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Open and cut: allosteric motion and membrane fission by dynamin superfamily proteins

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ABSTRACT Cells have evolved diverse protein-based machinery to reshape, cut, or fuse their membrane-delimited compartments. Dynamin superfamily proteins are principal components of this machinery and use their ability to hydrolyze GTP and to polymerize into helices and rings to achieve these goals. Nucleotide-binding, hydrolysis, and exchange reactions drive significant conformational changes across the dynamin family, and these changes alter the shape and stability of supramolecular dynamin oligomers, as well as the ability of dynamins to bind receptors and membranes. Mutations that interfere with the conformational repertoire of these enzymes, and hence with membrane fission, exist in several inherited human diseases. Here, we discuss insights from new x-ray crystal structures and cryo-EM reconstructions that have enabled us to infer some of the allosteric dynamics for these proteins. Together, these studies help us to understand how dynamins perform mechanical work, as well as how specific mutants of dynamin family proteins exhibit pathogenic properties.

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EUKARYOTIC LIFE AND THE EVOLUTION OF MEMBRANE-BOUND COMPARTMENTS

Membrane-bound cellular compartments are a hallmark of eukaryotic evolution and underlie the efficient division and specialization of work within the cell. Simple compartments feature small vesicles that form at the plasma membrane for the uptake of nutrients and neurotransmitters. More complex compartments include organelles such as mitochondria, which harbor simple genomes that encode some of their own proteins. Each compartment is actively remodeled during endocytosis or organelle division events, respectively. The timing and location of such remodeling activity, in turn, determines the efficiency of critical cellular functions and developmental processes. The endocytic membranes, for example, have to be remodeled and detached from the plasma membrane for neurotransmitter uptake in neurons and are therefore fundamental to nervous system functions (Ferguson and De Camilli, 2012).

Similarly, mitochondrial division requires membrane scission, which ensures the distribution of both catabolic and anabolic capacity and the inheritance of the correct complement of mitochondria by daughter cells (Mishra and Chan, 2014). Endocytic and organelle division events require dedicated protein-based machinery that can remodel and cut membranes. The structure and allosteric domain movements within these remodelers are critical to their activity. We discuss recent developments in this field below.

FISSION GTPASES OF THE DYNAMIN SUPERFAMILY OF PROTEINS

The dynamin superfamily of proteins (DSPs) catalyze membrane fission and fusion reactions during processes ranging from endocytosis in eukaryotes to cell division in certain bacteria (Jimah and Hinshaw, 2019). They play critical roles in organelle homeostasis and as innate immunity effectors that restrict certain pathogens (Bui and Shaw, 2013; Haller *et al.*, 2015). Independent of their membrane binding activity, DSPs also play an increasingly appreciated role as regulators and organizers of both the actin- and microtubule-based cytoskeletons (Strack *et al.*, 2013; Hatch *et al.*, 2014; Zhang *et al.*, 2019).

Endocytic dynamin knockout mice display neuronal dysfunction and, depending on the dynamin isoform, either are embryonic-lethal or have a lifespan limited to days or hours (Park *et al.*, 2013). Similarly, knockout mice for the mitochondrial fission dynamin DRP1

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Abbreviations used: BSE, bundle signaling element; DRP1, dynamin-related protein 1; DSP, dynamin superfamily protein; GMPPCP, β , γ -methyleneinosine 5'-triphosphate; GTP, guanosine 5'-triphosphate; K_{cat} , turnover number; K_m , Michaelis-Menten constant.

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(dynamamin-related protein 1) are embryonic-lethal (Wakabayashi et al., 2009). In cells, dynamamin knockouts result in clathrin-coated pits stuck to the plasma membrane, characterized by long tubules projecting into the cell (Ferguson et al., 2009). DRP1 down-regulation similarly results in undivided and hyperelongated mitochondria (Otsuga et al., 1998). In this review, we focus on comparing the plasma membrane-active endocytic, or “modern,” dynamamins with the mitochondrial and peroxisomal membrane-active “ancient” or dynamamin-related proteins to highlight our current understanding of their macromolecular motions, their assembly determinants, and the structural basis of their membrane fission function (Ramachandran and Schmid, 2018)

