Membrane fission by dynamin: what we know and what we need to know

Review

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Membrane fission by dynamin: what we know and what we need to know

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Abstract

The large GTPase dynamin is the first protein shown to catalyze membrane fission. Dynamin and its related proteins are essential to many cell functions, from endocytosis to organelle division and fusion, and it plays a critical role in many physiological functions such as synaptic transmission and muscle contraction. Research of the past three decades has focused on understanding how dynamin works. In this review, we present the basis for an emerging consensus on how dynamin functions. Three properties of dynamin are strongly supported by experimental data: first, dynamin oligomerizes into a helical polymer; second, dynamin oligomer constricts in the presence of GTP; and third, dynamin catalyzes membrane fission upon GTP hydrolysis. We present the two current models for fission, essentially diverging in how GTP energy is spent. We further discuss how future research might solve the remaining open questions presently under discussion.

Keywords dynamin; membrane fission; endocytosis; GTPase; molecular motor
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Introduction

Membrane vesicles carry cargo between cellular organelles, into and out of the cell. In the final step of endocytic vesicle biogenesis, the sides of a tubular membrane are brought into close apposition, leading to an amazing choreography of events, including the recruitment, assembly, and activation of numerous endocytic proteins that ultimately catalyze membrane fission. This process has fascinated cell biologists, biochemists, and physicists alike, due to its central importance to cell function.

In 1989, a GTPase called dynamin was discovered (Shpetner & Vallee, 1989) that functions at the heart of endocytic vesicle fission in plant and animal cells. Dynamin possesses the remarkable property of assembling into contractile helical polymers that wrap around the neck of a budding vesicle. The field has focused on how constriction of this helix contributes to severing the membrane to release the vesicle. Experimental validation of this hypothesis was more complex than expected, so many variations to this first, simple

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constriction model have been proposed and debated to explain exactly how dynamin performs its function.

A large family of related enzymes, including some in prokaryotes, participates in membrane remodeling events. For example, Vps1 in yeast is thought to catalyze fission of endosomal membrane tubes (Chi *et al*, 2014) and may, in addition, act in endocytic events (Smaczynska-de *et al*, 2010). Dynamin-related protein 1 (DRP1) and its yeast homolog DNM1 are key molecules involved in mitochondrial scission (Legesse-Miller *et al*, 2003; Ingerman *et al*, 2005; Mears *et al*, 2011; Koirala *et al*, 2013). The topologically opposite reaction—membrane fusion—is mediated by many dynamin-like proteins (Praefcke & McMahon, 2004; van der Bliek *et al*, 2013): Mitofusins drive mitochondrial outer membrane fusion and optic atrophy 1 (OPA1) fusion of the inner membrane (Ban *et al*, 2010); atlastins catalyze fusion of the ER membrane (Hu *et al*, 2009; Orso *et al*, 2009). Most of the dynamin-like proteins catalyze either fission or fusion, but Vps1, a fission catalyzer, was proposed to be bifunctional and to also catalyze fusion (Peters *et al*, 2004). Also in plants, dynamin-related proteins have been implicated in cell division (Kang *et al*, 2003), organelle division (Arimura & Tsutsumi, 2002; Gao *et al*, 2003; Miyagishima *et al*, 2003), and endocytosis (Fujimoto *et al*, 2010). In prokaryotes, functions related to the membrane stress response (Sawant *et al*, 2015) or the shedding of vesicles to the environment (Michie *et al*, 2014) were proposed to rely on dynamin-related proteins.

After almost 30 years of research on dynamin, recent structural analyses of dynamin family members and *in vivo* and *in vitro* data on dynamin activities help to better understand the mechanism by which dynamin promotes membrane fission. We decided to write this review article with the aim to first present the current state of the field and to then outline where the field is heading and which issues on dynamin function are still being discussed.

What we know: dynamin is a GTP-dependent fission machine that constricts membrane necks

Dynamin is a 100 kDa GTPase composed of the GTPase domain, the stalk consisting of a long four helix bundle, a bundle signaling element (BSE), which is a flexible connector between the GTPase domain and the stalk, a phosphoinositide-4,5-bisphosphate (PIP_2)-binding pleckstrin homology (PH) domain, which is connected to the other tip of the stalk, and a proline-rich domain (PRD) (see Fig 1). Dynamin partners that have SH3 domains bind specifically the PRD. The unstructured PRD is connected to the BSE and extends beyond the GTPase domain. Dynamin has three isoforms in mammals: dynamins 1 and 3, which are highly expressed in neurons, where dynamin 1 represents by far the predominant isoform, and dynamin 2, which is ubiquitously expressed. Most of the findings below have been shown for dynamins 1 and 2.

Dynamins have three well-established properties. (i) They self-oligomerize into helices, surrounding a membrane tube. (ii) Nucleotide-driven conformational changes lead to a constriction of the polymer and of the membrane beneath. (iii) Dynamins induce fission of the membrane necks in a manner dependent on GTP hydrolysis.

Below, we briefly describe the major findings related to these three properties.

