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Review

Exploring the links between lipid geometry and mitochondrial fission: Emerging concepts

Ashutosh Agrawal[a,*], Rajesh Ramachandran[b,*]

[a] Department of Mechanical Engineering, University of Houston, Houston, TX 77204, United States of America
[b] Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, United States of America

ABSTRACT

Mitochondria, the double membrane-walled powerhouses of the eukaryotic cell, are also the seats of synthesis of two critical yet prevalent nonbilayer-prone phospholipids, namely phosphatidylethanolamine (PE) and cardiolipin (CL). Besides their established biochemical roles in the regulation of partner protein function, PE and CL are also key protagonists in the biophysics of mitochondrial membrane remodeling and dynamics. In this review, we address lipid geometry and behavior at the single-molecule level as well as their intimate coupling to whole organelle morphology and remodeling during the concerted events of mitochondrial fission. We present evidence from recent experimental measurements ably supported and validated by computational modeling studies to support our notion that conical lipids play a catalytic as well as a structural role in mitochondrial fission.

1. Introduction

All biological membranes comprise a ~30 Å hydrophobic barrier in the form of a lipid bilayer (van Meer et al., 2008; White and Wimley, 1999). Yet, each discrete membrane is composed of an astonishing array of lipids with distinctive biochemical composition and biophysical properties, which in conjunction with the non-uniform distribution and sorting of select lipid species confer both global and local characteristics to that membrane (van Meer et al., 2008; van Meer and de Kroon, 2011). The mitochondria, bounded by two compositionally and morphologically disparate membrane bilayers, namely the outer (OMM) and the inner mitochondrial membrane (IMM), best exemplify this (Osman et al., 2011; Daum, 1985). In this review, we will explore how the distinct lipid composition of mitochondrial membranes, and importantly, local variations therein, affect the biophysical and mechanical properties of the membrane in order to facilitate and catalyze the essential but still poorly understood process of mitochondrial fission.

Of endosymbiotic origin from ancient bacteria, the modern day mitochondria of eukaryotic cells seldom exist as discrete 'bean-shaped' entities as commonly illustrated in textbooks. Instead, they form elaborate, interconnected and dynamic networks that continuously undergo coordinated cycles of fission and fusion (Chan, 2012; Richter et al., 2015; Labbe et al., 2014). Mitochondria, unlike intracellular transport vesicles, cannot be created de novo. Therefore, new mitochondria originate from preexisting ones through the coordinated processes of mitochondrial growth, elongation and division (Fig. 1a). In this regard, mitochondrial fission essentially recapitulates the binary fission of its ancestors. Mitochondrial dynamics and morphology are inextricably linked to the organelle's myriad cellular functions including ATP production, regulation of apoptotic cell death, calcium homeostasis, and control of reactive oxygen species (ROS) (Roy et al., 2015; Wai and Langer, 2016). Mitochondrial fission is essential for the partitioning and inheritance of mitochondria during cell division, the transport and distribution of mitochondria to intracellular regions of high energy demand (e.g. the neuronal synapse), the clearance of damaged mitochondrial segments via mitophagy, and the release of pro-apoptotic factors (e.g. cytochrome *c*) into the cytosol during programmed cell death. Conversely, mitochondrial fusion is critical for the maintenance of mitochondrial content homogeneity in the cell, and complementation of damaged mitochondrial DNA that ensues from environmental insults (Westermann, 2010; Youle and van der Bliek, 2012). The constant flux and counterbalance of mitochondria between fused and fragmented states maintains cellular homeostasis and ensures cell survival.

Defects in mitochondrial dynamics are causally linked to numerous human neurological disorders, including Alzheimer's, Parkinson's and Huntington's diseases, hereditary ataxia, dominant optic atrophy and Charcot-Marie-Tooth disease (Chen and Chan, 2009; Nunnari and Suomalainen, 2012; Girard et al., 2012; Niemann et al., 2005; Kandimalla and Hemachandra Reddy, 2015; Burte et al., 2015). Defects in mitochondrial dynamics also affect various aspects of cardiovascular biology, including cardiac development, response to ischemia-reperfusion injury, and heart failure (Ong et al., 2010; Ong and Hausenloy,

* Corresponding authors.
E-mail addresses: ashutosh@uh.edu (A. Agrawal), rxr275@case.edu (R. Ramachandran).

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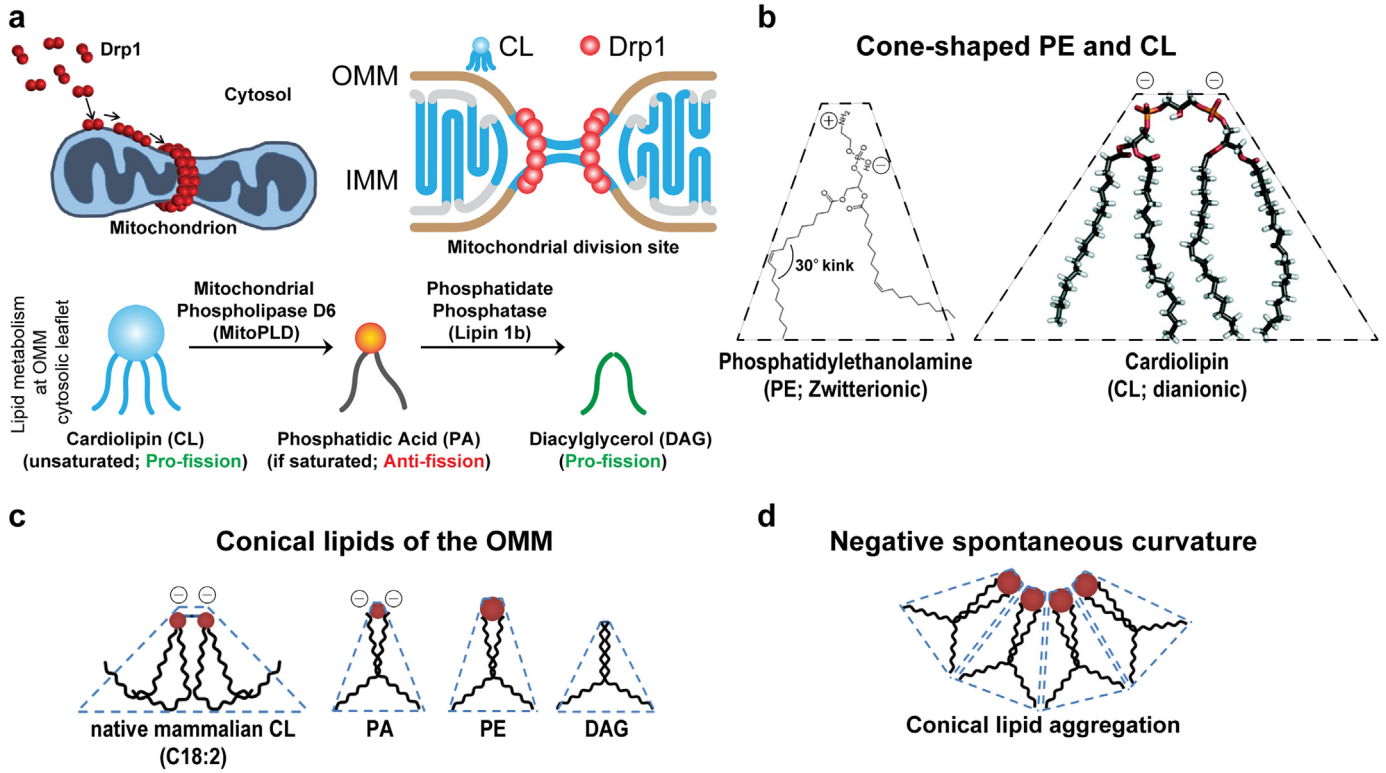