FISSION DYNAMIN STRUCTURES INCLUDE TWO HINGES

Both the modern and ancient dynamamin family proteins considered here are “large” GTPases that utilize the energy of nucleotide hydrolysis to fission membranes. Structural elements include the highly conserved N-terminal GTPase (G) domain that binds GTP with low affinity ($K_m \sim 15 \mu\text{M}$) and hydrolyzes it with a high basal hydrolysis rate ($K_{\text{cat}} \sim 0.3 \text{ s}^{-1}$ for unpolymerized modern dynamamins) that can be stimulated ~ 1000 -fold upon exposure to lipids and subsequent oligomerization (Stowell et al., 1999). The G-domain is followed by a three-helix bundle called the bundle signaling element (BSE) connected to the G-domain via a flexible hinge (hereafter referred to as the GTPase hinge). A second hinge (hereafter referred to as the Stalk hinge) connects the BSE to a four-helix bundle called the stalk (Figure 1, a–c). All known fission dynamamins are obligate dimers, and the dimeric interface (interface-2) resides within the four-helix stalk region (Figure 1d). Additional interactions at the tips of the stalk regions (including interface 1 at the “top” of the stalk and interface 3 at the “bottom” of the stalk) promote assembly of dynamamins into tetramers and higher-order oligomers (Gao et al., 2010; Faelber et al., 2011; Ford et al., 2011). Indeed, all dynamamins purified to date purify as dimers and/or tetrameric oligomers, and their structures have revealed a range of open versus closed states for the two hinges (Faelber et al., 2011; Ford et al., 2011; Gao et al., 2011; Reubold et al., 2015; Bohuszewicz and Low, 2018; Kalia et al., 2018; Kong et al., 2018).

Modern dynamamins also have a pleckstrin homology (PH) domain that interacts with the phospholipid headgroups of phosphatidylinositol (4,5) bisphosphate (PI(4,5)P₂) for membrane localization (Ferguson et al., 1994; Zheng et al., 1996). While the PH domain is absent from ancient dynamamins such as DNM1/DRP1, these enzymes harbor an ~ 100 -amino acid unstructured region in its place, the B-insert, which binds to other anionic lipids such as phosphatidic acid (Adachi et al., 2016) and cardiolipin on mitochondrial membranes (Bustillo-Zabalbeitia et al., 2014; Francy et al., 2017). Fission dynamamins utilize these membrane-binding elements to form polymeric assemblies that perform mechanical work on the underlying membrane, and these assemblies on lipid templates are often helical or ringlike oligomers that can encircle and constrict membrane tubes of variable dimensions in vitro (Marks et al., 2001; Roux et al., 2006; Mears et al., 2011; Kalia et al., 2015; Kong et al., 2018).

CONFORMATION-DEPENDENT REMODELING OF MEMBRANES BY DSPS

Remodeling of membranes by DSPs was first suggested by *Drosophila* shibire mutants that became paralyzed at nonpermissive temperatures. Electron micrographs of sections through their synapses revealed unreleased synaptic vesicles, the necks of which immunostained positive for dynamamin (Koenig and Ikeda, 1989; Takei et al., 1995). Similarly, assemblies of DRP1 have been observed to

constrict and catalyze fission at the mitochondria (Labrousse et al., 1999; Legesse-Miller et al., 2003). Consistent with membrane remodeling in vivo, assemblies of purified DSPs over a diverse range of templates such as liposomes (Sweitzer and Hinshaw, 1998; Takei et al., 1998), microtubules (Shpetner and Vallee, 1989), and lipid nanotubes (Stowell et al., 1999; Marks et al., 2001) have also been described in vitro. In particular, modern dynamamins are known to tubulate lipid vesicles containing PI(4,5)P₂, and these tubules can be further constricted, and even vesiculated, upon addition of GTP in the reaction mixtures (Roux et al., 2006; Pucadyil and Schmid, 2008). PI(4,5)P₂ seems to be important in particular to stimulate GTPase activity of PH domain-containing dynamamins, since additional amounts of other anionic lipids on lipid templates have little or no effect on GTPase activity (Stowell et al., 1999). Ancient dynamamins such as DRP1, on the other hand, are known to tubulate liposomes that have an artificially high, up to 100% content of anionic lipids such as phosphatidylserine (PS), suggesting a more nonspecific interaction between the B-insert and the underlying phospholipids in the membrane (Lackner et al., 2009; Mears et al., 2011). In addition to the lipid content, the tension in the underlying membrane is also considered an important determinant for fission, and some reports indicate that tension on lipid templates is needed to proceed toward complete fission, and loss of such membrane tension in cultured cells results in trapped clathrin-coated pits at late stages of endocytosis (Roux et al., 2006; Morlot et al., 2012).