Dynamin oligomerizes at the surface of membranes into helices

The first essential property of dynamin is its capacity to oligomerize into lock-washer-like rings or a cylindrical helix (Hinshaw & Schmid, 1995). Such oligomers were first observed at the non-permissive temperature in electron micrographs around the neck of plasma membrane buds in the temperature-sensitive *shibire* mutant in *Drosophila* (Koenig & Ikeda, 1989). These structures were shown to be made of dynamin by immune staining of synaptosomes treated with GTPγS (Takei *et al*, 1995). This oligomerization explains the membrane tubulation activity of dynamin (Sweitzer & Hinshaw, 1998; Takei *et al*, 1999), as well as the property to associate with tubular templates, such as narrow membrane tubes (Roux *et al*, 2010), microtubules (Shpetner & Vallee, 1989), and lipid nanorods (Stowell *et al*, 1999; Marks *et al*, 2001), which facilitate its assembly. This tubulation activity of dynamin is proposed to promote membrane curvature at the endocytic pits, as clathrin-coated pit necks are larger when dynamin recruitment is inhibited (Shupliakov *et al*, 1997; Newton *et al*, 2006). Dynamin oligomerization in solution is favored by binding to non-hydrolyzable analogs of GTP, such as GMPPCP, GTPγS (Warnock *et al*, 1996), or GDP•AlF_4^- (Carr & Hinshaw, 1997), while GTP hydrolysis favors disassembly of the dynamin oligomers and release of its subunits from the membrane (Warnock *et al*, 1996; Marks *et al*, 2001; Danino *et al*, 2004).

In the absence of nucleotide, dynamin assembles into a helical coat of 50 nm outer diameter with a helical pitch between 10 and 20 nm (see Figs 1 and 2), surrounding a membrane tubule of 10 nm radius (at the mid-plane of the membrane) (Sweitzer & Hinshaw, 1998; Takei *et al*, 1998, 1999; Chen *et al*, 2004; Danino *et al*, 2004). The polymer has an outer diameter of approximately 50 nm, with a helical pitch between 10 and 20 nm (see Figs 1 and 2). Cryo-EM revealed that the dynamin polymer unit is an anti-parallel dimer, with the GTPase domains facing outside and the PH domains on the inside, bound to the membrane (Zhang & Hinshaw, 2001; Chen *et al*, 2004; Mears *et al*, 2007). Crystallographic data also support this picture (see Fig 1A). Non-oligomerizing mutants could be crystallized in an anti-parallel dimeric form (Faelber *et al*, 2011; Ford *et al*, 2011). Dimerization is mediated by the stalks, which form a cross (see Fig 1A). The two GTPase domains are linked to one side of the cross whose other side is linked to the PH domains. Interactions between the stalk dimers drive the assembly into the helical polymer of the expected size, as seen by molecular dynamics of the assembly process (Faelber *et al*, 2011), identification of the position of mutated residues in non-oligomerizing mutants (Faelber *et al*, 2011; Ford *et al*, 2011), and structural insight into the tetrameric form of dynamin 3 (Reubold *et al*, 2015). Recent quantitative *in vivo* data also show that dynamin polymerizes into oligomers of varying size at the neck of clathrin-coated pits (Cocucci *et al*, 2014; Grassart *et al*, 2014).

Even though the exact interactions between subunits are not conserved throughout the dynamin superfamily, the basic assembly properties (formation of helical polymers) are shared by members of the dynamin superfamily from bacteria to mammals, as revealed by structural studies of dynamin-like proteins such as BDLP, Drp1/Dnm1, and Mgm1/OPA1 (Low & Löwe, 2006; Low *et al*, 2009; Ban *et al*, 2010; Mears *et al*, 2011; Abutbul-Ionita *et al*, 2012; Frohlich *et al*, 2013).