Fig. 1. Lipid protagonists of mitochondrial fission. a) *Top left.* Schematic of Drp1 recruitment to mitochondria and helical self-assembly around preconstricted division sites. *Top right.* Cartoon illustration of cardiolipin (CL) distribution in mitochondrial membranes and Drp1-mediated CL clustering at fission sites. *Bottom.* Schematic of CL metabolism at the OMM. CL is degraded to phosphatidic acid (PA) and then to diacylglycerol (DAG) by the sequential actions of phospholipases - MitoPLD and lipin 1b. Lipids containing saturated acyl chains, especially PA, are inhibitory to Drp1-catalyzed mitochondrial fission, whereas native mammalian CL that predominantly bears C18:2 unsaturated acyl chains and DAG promote fission. b) Cartoon illustration of the overall conical shapes of zwitterionic PE and negatively charged CL. Each double bond in the fatty acyl chain, as depicted here for dioleoyl PE or DOPE, introduces a rigid 30° kink that tends to increase the cross-sectional area of the phospholipid hydrocarbon tails relative to the polar headgroups. c) Assortment of cone-shaped lipids present in the OMM. d) Clustering or sequestration of conical lipids spontaneously favors generation of negative membrane curvature as illustrated.

2010; Kane and Youle, 2010; Giedt et al., 2012; Parra et al., 2008; Knowlton and Liu, 2015). Furthermore, mitochondrial dynamics defects have been directly implicated in a variety of cancers (Kong et al., 2015; Mitra, 2013; Caino and Altieri, 2015; Ferreira-da-Silva et al., 2015). A molecular level understanding of how mitochondrial fission and fusion occur, and are regulated, is therefore vital for targeted therapeutic intervention.

1.1. Mitochondrial lipids – shape matters

Markedly distinct from other intracellular membranes, zwitterionic (electrically neutral) phospholipids phosphatidylcholine (PC; ~40%) and phosphatidylethanolamine (PE; ~35%) predominate in mitochondrial membranes and are present in nearly equal proportions constituting nearly 75% of the total lipids (van Meer et al., 2008; van Meer and de Kroon, 2011; Osman et al., 2011; Daum, 1985). The relative abundance of PE in the mitochondria can be traced back to its bacterial origins, wherein PE constitutes 60–80% of the total membrane phospholipids, whereas PC, preferred by eukaryotes, is virtually absent (Epand and Epand, 2011; Epand and Epand, 2009). Of note, mitochondria synthesize nearly half of the total cellular PE, highlighting the organelle's oft-underappreciated role in phospholipid metabolism (van Meer and de Kroon, 2011; Osman et al., 2011; Basu Ball et al., 2018). On the other hand, phosphatidylglycerol (PG) (Epand and Epand, 2011), a major anionic lipid of bacterial membranes (enriched especially in gram-positive bacteria), serves only as a precursor lipid in eukaryotes and is only a very minor component of mitochondrial membranes under normal circumstances (Osman et al., 2011). These differences aside, a hallmark feature of both bacterial and mitochondrial membranes is the copious presence of the atypical, negatively charged and dimeric phospholipid containing four acyl chains, namely cardiolipin (CL) (Osman et al., 2011; Daum, 1985) (Fig. 1b). CL, however, is located almost exclusively in the IMM, where it constitutes nearly 1 out of 4 lipid molecules (25%) (Ha and Frohman, 2014; Frohman, 2015; Horvath and Daum, 2013). Emerging evidence indicates that a small proportion of CL (3–10%) is also present at the OMM (Osman et al., 2011; Ha and Frohman, 2014; Horvath and Daum, 2013; Chu et al., 2013), where it functions both as a signaling molecule and as an effector of protein-mediated membrane remodeling (Frohman, 2015; Macdonald et al., 2014; Stepanyants et al., 2015; Adachi et al., 2016; Ugarte-Uribe et al., 2014; Ugarte-Uribe et al., 2018; Bustillo-Zabalbeitia et al., 2014; Li et al., 2015; Kamerkar et al., 2018). Mitochondria, in addition, feature OMM-IMM contact sites that may contain up to 25% CL (Osman et al., 2011; Ardail et al., 1990; Simbeni et al., 1991). Whether these OMM-IMM contact sites, originally identified by biochemical fractionation methods, strictly coincide with that of the more recently identified MICOS (mitochondrial contact site and cristae organizing system) protein complex that tethers the IMM to the OMM and also possesses inter-membrane phospholipid transfer activity, and/or with ER-mitochondria contact sites that mark sites of impending mitochondrial fission is currently unclear (Schorr and van der Laan, 2018; Tatsuta and Langer, 2017). The physical nature of these contact sites as well as lipid arrangement within these complex structures also remains to be elucidated. Nevertheless, as IMM constriction and fission precedes and occurs independently of OMM fission (Cho et al., 2017; Chakrabarti et al., 2017), the role of OMM-IMM contact sites in mitochondrial fission, if any, is likely restricted to phospholipid metabolism and CL redistribution to the OMM. Other mechanisms at the OMM,