Because the crystal structures of nearly full-length recombinant DSPs do not contain nucleotides, membranes, or binding partners, how nucleotide binding affects membrane interaction and structural dynamics of full-length dynamamins has remained an area of active study. In particular, nucleotide-based changes within the G-domain, and how these changes remodel the relationship and orientations of the G-BSE and the BSE-stalk regions via the flexible hinges are important details that may explain dynamamin assembly properties in detail. Moreover, how interfaces within dynamamin dimers or tetramers may change to accommodate membranes and binding partners is an important structural question. In the next sections, we discuss structural details that illustrate the differences in dynamamin conformations during this conversion from the cytosolic apo state to the membrane- or receptor-bound and nucleotide-activated states.

NUCLEOTIDE-INDUCED STRUCTURAL CHANGES

Soluble-state dynamamin structures

Early hints that the membrane fission activity of DSPs depended on conformational dynamics came from loss-of-function mutants that displayed defective oligomerization properties (Gao et al., 2010). Because polymerization of dynamamins hinders crystallization, these polymerization-incompetent mutants were sought and used to determine crystal structures of nearly full-length molecules (PDB ID's: 3SNH, 3ZVR, 4BEJ, and 53AF) (Faelber et al., 2011; Ford et al., 2011; Fröhlich et al., 2013; Reubold et al., 2015). The winning constructs typically carried mutations in the stalk region as well as deletions of the PRD or the B-insert. These structures revealed a closed GTPase hinge, such that the G-domain packed closely with the BSE. The Stalk hinge in these structures showed flexibility within the asymmetric units of the crystal, in that some asymmetric units showed more compaction of the G-BSE than the stalk. This flexibility suggests that the Stalk hinge samples a range of different conformations even in a defined nucleotide-free state (Faelber et al., 2011; Fröhlich et al., 2013).

A recent 7 Å-resolution crystal structure of the mitochondrial fission dynamamin from *Cyanidioschyzon merolae* (CmDnm1, PDB ID: 6FGZ) shows a new and wholly compacted state of the molecule