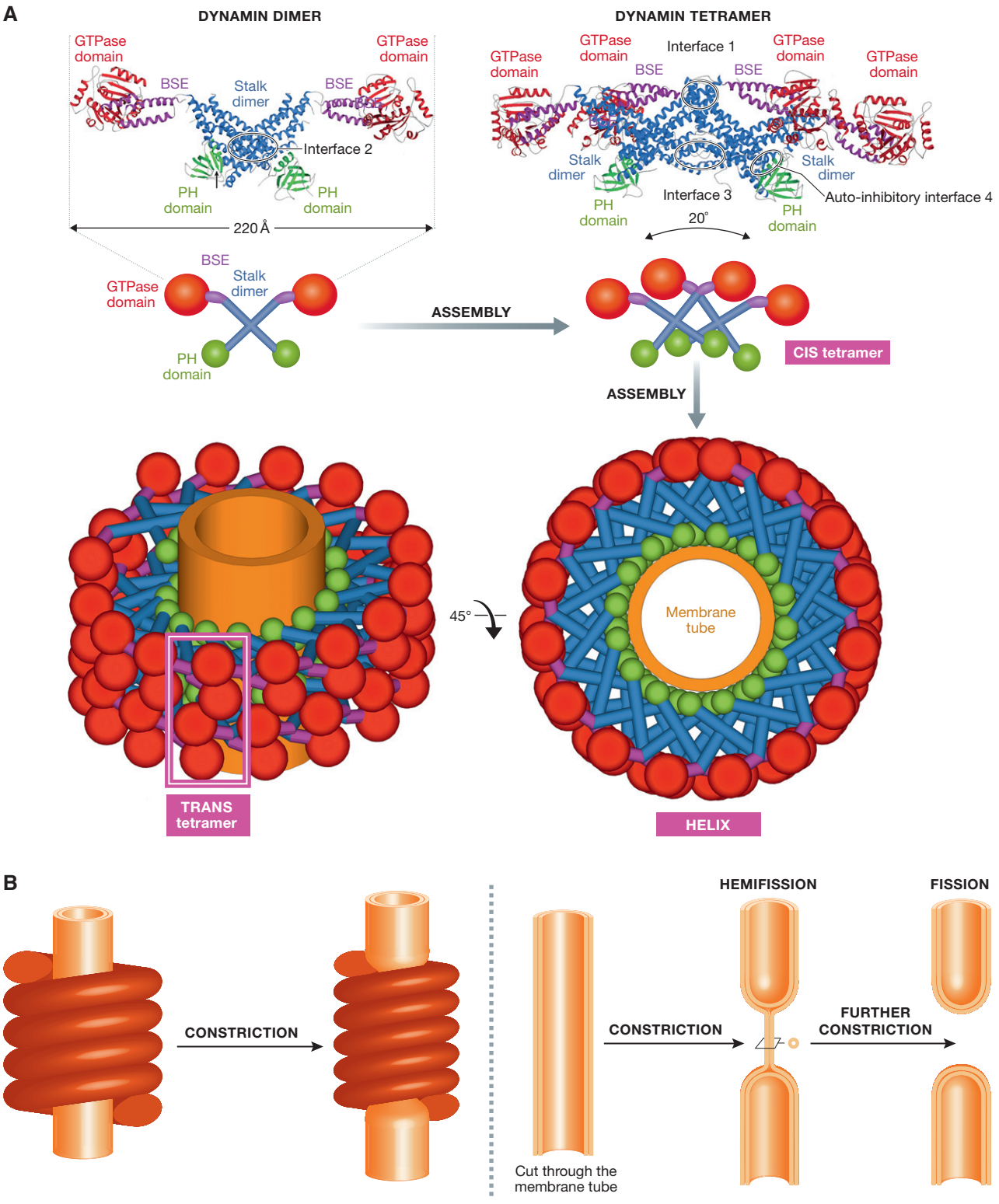

Figure 1. Structure and assembly of dynamin.
(A) Crystal structure of the dimer and of the tetramer, showing the interfaces required for assembly. A schematic representation shows how the tetramers further assemble into a helix, showing the basic CIS-tetramer and TRANS-tetramers. (B) The original constriction model for dynamin-mediated membrane fission, as suggested by the helical structure of dynamin.