as described below, likely concentrate CL locally to enable recruitment and functioning of the mitochondrial fission machinery. Interestingly, lipid microdomain ("raft')-forming sphingolipids and sterols that are found only in trace amounts in mitochondria, and that can locally enrich largely unsaturated lipids such as CL indirectly via phase separation (Stepanyants et al., 2015), are reportedly enriched at ER-mitochondria contact sites (Sorice et al., 2009; Hayashi and Fujimoto, 2010; Arasaki et al., 2015) - noted hotspots for both mitochondrial fission and fusion (Friedman et al., 2011).

PG and CL are not only synthesized exclusively in the mitochondria but are also confined there (van Meer and de Kroon, 2011; Osman et al., 2011). Mitochondrial membranes also contain other phospholipids, such as phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA), which together only constitute a relatively minor fraction (< 10%) of the total lipids. PS, transported from the ER, undergoes rapid enzymatic decarboxylation at the IMM to generate PE, whereas PA is either shuttled from the ER or is locally generated at the OMM de novo by the action of mitochondrial phospholipase D (MitoPLD or PLD6) on OMM-localized CL (Fig. 1a, *bottom panel*) (Osman et al., 2011; Choi et al., 2006). PA could also be further broken down to diacylglycerol (DAG – an "honorary" phosphate-less phospholipid) by the cytosolic PA phosphatase lipin 1b (Fig. 1a, *bottom panel*), with yet unexplored functions at the OMM (Huang et al., 2011).

Of importance, a significant fraction of the lipids present at the mitochondria (or in bacterial membranes for that matter) - PE, CL, PA and DAG - all deviate from the conventional cylindrical geometry of a typical phospholipid molecule (e.g. PC) (van Meer and de Kroon, 2011; Osman et al., 2011; Basu Ball et al., 2018). Instead, these particular lipids assume a patent conical shape owing to the smaller cross-sectional area (or volume) of their headgroups relative to their often unsaturated and kinked acyl chains (Fig. 1b, c). Therefore, these phospholipid molecules when distributed asymmetrically (disproportionately) between the monolayers of a lipid bilayer can generate and stabilize high negative membrane curvature (Fig. 1d) as well as assume a more energetically favorable nonbilayer configuration, such as the inverted hexagonal phase (H_{II} phase), upon sequestration (Basu Ball et al., 2018; Ortiz et al., 1999; Marrink and Mark, 2004). These curvatures likely help regulate the shapes of mitochondrial membranes during fission and fusion (Joshi et al., 2012; Burger, 2000). Nonbilayer phases form an integral part of the hemifission or hemifusion intermediate requisite for leak-free fission or fusion (Burger, 2000; Frolov et al., 2011). Whereas zwitterionic PE and DAG naturally tend to form nonbilayer phases on their own, dianionic CL and PA, owing to the electrostatic repulsion between their charged headgroups, necessitate charge neutralization (via hydrogen- or ionic-bonding) by partner proteins (or by divalent cations or changes in pH or ionic strength) to facilitate this phase transition (Ortiz et al., 1999; Burger, 2000). The conicity and nonbilayer propensity of these lipids is dictated further by their acyl chain composition – length and degree of unsaturation – properties that are also regulated by acyl chain remodeling enzymes as described below. The essential role of nonbilayer lipids in mitochondrial membrane remodeling is underscored by the fact that the simultaneous ablation of CL and PE synthesis in eukaryotes is not tolerated (Basu Ball et al., 2018; Gohil et al., 2005). Reductions in either CL or PE content alone also affect mitochondrial morphology (Basu Ball et al., 2018).

1.2. Protein-lipid cooperativity in mitochondrial membrane remodeling

Large self-assembling mechanoenzymatic GTPases of the dynamin superfamily (dynamin superfamily proteins or DSPs) catalyze both mitochondrial fission and fusion (Ramachandran, 2018; Ramachandran and Schmid, 2018). Dynamin-related protein 1 (Drp1), a soluble DSP located primarily in the cytosol catalyzes mitochondrial fission. In response to biochemical and biophysical cues, Drp1 is recruited from the cytosol onto the mitochondrial surface via interactions with various protein adaptors (e.g. Fis1, Mff, MiD49/51) localized at the OMM (Richter et al., 2015; Bui and Shaw, 2013). Fission occurs at division "hot spots" on the mitochondria where ER-derived membrane tubules and associated actin networks enwrap and locally constrict the mitochondria (down to ~150 nm diameter from ~500–1000 nm) to create a tubular (cylindrical) OMM template suitable for Drp1 assembly and amenable for fission (Friedman et al., 2011; Otera et al., 2013; Korobova et al., 2013; Ji et al., 2015). At these sites, Drp1 constitutes a dynamic helical polymer that self-assembles around the pre-constricted membrane tubule and employs GTP hydrolysis-dependent mechanochemical conformational changes to constrict the cylindrical membrane bilayer further toward fission (Stepanyants et al., 2015; Kamerkar et al., 2018; Friedman et al., 2011; Mears et al., 2011). Nevertheless, the existing paradigm does not invoke a role for direct Drp1-phospholipid interactions in fission (Bui and Shaw, 2013). We propose that this model is incomplete and that it is indeed specific and direct Drp1-phospholipid interactions at these sites, as discussed below, which remodels the membrane bilayer further for fission.