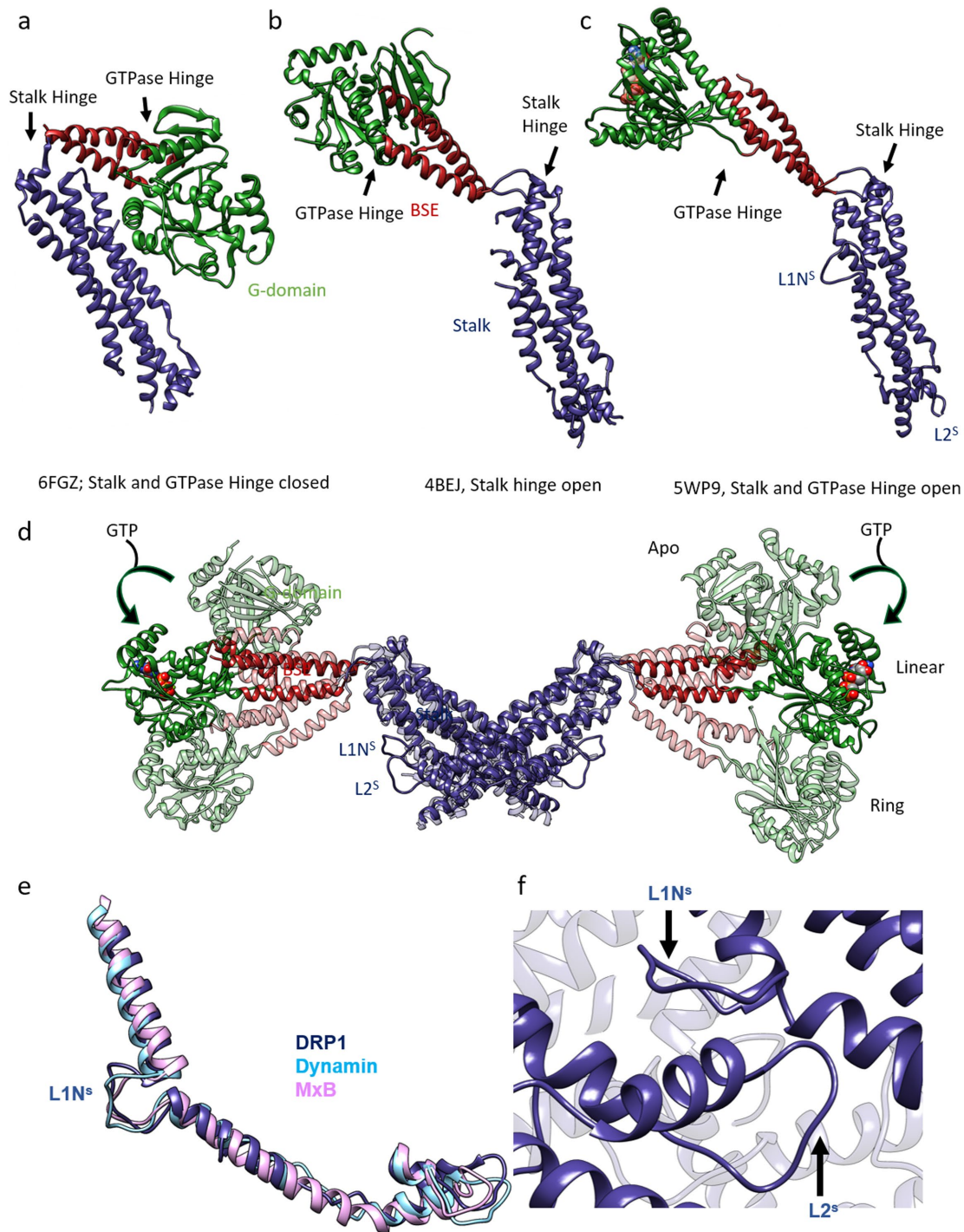


FIGURE 1: Structural rearrangements within the DRP1 molecules upon nucleotide binding: (a) *Cyandidioschyzon merolae* Dnm1 crystal structure depicts a closed state of a DRP1 molecule. (b) Crystal structure of human DRP1 shows an open GTPase hinge from chain (A) where both the loops that constitute the GTPase hinge were ordered. (c) Cryo-EM structure of DRP1 determined with nucleotide and the MID49 receptor. The G-domain is rotated and loops L1N^s and L2^s are stabilized and visible. (d) Overlay of DRP1 dimers in the apo state (4BEJ, light shade, Fröhlich *et al.*, 2013), the linear cryo-EM structure (5WP9, solid color), and the ring model (light shade, bent downward), as seen from Kalia *et al.* (2018), showing the range of movements exhibited by the G-BSE region relative to the stalk. The stalk is kept constant. (e, f) L1N^s and L2^s stabilization and interaction in dynamin family members: (e) Overlay of the region of the stalk that contains L1N^s and L2^s—from DRP1 cryo-EM structure (PDB ID: 5WP9), dynamin-3 crystal structure (PDB ID: 53AF), and MxB cryo-EM structure (PDB ID: 5UOT)—to depict the structural conservation of the region across dynamin family members. In each case, L1N^s and L2^s are stabilized by interdynamin and/or dynamin–receptor contacts. (f) L1N^s and L2^s mediate interdynamin contacts in the DRP1 cryo-EM structure (PDB ID: 5WP9).

(Bohuszewicz and Low, 2018). The apo-state tetramer contains a stalk interface 2 and a new interface 5 that mediates contacts between two adjacent stalk dimers. The authors argue that this is the fully closed, unassembled state of the molecule, which must open to form 15- to 18-membered rings around membranes—as observed in vitro at higher protein concentrations. Remarkably, both the Stalk hinge and the GTPase hinge are closed in this DRP1 ortholog structure (Figure 1a). The structure reinforces the importance of interface 2 in the dynamin superfamily, since this interface buries the largest surface area and occurs in this and all other structures of fission dynamins. Also, an overall resemblance of the structure to the fusion dynamins speaks to a shared evolutionary ancestry across diverse dynamin family members (Low *et al.*, 2009; Bohuszewicz *et al.*, 2016).

Nucleotide-bound minimal constructs

By contrast with the nucleotide-free crystal structures described above, x-ray structures of nucleotide-bound dynamins have primarily been studied in the context of minimized, engineered constructs that consist only of a G-domain or a fusion of the G-domain and an engineered BSE domain. For both endocytic and the mitochondrial dynamins, multiple crystal structures exist for these constructs in the GDP (PDB ID: 5D3Q), GMPPCP (PDB ID: 3ZYC, 3W6O), and GDP- AlF_4 (PDB ID: 2 \times 2E, 3W6P) bound states (Chappie *et al.*, 2010, 2011; Anand *et al.*, 2016). Importantly, all these structures reveal a dimeric assembly state formed via a direct G–G interaction, which is understood to stabilize the catalytic residues for nucleotide hydrolysis, including the main-chain carbon atoms that coordinate a hydrolyzing water molecule and a cation that participates in charge compensation in the transition state. Subtle changes in nucleotide coordination within the binding pocket, due to the nonhydrolyzable methylene in GMPPCP, revealed a crucial change in the GMPPCP-bound structure: while the rest of the catalytic site was similar to the GDP- AlF_4 state, the BSE in the GMPPCP state was in the open conformation, rotated $\sim 69^\circ$ away from the G-domain (Chappie *et al.*, 2011). This rotation depends on a conserved proline in this region (P294 in human dynamin-1) and leads to an opening of the GTPase hinge. The opening observed in these minimal constructs left open the question as to what happens to the Stalk hinge upon nucleotide binding, since the stalk and its associated hinge were both missing from these constructs. Cryo-EM structures have also suggested a G–G contact between adjacent rungs of dynamin helices, corroborating the idea of trans-G domain dimerization. Biochemical measurements support this idea, and dynamin-1, for example, shows dramatically increased GTPase activity with G–G dimerization—a phenomenon described as “assembly-stimulated GTP hydrolysis” (Chappie *et al.*, 2011; Chappie and Dyda, 2013; Mears *et al.*, 2011).