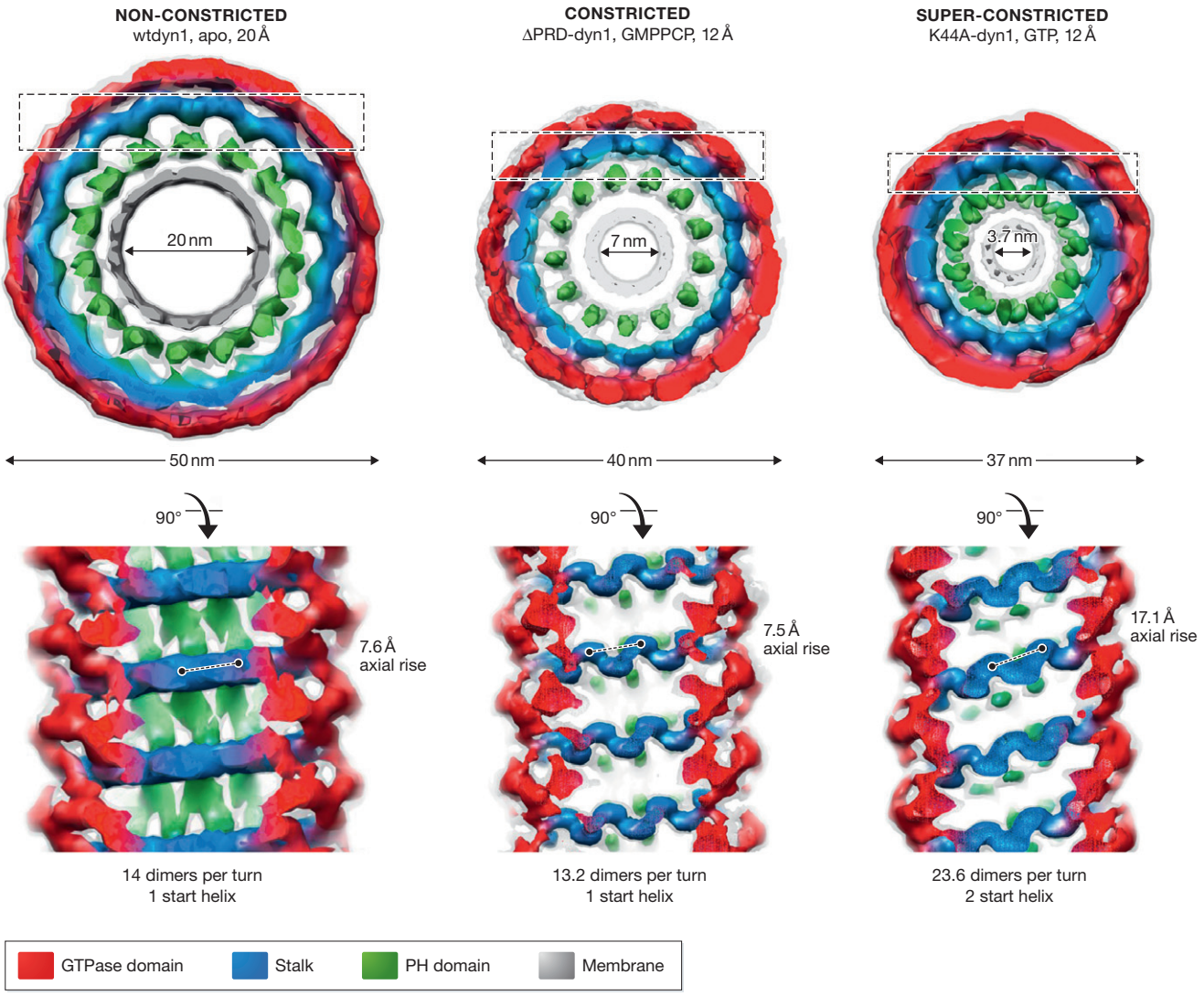

Figure 2. The three states of the dynamin helix observed by cryo-EM, with dimensions and angles.

Dynamin is a GTP hydrolysis-dependent, membrane fission catalyzer

The essential function of dynamin is to use energy from GTP hydrolysis to sever membrane tubules. Fission of clathrin-coated pits from the plasma membrane is defective at neuronal synapses of mice lacking dynamin 1, or both dynamins 1 and 3 (Ferguson *et al*, 2007; Raimondi *et al*, 2011) and in embryonic fibroblasts from mice with conditional double (Ferguson *et al*, 2009) or triple deletions of dynamin genes (Park *et al*, 2013). Mutants with reduced GTPase activity delay or block endocytosis of transferrin (Marks *et al*, 2001; Boll *et al*, 2004; Song *et al*, 2004) and prolong the residence time of clathrin/dynamin at the plasma membrane (Taylor *et al*, 2011; Kural *et al*, 2012). GTPase-defective mutants have dominant negative phenotypes when they co-assemble with wild-type proteins in overexpression experiments (Damke *et al*, 1994).

The mechanism of dynamin-mediated membrane fission has been studied by reconstitution with purified components. GTP hydrolysis is consistently required for membrane fission in these reconstituted systems (Sweitzer & Hinshaw, 1998; Roux *et al*, 2006; Bashkirov *et al*, 2008; Pucadyil & Schmid, 2008; Morlot *et al*, 2012; Shnyrova *et al*, 2013; Mattila *et al*, 2015). Membrane tension, which can be provided by adhesion of the membrane tubes to the substrate, facilitates the reaction (see also below) (Sweitzer & Hinshaw, 1998; Danino *et al*, 2004; Roux *et al*, 2006; Boulant *et al*, 2011; Morlot *et al*, 2012). As in membrane fusion, dynamin-mediated fission proceeds through a hemi-fission state where the inner leaflet of the tube disappears (see Fig 1B), leaving a connecting neck made of a single lipid monolayer wrapped in a cylindrical micelle (Bashkirov *et al*, 2008; Morlot *et al*, 2012; Shnyrova *et al*, 2013; Mattila *et al*, 2015).

Dynamin helices constrict in the presence of GTP

There is broad agreement that a key property of the dynamin helical oligomer is its ability to constrict in the presence of GTP. *In vivo*, inhibition of dynamin GTPase activity with chemicals or mutants

promotes the formation of elongated necks that have a membrane radius of ~10 nm (Takei *et al*, 1995; Marks *et al*, 2001; Liu *et al*, 2013), consistent with the size of oligomers in the absence of nucleotide (Sweitzer & Hinshaw, 1998; Chen *et al*, 2004; Danino *et al*, 2004; Roux *et al*, 2010). Membrane tubes enclosed in helices of dynamin are more constricted during GTP hydrolysis (see Fig 2) (Sweitzer & Hinshaw, 1998; Danino *et al*, 2004; Sundborger *et al*, 2014) or in the presence of non-hydrolyzable GTP analogs (Zhang & Hinshaw, 2001; Chen *et al*, 2004; Mears *et al*, 2007). The most constricted conformation is observed with GTP-loaded K44A mutant dynamin, which has a reduced affinity for GTP partially inhibiting its GTPase and fission activity. Under these conditions (i.e. super-saturating concentrations of GTP), K44A dynamin may be trapped in either a GTP bound state or an undefined GTP hydrolysis transition state. The membrane tube that is wrapped by this form of dynamin exhibits an inner luminal radius < 2 nm (see Fig 2) (Sundborger *et al*, 2014). This super-constricted state is achieved by assembling into a two-start helix and is also observed after short-time reactions containing wild-type dynamin and GTP (Sundborger *et al*, 2014). This constriction of the dynamin helix is consistent with a twisting of the helical collar that can be visualized by the rotation of beads attached to the dynamin coat (Morlot *et al*, 2010, 2012) or from the cryo-EM structures with 14 subunits per turn without nucleotide (Chen *et al*, 2004), 13 with GMP-PCP (Zhang & Hinshaw, 2001), and 11 with K44A and GTP (Sundborger *et al*, 2014). The same twisting activity could result in the elongation of the pitch observed in cases where the membrane template cannot be constricted (Stowell *et al*, 1999; Marks *et al*, 2001; Lenz *et al*, 2008; Faelber *et al*, 2011). This constriction ability seems to be shared among members of the dynamin family, as it is also observed for the dynamin-related protein, Dnm1 (Mears *et al*, 2011).

