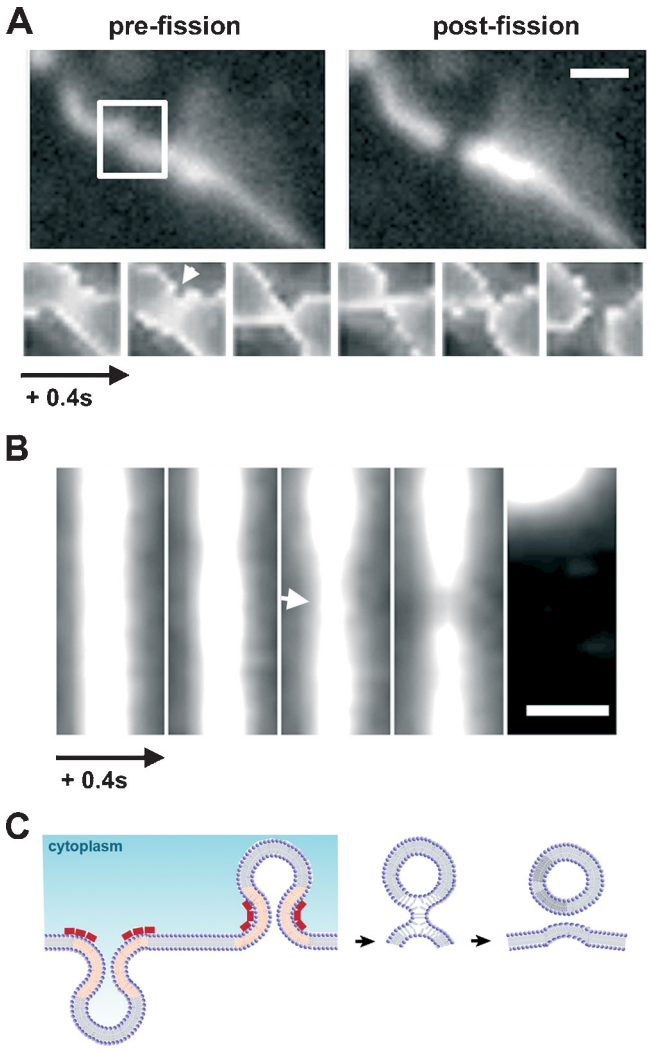

Fig. 1. Key geometries of membrane fission: thin membrane neck and hourglass hemifission intermediate. (A) Formation of a thin membrane neck precedes mitochondria fission. DiO marked membrane is observed, bar 1 μm. Time sequence shows the neck evolution toward fission. White arrow points to the neck. (B) Membrane neck formation and fission in a reconstructed system containing a lipid nanotube, dynamin-1 and GTP (Shnyrova et al., 2013). Rh-DOPE fluorescence is observed, bar 1 μm. White arrow points to the forming neck. (C) A cartoon illustrating the hypothetic "hemifission" pathway of neck breakup. Proteins driving the membrane remodeling (red squares) gather either on the internal or the external monolayer of the neck, dependently on the directionality of fission reaction (toward or outward the cytoplasm, left panel). Local "self-fusion" of the inner monolayer of the neck results in hemifission configuration (middle panel), which further decays to complete fission (right panel). Pink color outlines the area with negative Gaussian (saddle-shape) curvature.