DRP1 cryo-EM structure with MID49 and GMPPCP

As noted above for the visualization of G–G dimers in a reconstruction of dynamin-1 (Chappie *et al.*, 2011), cryo-EM has begun to reveal how nucleotide binding and hydrolysis lead to allosteric protein motions within full-length dynamins (Kalia *et al.*, 2018; Kong *et al.*, 2018). For DRP1, guanine nucleotide binding induces a 90° rotation of the G-domain via the GTPase hinge while orchestrating an $\sim 40\text{-\AA}$ movement of the G-domain toward the stalk via the Stalk hinge (Kalia *et al.*, 2018; Figure 1d). In the structure, both hinges are open, leading to 1) exposure of two of the four interfaces where DRP1 binds with its receptor MID49 or MID51 and 2) copolymerization of DRP1 with MID49/51, leading to a vast and highly avid DRP1 assembly network on mitochondrial membranes. Perhaps coassembly of DRP1 and

MID49/51 into these long and approximately linear polymers on the outer surfaces of mitochondria facilitates the recruitment of sufficient copies of DRP1 to appreciably decorate the much larger surface around a mitochondrial tubule, as compared with the thin plasma membrane necks that “modern” dynamins sever. Similar linear polymers have been observed for the assembly of DRP1 with MFF, but structural details of that assembly are unknown (Clinton *et al.*, 2016).

Part of the interaction network with the MID49/MID51 receptors included the conserved loops that interrupt the alpha helices of the stalk. The DRP1 L1N^S and L2^S loops involved in these receptor interfaces determine the geometry of assembly for other dynamin-family oligomers (Reubold *et al.*, 2015; Alvarez *et al.*, 2017). The conserved L1N^S loop is a 12-residue interruption of the first stalk helix and the site of several disease alleles that correlate with elongated mitochondria. These mutations include missense substitutions of the deeply conserved residues G362 and G363. A prior x-ray crystallography study established that this loop composes part of the intramolecular PH-domain binding site for a soluble conformation of endocytic dynamin tetramers (Reubold *et al.*, 2015). Indeed, the disease-associated G362D mutant of DRP1 failed to coassemble with MID49 and displayed both altered assembly and conformational properties (Kalia *et al.*, 2018). These observations are consistent with the phenotypes associated with this mutation: elongated mitochondria in cells and a defect in DRP1 recruitment to mitochondria (Sheffer *et al.*, 2016; Vanstone *et al.*, 2016).

The entire backbone of the DRP1 L1N^S loop was resolved in the cryo-EM structure with MiD49, while the same region in a crystal lattice was mostly disordered (Fröhlich *et al.*, 2013). The stability can be explained by the multivalent contacts that the loop made both with the receptor and with the L2^S loop of the next molecule over within the linear oligomer (Figure 1, e and f). This loop was observed to make similar critical contacts within the helical oligomer studied in a recent cryo-EM structure of MxB (Alvarez *et al.*, 2017) and the lattices of the dynamin-3 crystal structure (Reubold *et al.*, 2015; Figure 1e). Interestingly, only one residue change (G362D) within this loop was sufficient to break the interaction of DRP1 with MiD49 and was also able—via yet unknown allosteric motions—to shift the polymer architecture from linear strings to closed rings that compose twelve dimers of DRP1 in a new conformation (Kalia *et al.*, 2018).