The properties of dynamin described above are in agreement with the initial constriction model that dynamin breaks membrane by constriction during GTP hydrolysis (see Fig 1B). In the first description of this model, the dynamin helix would constrict until the membrane neck reaches the hemi-fission state and then is fully broken. However, two findings from *in vitro* experiments have been in apparent disagreement with this simplest view. First, constriction of dynamin is necessary, but not sufficient for fission. Second, GTP hydrolysis triggers partial depolymerization of the dynamin coat. In the following, we detail these findings and explain how they set the current debate about the dynamin mechanism.

What is being discussed: reconciling GTP-driven constriction, disassembly, and mechanics of the membrane

In this part, we will briefly discuss recent data on the role of mechanics of membrane on the fission reaction, and results on the role of disassembly in fission. Then, we discuss the two models that try to conciliate these data.

Contributions of membrane constriction and tension to fission

The first observations of dynamin-mediated fission *in vitro* showed that membrane tension was necessary for dynamin to break membranes. Nonetheless, this observation is consistent with the fact that the super-constricted state of dynamin does not constrict the membrane sufficiently to reach hemi-fission, leaving a lumen of 1.9 nm radius (see Fig 2) (Sundborger *et al*, 2014): In a case where dynamin would constrict the membrane enough to go beyond the hemi-fission state and break it completely, membrane tension would have no impact on the fission rate, as a membrane with low tension would be broken as efficiently as a membrane with high tension. However, fission occurs within minutes if membrane tension is low (Pucadyil & Schmid, 2008; Dar *et al*, 2015), whereas it takes a few seconds when membrane tension is high (Roux *et al*, 2006; Bashkirov *et al*, 2008; Morlot *et al*, 2012), showing indeed that tension has a direct impact on fission efficiency. Moreover, upon dynamin-mediated constriction, the hemi-fission state is reached stochastically (Shnyrova *et al*, 2013; Mattila *et al*, 2015) and is reversible, suggesting that once constricted, thermal fluctuations of the membrane are needed to reach the hemi-fission state.

Why would membrane tension be required? The solution came from membrane physics (Kozlovsky & Kozlov, 2003): Calculations showed that the elastic energy of a highly constricted membrane neck (down to a lumen of 3 nm, but prior to hemi-fission) was the same as the elastic energy of the hemi-fission intermediate. In this case, the calculations predict a low energy barrier, and thus, one expects the system to pass from the super-constricted to the hemi-fission state spontaneously and stochastically (i.e., by thermal fluctuation), and to be reversible, as observed by the Frolov group (Shnyrova *et al*, 2013; Mattila *et al*, 2015). From this conceptual framework, one thus expects the fission reaction to be stochastic. Moreover, because the elastic energy of the membrane depends on tension and rigidity, the rate of fission is also expected to depend on both, consistent with the early observation that membrane tension was required for fission (Danino *et al*, 2004; Roux *et al*, 2006). Quantitative measurements of fission rates with membrane tension and rigidity *in vitro* further confirmed theoretical predictions (Morlot *et al*, 2012). *In vivo*, the fission rate is similar to the fastest *in vitro* values (5–10 s), and the distribution is also stochastic (Merrifield *et al*, 2005; Cocucci *et al*, 2014). Importantly, consistent with the role of membrane elasticity in dynamin-mediated membrane fission, increased membrane rigidity reduces the rate of fission (Morlot *et al*, 2012), and the presence of polyunsaturated lipids, which reduces membrane rigidity, facilitates fission (Pinot *et al*, 2014). Thus, constriction by dynamin may not be sufficient to cause membrane fission, but rather dynamin would constrict the membrane tubule to a size that spontaneously reaches hemi-fission in a tension and rigidity-dependent manner.

Nucleotide-dependent disassembly of dynamin

As early as dynamin was found to oligomerize, it was observed that dynamin oligomers in solution would disassemble upon GTP hydrolysis (Warnock *et al*, 1996). This GTP-triggered disassembly was reported by many techniques (Sweitzer & Hinshaw, 1998; Bashkirov *et al*, 2008; Pucadyil & Schmid, 2008) but was absent in other reports (Stowell *et al*, 1999; Danino *et al*, 2004; Roux *et al*, 2006; Morlot *et al*, 2012), even though limited disassembly could not be excluded in these experiments. The discrepancy may reflect the nature of the lipid templates used (their lipid composition and intrinsic curvature), as well as the concentrations of dynamin and assays used to measure disassembly (fluorescence, sedimentation, FRET). Structural studies showing that the GMPPCP-bound form of

a G domain-GED dimer can be docked into cryo-EM structures, whereas the GDP−AlF_4^--bound state cannot (Chappie, Mears *et al*, 2011; Sundborger *et al*, 2014), may indicate that the helical scaffold is destabilized in the transition state. Moreover, GTP-triggered disassembly is consistent with the necessary recycling of dynamin observed *in vivo* (Merrifield *et al*, 2002; Doyon *et al*, 2011; Cocucci *et al*, 2014; Grassart *et al*, 2014). It is also consistent with the finding that fission may occur at the tip of the dynamin coat (Morlot *et al*, 2012), which would require that part of the coat be removed prior to fission. Different results have been obtained in a similar assay (Dar *et al*, 2015), but because light microscopy was used in both studies, the resolution may be insufficient to draw any clear conclusion.