DSPs mitofusin (Mfn1/2) and optic atrophy 1 (OPA1), which unlike Drp1 are both transmembrane domain-anchored integral membrane proteins of the OMM and IMM, respectively, catalyze the individual mergers (fusion) of these two separate membranes. Although the mechanisms of mitochondrial fusion, in comparison to fission, are much poorly understood, emerging evidence indicates that coordinated DSP interactions with cone-shaped, nonbilayer-prone lipids, specifically CL and PA, also play a crucial role in these processes (Choi et al., 2006; Ramachandran, 2018; Kameoka et al., 2018; Ban et al., 2017). Hereafter, we will restrict our discussion to the better mechanistically dissected process of mitochondrial membrane fission and the catalytic role of conical lipids.

1.2.1. Drp1-CL/PA interactions in mitochondrial fission

CL is the signature phospholipid of the mitochondria. As mentioned, enriched in the IMM, where it constitutes nearly 25% of the lipids, CL is nevertheless found in relatively minor but significant quantities (3–10%) in the OMM (Richter et al., 2015; Ha and Frohman, 2014). Furthermore, CL content can approach 25% at OMM-IMM contact sites that speckle the mitochondrial surface (Ardail et al., 1990; Simbeni et al., 1991). In mammalian cells, mitochondria surface-exposed CL appears to play a critical role in regulating mitochondrial fission (Adachi et al., 2016). CL promotes the helical self-assembly and enzymatic stimulation of Drp1 over membrane surfaces in vitro (Macdonald et al., 2014; Stepanyants et al., 2015; Adachi et al., 2016). The selective degradation of OMM-localized and cytosol-exposed CL to PA by mitochondrial phospholipase D (MitoPLD) localized at the mitochondrial surface (Choi et al., 2006) inhibits Drp1 polymers in fission, firmly establishing a role for OMM surface-localized CL in governing mitochondrial fission in vivo (Adachi et al., 2016). However, until recently, the mechanism by which CL fulfills this role has remained mysterious, given that the existing protein-centric paradigm does not invoke a role for direct Drp1-CL interactions in fission (Bui and Shaw, 2013). Drp1 binds and is also enzymatically stimulated by PA and PG, albeit to a much smaller extent relative to CL, even though this differential response to phospholipids is acutely sensitive to acyl chain composition with unsaturated chains that enhance lateral packing defects in membrane bilayers favored over saturated ones (Macdonald et al., 2014; Stepanyants et al., 2015; Adachi et al., 2016). Indeed, a combination of headgroup electrostatic interactions and coupled hydrophobic membrane insertion into the nonpolar acyl chain region is thought to be involved in specific Drp1-phospholipid binding, even though evidence for this remains indirect (Adachi et al., 2016).

Drp1 selectively binds phospholipids via its intrinsically disordered variable domain (VD) (Stepanyants et al., 2015; Bustillo-Zabalbeitia et al., 2014; Lu et al., 2018). Recent in vitro reconstitution experiments have shown that the Drp1 VD in isolation preferentially interacts with CL (Lu et al., 2018), and that in full-length Drp1, the VD functions to

cluster or sequester CL underneath the growing Drp1 helical scaffold (Stepanyants et al., 2015). Furthermore, Drp1 also enhances the nonbilayer propensity of cone-shaped lipids CL and PE in a GTP-dependent manner to create highly narrow and local membrane tubule constrictions prone to hemifission, and subsequently, to fission (Stepanyants et al., 2015). However, as no high-resolution information on VD-phospholipid interactions is currently available, the functional ramifications of VD-CL interactions in mitochondrial membrane remodeling and fission remain unclear. How Drp1 distinguishes between CL and PA, both cone-shaped, dianionic phospholipids, is also unknown. We suggest that differences in intrinsic curvature (DOPA has the highest known negative membrane curvature (Kooijman et al., 2005)) and conicity - a measure of the degree of acyl chain exposure to the lipid-water interface) underlies this difference. In other words, greater acyl chain interactions of the VD via membrane insertion may precipitate stronger Drp1-phospholipid binding and coupled enzymatic stimulation.

1.2.2. CL/PA metabolism and regulation of Drp1 function

OMM-localized CL, the presumed lipid target of Drp1 in fission, is also enzymatically degraded to PA by the action of MitoPLD, an integral membrane protein of the OMM whose active site faces the cytosol (Choi et al., 2006). Overexpression of MitoPLD and enhanced PA production inhibits fission (Adachi et al., 2016). Interestingly, Drp1 also interacts directly with MitoPLD suggesting that Drp1 modulation of MitoPLD activity and vice versa helps create a lipid microenvironment that regulates fission (Adachi et al., 2016). Interestingly, PA is further broken down to DAG by the action of a PA phosphatase, lipin 1b, a cytosolic protein recruited to the mitochondrial surface, whose activity conversely promotes fission (Huang et al., 2011). How DAG, an uncharged lipid that primarily functions as a second messenger in the cell, functions to regulate Drp1 function remains unclear. Yet, it is conceivable that the accumulation of cone-shaped DAG (Fig. 1c) around the fission apparatus induces local packing defects in the bilayer to enable Drp1 membrane insertion and facilitate the nonbilayer lipid topological transition essential for membrane hemifission, and subsequently, fission. Thus, CL, PA and DAG around the division apparatus likely regulate mitochondrial fission.

1.2.3. Role of acyl chain remodeling in mitochondrial fission

Enzymes that modify phospholipid acyl chains also regulate mitochondrial fission. Chemical inhibition of lipid desaturases, which convert saturated acyl chains to unsaturated forms, also inhibits mitochondrial fission in vivo (Adachi et al., 2016). Conversely, overexpression of a PA-preferring phospholipase A1 (PA-PLA1), which converts PA to lyso-PA (LPA), promotes fission presumably through the generation of LPA-facilitated high positive membrane curvature underneath the Drp1 scaffold (Baba et al., 2014). Indeed, in reconstitution experiments in vitro, native CL primarily bearing unsaturated C18:2 acyl chains (tetralinoleoyl CL or TLCL) is substantially more effective than C14:0 tetramyristoyl CL (TMCL) in promoting Drp1 helical self-assembly, CL sequestration under the growing Drp1 scaffold, stimulation of Drp1 GTPase activity, GTP-dependent nonbilayer propensity and membrane constriction (Stepanyants et al., 2015).