suggest that breakup proceeds through a hemifission stage (Bashkirov et al., 2008; Markvoort et al., 2007; Noguchi, 2012; Yamamoto and Hyodo, 2003). In hemifission the inner monolayer of the neck splits into two disconnected parts while the outer monolayer collapses into a micelle-like structure (Kozlovsky and Kozlov, 2003). Topologically, this structure resembles the hemifusion state, implying mechanistic similarities between fusion and fission (Chernomordik and Kozlov, 2003; Frolov and Zimmerberg, 2010; Markvoort et al., 2010). Theoretical analysis has identified the preferable geometry of hemifusion state to be an hour-glass monolayer stalk (Kozlovsky and Kozlov, 2002; Kuzmin et al., 2001), whose shape has been recently confirmed in experiments (Aeffner et al., 2012; Yang and Huang, 2002). Similar monolayer stalk has been postulated to be the core hemifission intermediate, as this structure is the most energetically feasible (Kozlov et al., 2010; Kozlovsky and Kozlov, 2003). The shape and stability of the hemifission intermediate depends on its composition. While it is thought to spontaneously translate into complete fission in pure lipid systems (Kozlovsky and Kozlov 2003; Markvoort et al., 2007), it can stochastically transform back to the constricted neck if enclosed into a protein cage (Shnyrova et al., 2013). However, proteins can alleviate the elastic stresses in cylindrical micelle configuration, thus potentially stabilizing the hemifission topology (Mizuno et al., 2010, 2012).

As a rule, creation of bulk and local geometries of cellular membrane fission is a function of specialized protein complexes. Despite different structural and self-organizing principles many of such proteins implement remarkably similar mechanisms of local membrane deformations (Kozlov et al., 2010), which might have constituted the basis for the evolutionary development of fission machinery. Therefore, this review is focused on the fundamental mechanisms that proteins implement to control the geometry of fission at its different length scales. We will highlight the results from in vitro experiments and theoretical modeling that have been instrumental in revealing force factors and geometries of fission and illustrate protein specialization and catalytic approaches to hemifission.

2. Molecular geometry and fission

Membrane fission results from the interaction of peripheral membrane proteins with the cytoplasmic side of cellular membranes (Campelo and Malhotra, 2012; Kozlov et al., 2010; McCullough et al., 2013; Rossman and Lamb, 2013). Thus, the topology of fission reaction (Fig. 1C) defines whether the protein machineries assemble on the inner or the outer part of dividing membrane compartments. For example, proteins mediating fission in vesicular transport assemble from the external part of dividing vesicle (Antonny, 2006; Gurkan et al., 2006), while those involved in fission of viruses or luminal vesicles in multivesicular body act from the inside (McCullough et al., 2013; Rossman and Lamb, 2013). As both approaches involve multiple protein species converging to the dividing cellular area, a minimal set of components required to produce fission is generally determined via in vitro reconstitution with minimal systems containing protein(s) of interest and a lipid template.

Such in vitro systems have allowed identifying several groups of proteins and protein domains capable of mediating fission by acting on the external monolayer of the membrane. They include COPI and COPII complexes (Bremser et al., 1999; Matsuoka et al., 1998; Pucadyil and Schmid, 2009), dynamin-1 (Roux et al., 2006; Sweitzer and Hinshaw, 1998) and 2 (Liu et al., 2011), the endocytic proteins epsin and its ENTH domain (Boucrot et al., 2012; Neumann and Schmid, 2013), amphiphysin, endophylin A1 and A3 and their N-BAR domains (Boucrot et al., 2012; Gallop et al., 2006; Peter et al., 2004). These proteins and their domains universally contain limited membrane-inserting parts, which are often described as "wedges" driven into the lipid monolayer by hydrophobic and electrostatic forces (Blood and Voth, 2006; Drin and Antonny, 2010; Zimmerberg and Kozlov, 2006). The wedge shape, evoking the conical shape of lipids with positive spontaneous curvature, has become a protagonist in molecular geometry of fission arranged from the external monolayer of the neck (Auth and Gompper, 2009; Kozlov et al., 2010).

So far two types of protein "wedges" have been directly associated with fission: the amphipathic helix (AH) omnipresent in different protein systems and small-unstructured hydrophobic loops of plekstrin homology domain (PHD). AH motif generally acquires its helical structure upon protein binding to the membrane, the process sensitive to lipid packaging defects, membrane curvature and charge (Bhatia et al., 2010; Drin and