The current models for dynamin's fission mechanisms

A two-stage model for dynamin-catalyzed fission (Fig 3A) This model reconciles the fact that dynamin disassembles upon GTP hydrolysis with the need for assembled dynamin to constrict the membrane, by suggesting that these two stages are temporally distinct. Thus, while dynamin scaffolds are needed to constrict the membrane, these same scaffolds could stabilize the underlying tubule and inhibit fission (Bashkirov *et al*, 2008; Pucadyil & Schmid, 2008; Boucrot *et al*, 2012). This model suggests that in stage one, assembled dynamin in a specific nucleotide loaded conformation adopts a super-constricted state enabling the formation of hemi-fission intermediates. Based on *in vitro* data discussed above, this likely corresponds to a GDP+Pi transition state (mimicked by GDP•AlF_4^- binding), when G domains across adjacent rungs form their highest affinity interactions (Chappie *et al*, 2011). Subsequent release of Pi to the GDP-bound state would loosen the scaffold, as seen by negative-stain EM (Stowell *et al*, 1999; Danino *et al*, 2004; Mattila *et al*, 2015), allowing for the hemi-fission intermediates to proceed to complete fission. Importantly, formation of the transition state requires interactions of G domains between adjacent rungs of a dynamin helix. Indeed, *in vitro* (Shnyrova *et al*, 2013) and *in vivo* (Cocucci *et al*, 2014) data suggest that the minimum fission apparatus is slightly larger than one rung in the super-constricted state (25-30 monomers, 11 dimers per turn).

The two main points of this model under discussion are the following:

- First, the two-stage model requires that all dimers in a rung are in the same nucleotide state and thus a high degree of cooperativity of the dimers for GTPase hydrolysis. This seems inconsistent with what is known about the GTPase activity of dynamin. The Hill coefficient of dynamin against GTP is one in the assembled state (Tuma & Collins, 1994), which means that there is no cooperativity of dimers in GTP hydrolysis. Also, GTPase domains should stay in the transition (GTP + Pi) state long enough for hemi-fission and fission to occur, which takes 5–10 s, whereas the GTPase rate in the assembled state is a few GTP per second per monomer of dynamin. Thus, the kinetics of dynamin GTP hydrolysis seems inconsistent with the two-stage model.
- Second, how induction of hemi-fission is coupled to disassembly is essential in the two-stage model, as the super-constricted state of dynamin does not reach hemi-fission. The original proposition was that disassembly was fast enough to destabilize the membrane and drive hemi-fission. However, the membrane is very fluid, with a viscoelastic time less than 10 milliseconds (Camley & Brown, 2011). It means that any deformation occurring slower than this time would be followed smoothly by the flow of membrane. No viscoelastic stress will thus appear, and the behavior of the membrane will be dictated by equilibrium mechanics. The dynamin disassembly rate is typically in the order of a few tens to a few hundreds of milliseconds, up to a few seconds to fully disassemble *in vivo* (Cocucci *et al*, 2014). This is much slower than the membrane viscoelastic time, and thus, complete disassembly of the dynamin coat circling a non-hemi-fissioned tubule of membrane is expected to lead to tubule widening rather than collapse and break.

But other sources of destabilization may be at work (see Fig 3A): It was shown that the PH domain of dynamin contains a rather short amphipathic loop that could wedge itself into the membrane to constrict it further (Ramachandran *et al*, 2009). Indeed, biochemistry experiments show that the residues of this helix insert deeper in the leaflet in a nucleotide-dependent manner (Mehrotra *et al*, 2014; Mattila *et al*, 2015). However, this hypothesis has received some skepticism, as the position of this loop, away from the PIP_2 binding pocket in the PH structure, does not allow for insertion in the membrane without releasing its link to PIP_2. Moreover, the loop (a few amino acids) is so short that one can question the fact that it could generate enough curvature to constrict further the membrane.

A solution might come from the fact the PH domains would tilt when dynamin is constricted (Shnyrova *et al*, 2013) (see Fig 3A). In the super-constricted state, one PH domain per dimer seems tilted in the cryo-EM data, which could indeed push the helix further in the leaflet (Sundborger *et al*, 2014). However, the resolution of the currently available cryo-EM data is too limited in order to confirm tilting. Whether this loop insertion is sufficient to create curvature, and whether it keeps its link to PIP_2 is still unclear.

The constrictase/ratchet model (see Fig 3B) The constrictase/ratchet model is a refined constriction model that proposes that dynamin acts as a motor. GTP hydrolysis energy would be spent in mechanical work to slide adjacent turns of the helix. In this model, GTPase domains, which are linking dynamin turns through direct interactions, could act as molecular motors, and by cycles of association/powerstroke/dissociation powered by several GTP hydrolysis cycles (see Figs 3B and 4B), would trigger relative sliding of the helical turn, leading to constriction and twisting of the helix. This model is analogous to the mechanism of myosin movement on actin filaments, but with dynamin playing the role of myosin and actin at the same time.