1.2.4. Consensus roles of lipids in mitochondrial fission

Taking into account all of the above observations, it appears that a combination of protein-mediated membrane curvature stress with a coincident internal membrane strain induced by the concentration of curvature-prone lipid species helps destabilize membranes for fission. This model is derived from a synthesis of a number of key observations as outlined above, which all indicate for the critical role of lipid shape, lipid packing, membrane structure, and the careful regulation thereof. Thus, the presence of highly conical and unsaturated CL, PA and DAG species, at high local concentrations appears to place the lipid bilayer under a curvature strain, thus generating local hydrophobic packing defects in the membrane.

We speculate that the hydrophobic packing defects exposed by the more prominently cone-shaped unsaturated CL, PA and DOG species allow for a more dynamic and reversible interaction of the Drp1 VD with the membrane bilayer core. Conversely, we surmise that saturated acyl chain-bearing CL, PA and DAG either elicit an irreversible insertion of Drp1 VD into the membrane bilayer core stabilized by strong hydrophobic acyl chain interactions, or alternatively prevent Drp1 VD membrane insertion and associated Drp1 conformational rearrangements due to the tighter lipid packing. Indeed, experimental evidence clearly indicates the stronger lateral packing interactions between the saturated acyl chains prevent these phospholipids from being reorganized (mobilized) and laterally sequestered during Drp1 self-assembly (Stepanyants et al., 2015).

How adaptor proteins and phospholipids cooperate with Drp1, mechanistically, to mediate mitochondrial fission remains an unresolved mystery, and poses a fundamental gap in our understanding of mitochondrial dynamics.

In the following sections, we delve into the mechanics of protein-lipid cooperativity in mitochondrial fission based on membrane energetics and computational modeling approaches.

1.3. Membrane energetics: computational modeling and predictions

Mitochondrial dynamics entail largescale deformations of the bounding membrane bilayers. In effect, these membranes can be considered and modeled as 2D surfaces since the average bilayer thickness of ~5 nm is significantly smaller than their in-plane dimensions (Boal and Boal, 2012). A key feature of lipid bilayers is that they behave as elastic surfaces when they bend and stretch. Remarkably, however, these bilayers behave as 'in-plane fluids' as the constituent lipid molecules possess the translational freedom to move around in stark contrast to solid surfaces. The primary energetic cost to deform lipid bilayers therefore comes from the bending and stretching of lipids. These modes of deformation alter the separation between polar lipid headgroups and regulate the amount of exposure of nonpolar lipid hydrocarbon tails to the aqueous medium, which also changes the energetic state of the lipids (Boal and Boal, 2012).

The cost to bend a bilayer in essence relies on two curvatures - the mean curvature and the Gaussian curvature (Canham, 1970; Helfrich, 1973). In the context of mitochondrial fission, these curvatures are better understood by analyzing the shape of a spherocylinder (Fig. 2) – a cylinder capped by hemispheres at either end - the typical shape of the mitochondria. For the purposes of the forthcoming arguments and ease of understanding, let us first consider an equatorial ring on the hemispherical surface (Fig. 2). The curvature along this ring is given by the rate of change of the unit surface normal along the curve. Let us then consider an arc that subtends an angle θ at the center of the ring. The orientation of the unit normals at the two end points differ by the angle θ. Thus, the rate of change of the unit normal vector over the arc is given by $\theta/(R\,\theta) = 1/R$. Hence, the curvature along the ring is equal to 1/R, where R is the radius of curvature. Next, we consider a perpendicular ring. Since the hemisphere possesses rotational symmetry about the longitudinal axis, the rate of change of unit normal along this curve is also equal to 1/R. With these curvatures along the two perpendicular rings, we can now compute the mean and Gaussian curvatures at the point of intersection. The mean curvature is given by the average ½(1/R + 1/R), which is equal to 1/R, and the Gaussian curvature is given by the product of (1/R)*(1/R), which is equal to $1/R^2$. Next, we consider the cylindrical domain. First, we consider a ring in the circumferential direction (green curve) (Fig. 2). This ring has the same geometrical properties as that of the ring along the hemisphere, and hence has a curvature equal to 1/R. Next, we consider a straight line along the longitudinal direction (magenta curve) (Fig. 2) and perpendicular to the circumferential ring. As we move along the straight line, the unit normal does not change, and hence, both the rate of change of the unit