Interestingly, wild-type DRP1 could also form closed rings, but only through a path-dependent reaction that depended on the formation of a linear oligomer *first* with MID49. Nucleotide exchange from GMPPCP to GTP then drove the linear oligomer to dissociate from MID49/51 and to curl into closed rings. Complete hydrolysis of the GTP in solution led to complete disassembly of the rings. L1N^S also maintains critical intermolecular contacts in the recent dynamin-on-lipid cryo-EM structure discussed below (Kong *et al.*, 2018). Based on its location, we predict that this loop must undergo conformational changes that underlie the structural basis of the linear filament to ring (or helix) conversion. Finally, while L1N^S is vital for the allostery that mediates the filament to ring formation, it may not be the only determinant of such changes. We also noted that a G-domain mutant—D221A, which is remote from the L1N^S loop—also induces DRP1 to form rings with GMPPCP and MID49, rather than linear filaments. Further structural studies of the closed ring state of DRP1 will help explain the short- and long-range allosteric motions that are responsible for DRP1 curling into closed rings (Srinivasan *et al.*, 2016).

Another important aspect of this structure is the L2^S loop (residues 402–409 in human DRP1). This loop resides at the tip of the stalk (Figure 1e) and mediates contact between dynamin tetramers by participating in the formation of stalk interface-3. Previous

structures (PDB ID's 5UOT, 53AF) have also shown this loop in a similar configuration (Reubold *et al.*, 2015; Alvarez *et al.*, 2017). Similarly to L1N^S, the reason for the stabilization of this loop in these structures is the interaction with L1N^S of a neighboring dynamin (Figure 1, d–f). This loop also harbors a disease allele, Arg403, and patients harboring the R403C mutation show symptoms that include epilepsy and encephalopathy, possibly due to defective DRP1 oligomerization (Fahrner *et al.*, 2016).

These *in vitro* observations raise new questions about DRP1-based mitochondrial constriction: 1) Are the DRP1 rings sufficient to constrict mitochondria? In the Kalia *et al.* (2018) study, the rings formed by the wild-type protein after disengaging from the MID49/51 receptors ranged in size from 11 to 16 dimers of DRP1, and, interestingly, some reports have indicated that efficient membrane fission could be driven by oligomers of human dynamin-2 corresponding to this size (Cocucci *et al.*, 2014). 2) How are the receptors modified or removed to enable DRP1 to disengage and curl up into rings? If the MID49/51 receptors are always around, a linear, rather than a ring state of DRP1, will be favored—precluding constriction and most likely explaining the overexpression phenotype for transgenic MID49/51 receptors (Zhao *et al.*, 2011). Interestingly, MARCH-V, a ubiquitin ligase that ubiquitylates MID49, supports mitochondrial division (Xu *et al.*, 2016), which suggests that ubiquitination of receptors may be a step in the division process.

New views of dynamin-1 around a lipid tubule

A recent study has also reported high-resolution structures of helical assemblies of endocytic dynamin on a lipid template (Kong *et al.*, 2018). While low-resolution structures of dynamins around membrane cylinders were known (Chappie *et al.*, 2011; Mears *et al.*, 2011; Sundborger *et al.*, 2014), this study reports near-atomic resolution snapshots of dynamin bound to lipid tubules in a GMPPCP bound state. The nucleotide-based conformational changes here closely resemble those seen in the case of the minimal constructs with GMPPCP (Chappie *et al.*, 2011) and the DRP1 study mentioned above (Kalia *et al.*, 2018). While G–G dimerization observed in this study strengthens the concept of assembly-mediated hydrolysis for certain members of the dynamin family, fundamental questions remain as to the steps that follow and lead to complete fission. While the initial assembly appears to be a one-start helix, the addition of GTP leads to nucleotide hydrolysis and a conversion into a pitch-expanded two-start helix with greater constriction, perhaps due to the force exerted on the membrane via the PH domains (Figure 2, a and b, Marks *et al.*, 2001; Figure 2d, Kong *et al.*, 2018).

Interestingly, the monomers within the dimeric subunit of this helical oligomer exhibit an asymmetry in the GTPase hinge. While one monomer appeared to have a completely open GTPase hinge, another displayed a kink in this area (residues 292–294). Consequently, the PH domain of the bent dynamin was also better defined in the structure, leading the authors to propose that the bent dynamin is stabilized by a transfer of force to the lipid membrane. Indeed, in terms of the secondary structure, mutants that increase the helical propensity of this region, and thereby prevent kinking, display reduced transferrin uptake in cell cultures, consistent with decreased dynamin-mediated endocytosis. Because such kinking may underlie a mechanism for the relay of the force generated by polymeric assembly via allosteric coupling between the G-BSE and PH domains upon nucleotide binding and hydrolysis, this may be a way to generate torque for membrane fission, as has been hypothesized elsewhere (Pannuzzo *et al.*, 2018). Overall, these observations are consistent with previous biophysical and crystallographic predictions regarding the conformational opening of the PH

domains with respect to the stalk and the involvement of L1N^S and the L2^S loops in maintaining inter-dynamin contacts (Reubold *et al.*, 2015; Srinivasan *et al.*, 2016).