The biochemistry of the GTPase activity is indeed consistent with such motor activity: It has a fairly low affinity for nucleotides and a high GTPase rate (at least when activated through assembly) (Praefcke & McMahon, 2004). The model is also supported by structural studies: The global architecture of dynamin is very similar to myosin or kinesin: It has a stalk, which is connected to the GTPase domain through a flexible hinge to the BSE. GTP binding was shown to induce trans-dimerization (between helical turns) of the GTPase domains via an interface across the nucleotide-binding site (Chappie *et al*, 2011). Structural studies indicated that the BSE senses the nucleotide loading status of the GTPase domain (Chappie *et al*,

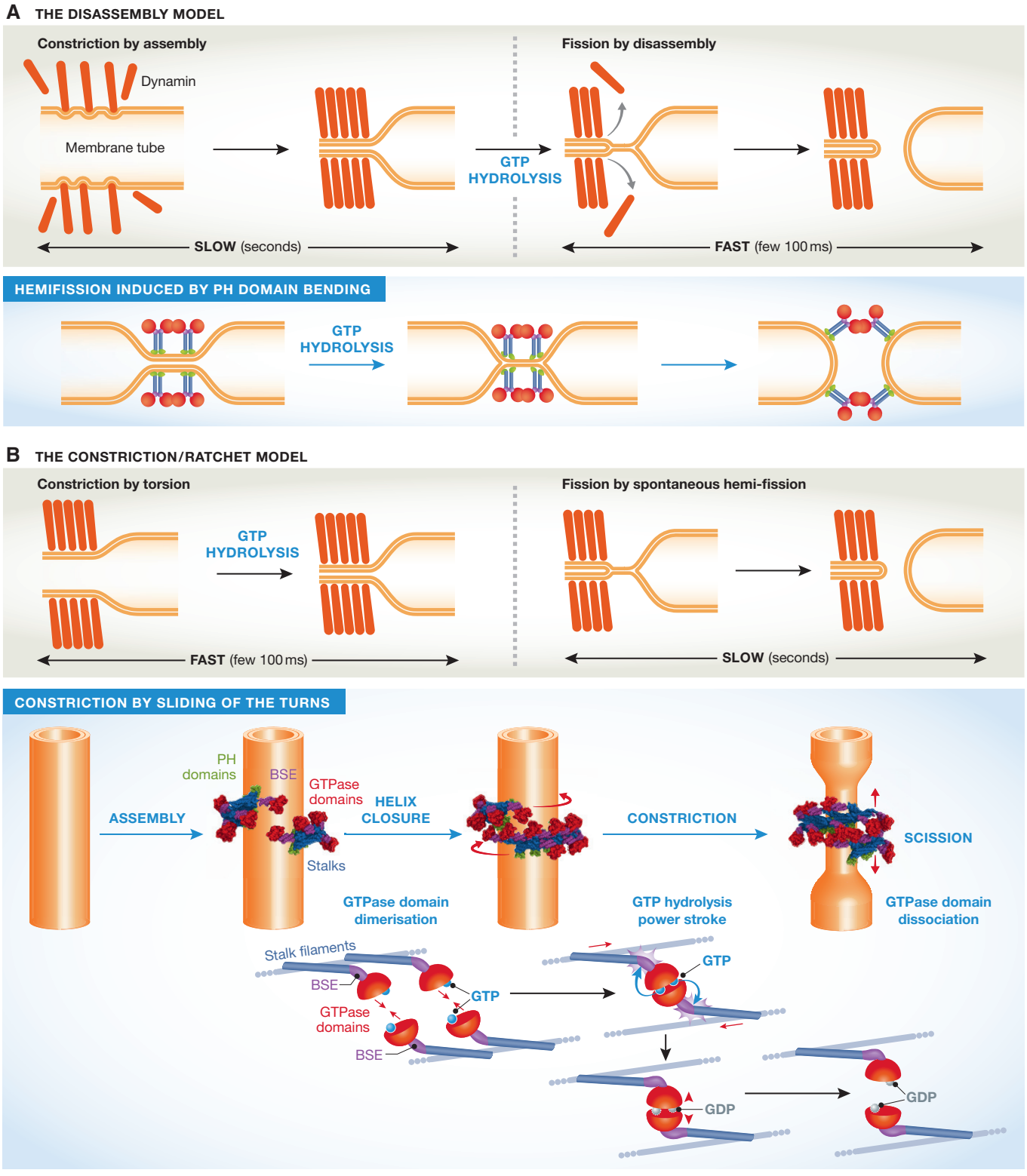

Figure 3. The two models of dynamin-mediated membrane fission.
(A) The two-stage model, where constriction is mediated by assembly, and fission by disassembly. (B) The constriction/ratchet model in which constriction is realized by active sliding of the helical turns and fission by spontaneous fusion of the membrane. The one ring state presented here is proposed to be the most common *in vivo* (see text).