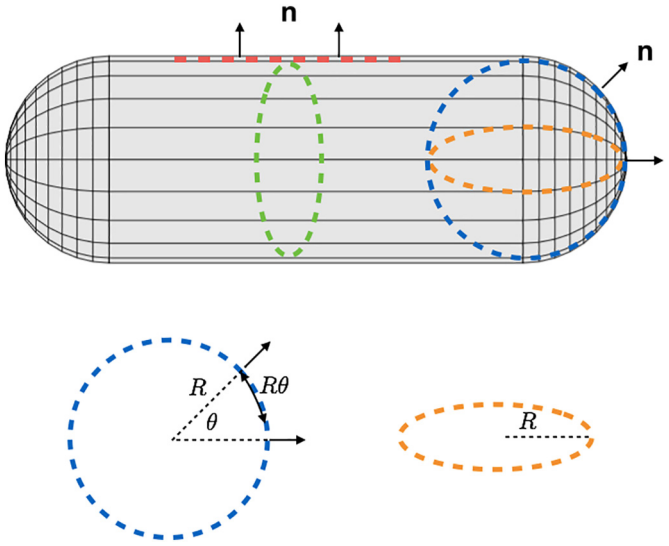

Fig. 2. Curvatures of lipid bilayers. The energy to bend mitochondrial membranes is determined by two curvatures: the mean curvature and the Gaussian curvature. We discuss these curvatures for the hemispherical and cylindrical domains of a mitochondrion-like surface. The curvature along a curve is given by the rate of change of the unit normal vector along the curve. The black arrows show the orientations of two unit normals along a ring with radius R (blue curve). These vectors make an angle θ with respect to each other, and therefore, the rate of change of unit normal can be computed as: $\frac{\theta}{R\theta} = \frac{1}{R}$. Since the sphere has rotational symmetry, a perpendicular ring (orange curve) also has the same curvature $\frac{1}{R}$. Thus, for the hemispherical domain, the mean curvature is given by $\frac{1}{2}\left(\frac{1}{R} + \frac{1}{R}\right) = \frac{1}{R}$, and the Gaussian curvature is given by $\frac{1}{R} \times \frac{1}{R} = \frac{1}{R^2}$. For the cylindrical domain, the circumferential ring (green curve) has the same curvature $\frac{1}{R}$ as that for the hemispherical rings. For the perpendicular curve (red curve), which is a straight line, the unit normal remains unchanged and hence, the curvature in the longitudinal direction is zero. Therefore, for the cylindrical domain, the mean curvature is given by $\frac{1}{2}\left(\frac{1}{R} + 0\right) = \frac{1}{2R}$, and the Gaussian curvature is given by $\frac{1}{R} \times 0 = 0$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

normal and the curvature along the longitudinal direction vanish. Thus, for a cylinder, the mean curvature is given by ½(1/R + 0) = 1/2R, and the Gaussian curvature is given by (1/R)*(0) = 0.

Using these curvatures, the energy to bend a membrane can be represented by the most commonly used model, called the Helfrich-Canham model (Canham, 1970; Helfrich, 1973). In this model, the bending energy per unit surface area of the membrane is given by:

$$W_b = \frac{\kappa(2H - 2H_0)^2}{2} + \bar{\kappa}\mathrm{K} \tag{1}$$

where κ, $\bar{\kappa}$ are bending moduli and H_0 is the preferred curvature of lipids, called the spontaneous mean curvature. For the majority of lipids, membranes have a κ of around 20 k_BT and $\bar{\kappa}$ is around -20 k_BT (Hu et al., 2012), where k_B is the Boltzmann's constant and T is absolute temperature. While κ has been estimated from experimental methods, experimental validation of $\bar{\kappa}$ is still pending. The H_0 represents an effective curvature that membranes might possess depending on the composition of the lipids. It depends on two key factors. First, is the shape of the lipids. If the lipids have a conical shape and are present in different concentrations in the two bilayer leaflets, they would have a tendency to bend the membrane and would hence give rise to a non-zero H_0. Second, the propensity to bend the membrane depends on the density of such cone-shaped lipids. A higher density of cone-shaped lipids would lead to a larger H_0 forcing the membrane to bend more. The energy per unit area to stretch lipid membranes is given by:

$$W_s = \kappa_a \frac{\phi^2}{2} \tag{2}$$

where, ϕ is the dimensionless areal stretch and κ_a is the stretch modulus. For lipid membranes, κ_a is around 55–70 k_BT/nm^2 or 230–290 mN/m (Phillips et al., 2012).

The above mathematical theory, depending on the local curvatures and dilation, accounts for the macroscopic features of membranes. However, in certain biological processes, internal structure of the membrane characterized by the tilting of lipids with respect to surface normal can become a critical player. This need primarily arises in the presence of geometrical constraints imposed onto a bilayer by embedded proteins and/or membrane morphology. For example, the presence of membrane-inserted protein structures could regulate the orientation of the adjacent lipids. If these protein segments are tilted in some fashion relative to the plane of the bilayer, the neighboring lipids would exhibit a similar tilt. Alternatively, the extreme bending of bilayer leaflets during membrane fission and fusion events could force lipids to tilt in order to minimize hydrophobic packing defects. The energetics of such deformations therefore necessitate a revised membrane model that accounts for the energetic cost of lipid tilting and bending, and the interplay between them (Hamm and Kozlov, 2000; Fournier, 1999; Terzi and Deserno, 2017).

1.3.1. The mechanics of mitochondrial fission

1.3.1.1. Mitochondrial squeezing and creation of membrane superconstrictions. The Helfrich-Canham model has been previously employed to investigate the pre-constriction of the OMM in the presence of ER-associated actin forces (Manor et al., 2015). This study showed that a force of 10–20 pN force from actin filaments would be required to constrict the OMM, a prediction that is in the physiological range. Our own recent studies based on an extension of Helfrich-Canham model illustrated the potential role of cone-shaped lipids in mitochondrial fission (Irajizad et al., 2019). Our study simulated the shape transition of the OMM from the initial spherocylindrical geometry to a squeezed shape with a central superconstriction mimicking the mitochondrial division site (Fig. 3). This study revealed that cone-shaped lipids such as CL, PE and DAG could have a significant impact on the constriction of mitochondria. Based on lipid geometry, the study assumed that the cone-shaped lipids preferentially aggregated in the negatively curved inner (IMS-facing) leaflet of the OMM immediately adjacent to where Drp1 self-assembles. Invoking a spontaneous curvature field from the cone-shaped lipids led to a geometric instability that enabled a rapid shape transition from a tubular shape to a higher constricted shape (Fig. 3). This cooperativity between cone-shaped lipids and Drp1 to induce instability was observed during both the actin-driven and Drp-driven constriction phases. For the case of Drp1-mediated fission, incorporation of CL-induced negative curvature underneath the protein coat further facilitated the instability and constriction (Fig. 3). In addition to precipitating constriction, the study also showed that cone-shaped lipids induce structural plasticity during shape transition. In the absence of lipid aggregation, the shape change of a spherocylidner exhibited an elastic response. In other words, if the protein forces or curvatures were removed, which would represent their depolymerization, the shape elastically rebounded back to the original undeformed spherocylindrical geometry. However, if the cone-shaped lipids were allowed to aggregate, as assumed in the model, the spherocylinder showed a plastic response and continued to remain in the superconstricted state, even upon the removal of protein forces and curvatures. This plasticity induced by cone-shaped lipids could play an important role in imparting robustness to the fission reaction.