Helices versus closed rings

The steps leading to complete membrane fission by DSPs have been a subject of intense study. GTP exposure to preassembled dynamin polymers results in constricted assemblies—a two-start helix for dynamin and rings for DRP1. Given the differences in the states of the polymers in the dynamin-1 vs. DRP1 structures, what is the role of G–G contacts in DRP1-based constriction? While no G–G contacts were seen within DRP1 rings, “stacked” or adjacent rings could form G–G contacts in *trans* on lipid surfaces, leading to assembly-stimulated hydrolysis of GTP and generation of torque on the underlying membrane. Alternatively, the oligomeric state could be different in the absence of a membrane template and, perhaps, become helical when assembled on a lipid surface. While DRP1 helical oligomers have not been observed over a “mitochondrial” lipid mix, pure DOPS liposomes can be tubulated by DRP1, which forms loosely organized helical assemblies around such membranes (Lackner *et al.*, 2009; Mears *et al.*, 2011). Intriguingly, it has been demonstrated that incubation of such mixtures with GTP leads to structures that resemble rings, or at least helical oligomers, with a very large pitch or interring distance (Figure 2b; Mears *et al.*, 2011).

Moreover, rings have been observed on lipid templates in the case of the antiviral dynamin MxA (Figure 2, c and f; Kochs *et al.*, 2005; Haller *et al.*, 2010) and even dynamin-1 on lipid nanotubes, after these proteins were incubated with lipid templates and GTP (Figure 2, a and b; Marks *et al.*, 2001). Such observations suggest that closed rings may be an overlooked state of dynamin-based, or at least DRP1-based, polymerization, constriction, and eventual fission cycle and that their structure and function deserve more in-depth study. Kalia *et al.* (2018) noted that rings with an ~16-nm inner diameter might not be constricted enough to fission the double-membrane mitochondria (Figure 2e). Curiously, dynamin-2 may also play a role in mitochondrial division downstream of DRP1, which may explain how DRP1 could act as a “constrictase” by forming closed rings, while leaving the final fission step to a “modern” dynamin that is tuned to the dimensions of the DRP1-constricted ring (Lee *et al.*, 2016). Alternatively, by destabilizing the inner membrane of the mitochondria, the rings themselves may induce membrane destabilization and fission. In support of this idea, a recent report suggests that DRP1 is sufficient for mitochondrial fission in murine embryonic fibroblasts that lack all dynamins-1, -2, and -3 (Kamerkar *et al.*, 2018). This makes the rings on lipid membranes an important candidate for further biophysical and structural characterization. Finally, the mechanisms of membrane scission may be disparate or context-dependent for different dynamin family members, proceeding via helical oligomers for some, and via ring-shaped assemblies for others.

Together, new views of dynamins have illustrated ways in which DSPs bind nucleotides, undergo conformational changes, and remodel target membranes. Nucleotide binding in the G-domains opens up dynamins to assemble via stalks—and these movements are orchestrated by the conformational flexibility in the Stalk hinge and the GTPase hinge. As noted above, fascinating structural loops such as the L1N^S and L2^S also coordinate allosteric changes that enable dynamin fission-based functions. Further understanding of the structural changes within these elements in dynamins may enable us to understand membrane fission better and to develop targeted therapies for dynamin/DRP1-based pathogenesis. In disease systems such as encephalopathy, microcephaly, optic