In the light of these findings, a natural and critical question arises: what is the extent to which cone-shaped lipids should aggregate in these regions in order to catalyze buckling and constriction? Based on

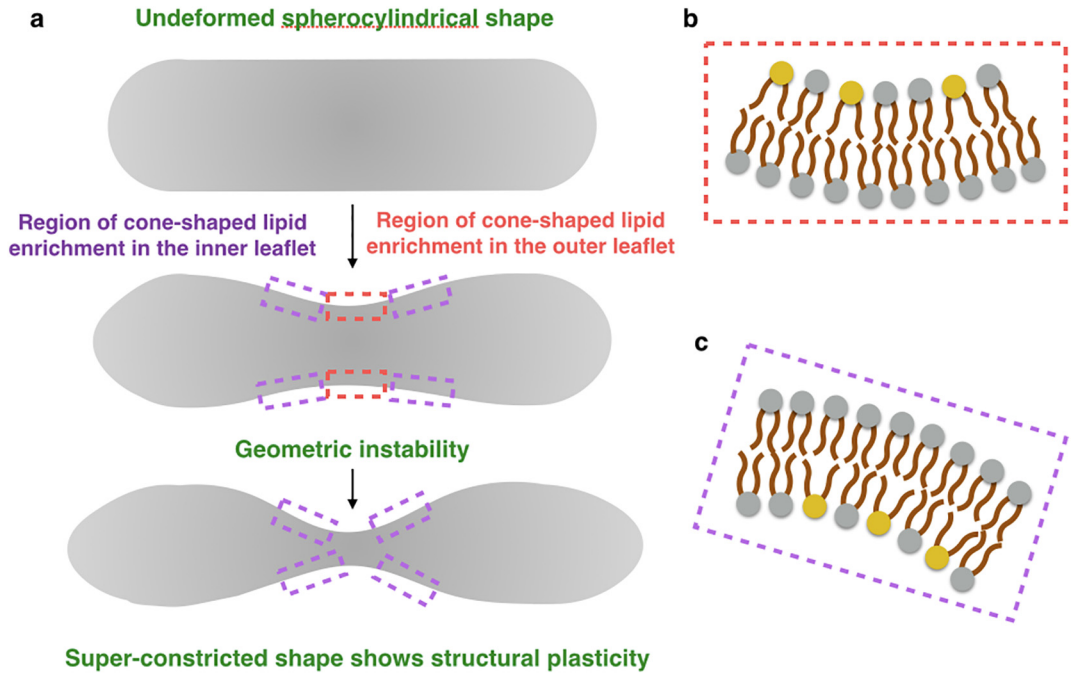

Fig. 3. Lipid-catalyzed mitochondrial constriction. a) The undeformed shape of a mitochondrion resembles a spherocylinder. Under the action of ER/actin forces and helical Drp1 self-assembly (red boxes), the mitochondrion undergoes constriction. However, if the aggregation and curvature generation from conical lipids is assumed in the adjacent domains (magenta boxes), the mitochondrion undergoes dramatic geometric instabilities that induce a snap-through shape transition and constriction. The aggregation of cone-shaped lipids (yellow heads) further imparts plasticity to the superconstricted geometry, preventing the elastic response of reverting to the original shape even upon transient removal of protein forces and curvatures. b and c) Localization of cone-shaped lipids in the outer (cytosol-facing) leaflet underneath the Drp1 scaffold and in the inner (IMS-facing) leaflet in the adjacent regions with negative spontaneous curvatures catalyzes buckling and constriction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

known estimates of the preferred curvatures of PE and CL, the study estimated that not more than a 4% increase in the concentration of cone-shaped lipids in the internal leaflet of the OMM (Fig. 3; magenta boxes) immediately adjacent to the Drp1-constricted membrane region is required to induce instability (Irajizad et al., 2019). This could possibly occur due to multiple factors. One possibility is that cone-shaped lipids owing to their intrinsic negative curvature could mobilize to these regions flanking the Drp1 polymer. Curvature-mediated lipid dynamics has indeed been revealed in several experimental and theoretical studies (Mukhopadhyay et al., 2008; Kamal et al., 2009; Renner and Weibel, 2011; Sakuma et al., 2011; Koldso et al., 2014; Boyd et al., 2017). While lipid partitioning due to curvature is estimated to be weak because of entropic effects (Kamal et al., 2009), the minimal segregation could be sufficient to trigger instability and remodel membranes as indicated in the computational study (Irajizad et al., 2019). With regard to the external leaflet of the OMM, Drp1 was demonstrated to cluster CL underneath its helical scaffold during self-assembly (Stepanyants et al., 2015). Drp1-mediated CL clustering was also shown to be essential for the formation of highly narrow membrane constrictions (Stepanyants et al., 2015).

1.3.1.2. Events that likely precipitate mitochondrial fission. While cone-shaped lipids could function in concert with membrane remodeling proteins to play a structural role in mitochondrial constriction, they may possibly also play an even greater role in the catalysis (acceleration) of mitochondrial fission. Fission entails the leak-free merger of the two highly constricted tubular bilayer leaflets via a nonbilayer hemifission intermediate, and the concomitant separation of the newly formed mitochondrial poles to create two daughter mitochondria.

The effect of cone-shaped lipids and spontaneous curvature on the stability of membrane necks has been analyzed in detail in ref. (Kozlov, 2001). This study suggests that presence of cone-shaped lipids in the opposite leaflet, which generates a negative spontaneous curvature, destabilizes the neck and promotes membrane fission. Thus, the presence of conical lipids immediately adjacent to the protein-constricted membrane region as described above could further play a crucial role in resolving the metastable nonbilayer hemifission intermediate and help dissociate the newly formed daughter mitochondrial poles. The transition pathway that precipitates leak-free fission of the OMM is illustrated in Fig. 4 (Kozlovsky and Kozlov, 2002; Kozlovsky et al., 2002). The first step involves the formation of the pre-fusion state, where the highly compressed inner monolayer of the constricted membrane tubule makes physical self-contact. The next step is a rapid transition to a metastable nonbilayer hemifission intermediate state in which the lipids of the inner monolayer undergo mixing leaving the outer monolayers intact. The final step involves the energetically favorable dissociation of the negative curvature-strained connecting diaphragm formed by the outer monolayers eventually giving rise to two separated bilayers. It is important to note that while this fission mechanism may sound similar to that of the fission of endocytic vesicle necks at the plasma membrane, there are some clear differences. In the case of mitochondrial fission, the transition proceeds from a region of high positive radial curvature underneath the Drp1 scaffold toward high negative curvature in the flanking regions. Hence, the presence of cone-shaped lipids in the leaflet that generates negative spontaneous curvature would tend to catalyze fission. By contrast, as demonstrated in the case of endocytic vesicle fission (Shnyrova et al., 2013; Mattila et al., 2015; Bashkirov et al., 2008), the generation of positive spontaneous curvature alone is sufficient to promote fission (Kozlovsky and Kozlov, 2003). This is so because, in the case of vesicle fission, the shape transition occurs in the reverse order from (c) to (a) as illustrated in Fig. 4.

The role of lipid composition in promoting membrane fission has also been investigated in ref. (Chen et al., 1997). This study explored the link between lipid composition and Gaussian curvature, and

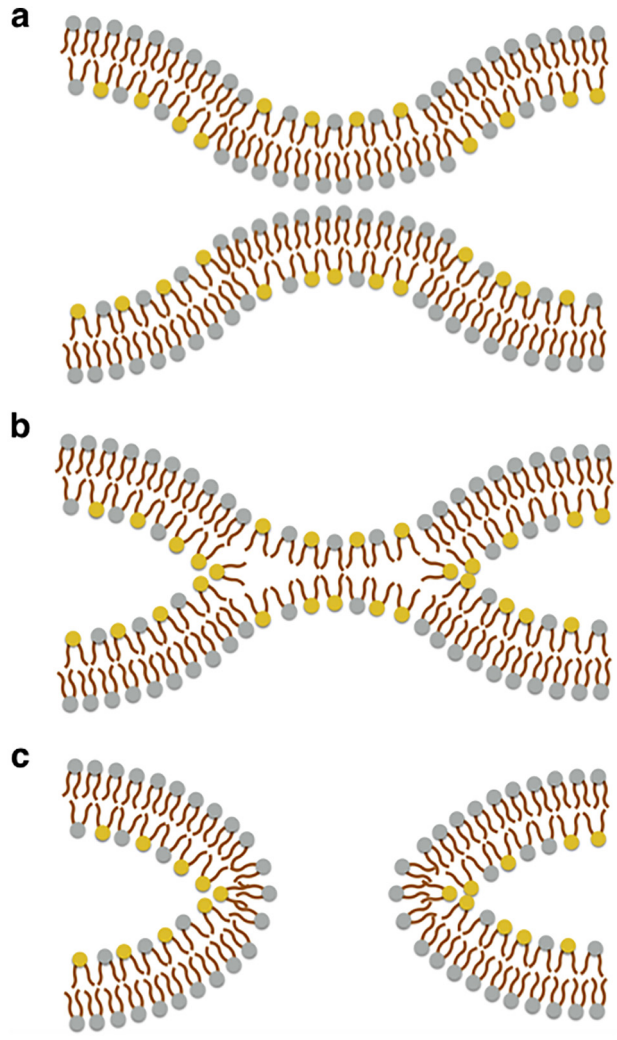

Fig. 4. Pathway to OMM fission. a) The superconstricted geometry where opposing sides of the OMM bilayer come into close contact. IMM fission precedes OMM fission and occurs independently of Drp1. Cone-shaped lipids of the OMM are depicted in yellow. The positive curvature in the radial dimension in the center and the negative curvature in the adjacent domains help in reaching the pre-fusion state. b) An intermediate hemifission state created by fusion of opposed inner leaflet regions, which leads to lipid mixing and creation of a lipid diaphragm. c) The final state where the hemifission intermediate is resolved to create two daughter mitochondria. Presence of conical lipids in the outer and inner leaflets generates spontaneous curvatures that energetically favor membrane fission. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

predicted its impact on membrane fission. The study found that the presence of two lipid species of disparate densities (concentration) lowers the Gaussian rigidity in the neck region if the lipid species of lower density (in our case, CL) aggregates in the region of positive Gaussian curvature (underneath the Drp1 scaffold). This localization is predicted to destabilize the neck and promote fission. In multi-lipid systems, the presence of cone-shaped (CL and PE) and cylindrical lipids (PC) together can also promote lipid tilting and create an energetically less costly pathway toward fission.

Another factor that can promote fission is membrane tension. Presence of tension can facilitate disintegration of the lipid diaphragm shown in panel b (Fig. 4). Indeed, a sufficient increase in tension was estimated to spontaneously develop at the rim of this central lipid diaphragm (Kozlovsky et al., 2002). Other key sources of tension in biological membranes include external pushing and pulling forces from cytoskeletal elements – actin (pushing force) (Korobova et al., 2013; Manor et al., 2015) and microtubules (pulling force) (Ramachandran, 2018; Moore and Holzbaur, 2018) in the case of mitochondrial fission – that could be coupled to the aggregation of curvature-strained conical lipids. Heterogeneity in membrane composition has indeed been shown to alter membrane tension (Agrawal and Steigmann, 2009). Spatial variations in membrane tension have also been confirmed by recent experimental studies (Shi et al., 2018).

2. Conclusions and perspectives

It is increasingly clear that lipids, especially asymmetrically shaped ones such as PE, are not simply passive constituents of membranes, but are instead active drivers, both catalytically and structurally, of dynamic membrane remodeling events that culminate in fission. Lipid-centric biophysical studies are therefore essential to explore the molecular link between lipid and protein dynamics during membrane remodeling. It may also be insightful to quantify inter- and intra-leaflet lipid dynamics during mitochondrial fission both experimentally and computationally. It is important to note that similar to fission, lipid-protein cooperativity could also play a crucial role in mitochondrial fusion. Indeed, mitochondria, which contain varied membrane architectures and undergo dramatic membrane reshaping events, might yet prove to be the ideal system to reveal basic membrane mechanics that might be relevant to other membrane systems.

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