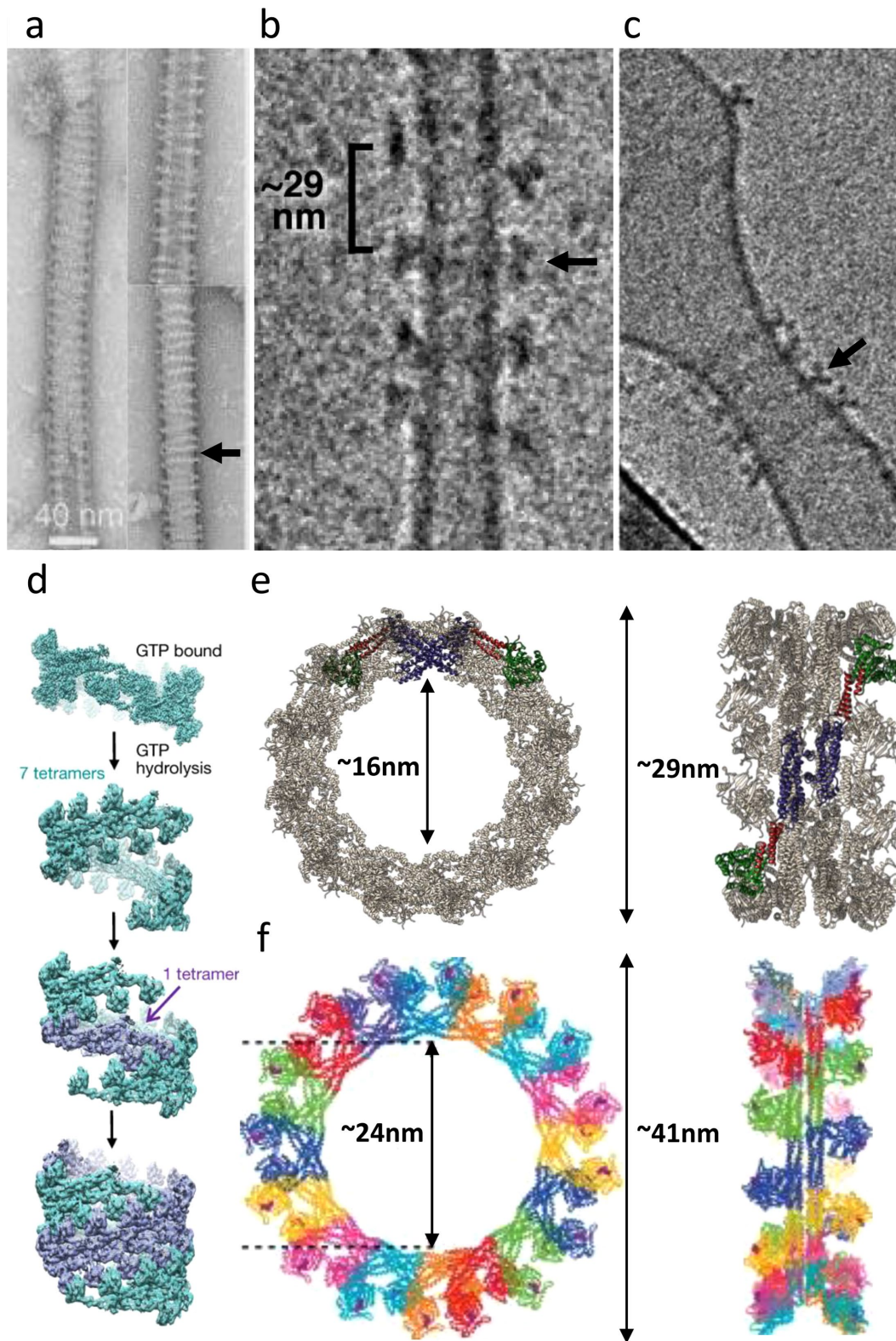


FIGURE 2: Dynamin helices and rings. (a) Left: A continuous dynamin-1 helix arranged on lipid nanotubes, in the presence of GTP- γ S (Marks *et al.*, 2001). Reproduced with permission from Nature Publishing Group. Right: same as left after incubation with 500 μ M GTP for 30 min. Note the increase in the helical pitch (arrows) and possible discontinuous helices/rings. (b) DRP1 helical polymers incubated with GTP, leading to an increase in helical pitch (Mears *et al.*, 2011). Reproduced with permission from Nature Publishing Group. These structures (arrow) could represent helices with a very elongated pitch, or rings of DRP1. (c) Rings of MxA observed on a lipid template, as seen in Kochs *et al.* (2005); Haller *et al.* (2010). Reproduced with permission from Academic Press and ASBMB. (d) A model for dynamin-1-based membrane fission, as described by Kong *et al.* (2018). Reproduced with permission from Nature Publishing Group. (e) Models for rings of DRP1 (Kalia *et al.*, 2018) and (f) of MxA (Haller *et al.*, 2010). Reproduced with permission from ASBMB.

atrophy, and Charcot–Marie–Tooth disease, where dynamin function is compromised, targeted therapies could enable efficient fission (Zuchner *et al.*, 2004; Waterham *et al.*, 2007; Chang *et al.*, 2010; Dhindsa *et al.*, 2015; Vanstone *et al.*, 2016). Conversely, systems where hyperfission enables diseases such as certain injuries related to hypoxia and reperfusion or specific malignancies, structure-based inhibition of dynamin/DRP1 assembly may be beneficial (Qian *et al.*, 2013; Ding *et al.*, 2017; Zhang *et al.*, 2018). With new and future structures, we look toward understanding the full conformational repertoire and dynamics of DSPs with membranes and binding partners—as well as structure-inspired therapeutic interventions to tackle disease.

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