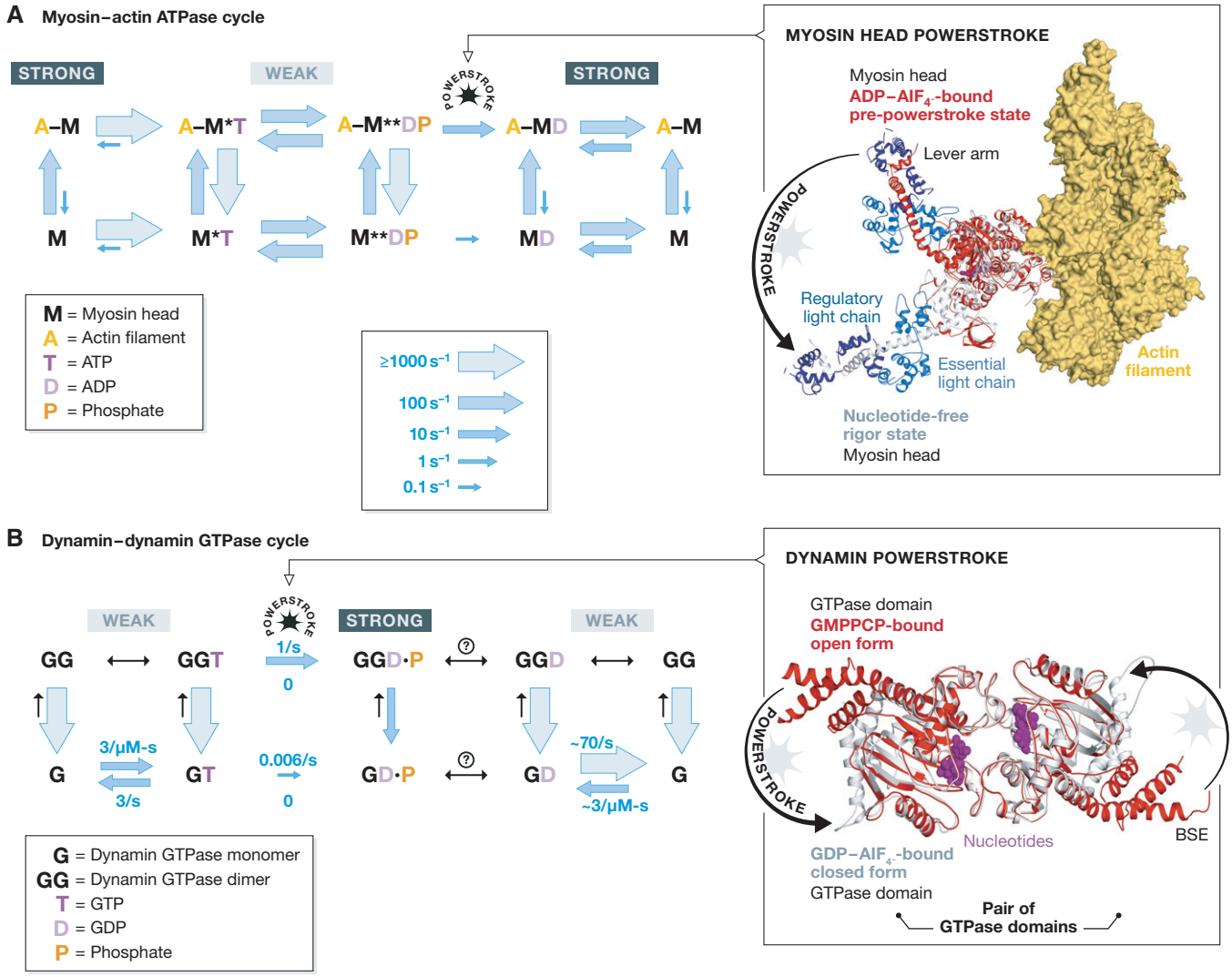

Figure 4. Comparison of the skeletal muscle myosin ATPase (A) with the dynamin GTPase (B) cycles.
Both reaction pathways are populated by chemical intermediates defined in the figure. High energy states are indicated with an asterisk. Arrows indicate the reactions between each pair of intermediates. The sizes of the blue arrows are proportional to the rates under physiological conditions (taking into account the concentrations for bimolecular reactions) as defined at the bottom right. The black arrows in (B) indicate unknown rates. The bottom rows in (A) and (B) are reactions of myosin (M) and dynamin (G) monomers. The top rows are reactions of myosin bound to an actin filament (AM) or dynamin dimers (GG). The vertical arrows indicate the rates of myosin binding actin filaments and dynamin forming dimers. In (A), the right panel represents a superposition of myosin in the nucleotide-free, pre-power stroke state (pdb 2mys, white) and the ADP-AlF_4^--bound rigor state (pdb 1br1, red). ADP-AlF_4^- is shown in magenta, and the two myosin light chains bound to the lever arm are shown in blue and dark blue. The positions of the second light chain and the distal end of the lever in pdb 1br1 were modeled based on pdb 2mys. Five actin molecules (yellow) are indicated (from pdb 5jlh). In (B), the right panel represents a superposition of the G domains in the dynamin GG construct in the GMPPCP-bound open (pdb 3zyk in red) and the GDP-AlF_4^--bound closed form (pdb 2x2e in white). Nucleotides are shown in magenta. Note the 70° rotation of the BSE relative to the G domain.

2009, 2010, 2011). It adopts an open conformation in the presence of GTP, whereas a 70° rotation to a closed state was observed in the presence of GDP–AlF_4^- (a transition state analog) or in the absence of nucleotide. This movement could act as a power stroke for dynamin during constriction (Chappie *et al*, 2011; Ford *et al*, 2011). This model is also consistent with cryo-EM studies showing that upon conversion from the non-constricted to the constricted state, there is a reduction in one dimer per helix turn (from 14 to 13).

However, there are several open questions for this model. First, the structural data obtained by X-ray diffraction on almost full length (Faelber *et al*, 2011; Ford *et al*, 2011; Reubold *et al*, 2015) or truncated (G domain-BSE, "G-G") dynamin constructs (Chappie *et al*, 2010) do not perfectly match the cryo-EM data (see Chappie *et al*, 2011; Sundborger *et al*, 2014 for details). In particular, the EM density maps of the stalks do not perfectly fit the stalk dimer observed in all published X-ray structures. This may indicate conformational rearrangements of the stalks upon oligomerization or flexibility of the stalk assembly not observed in low-resolution EM data. While the GMPPCP-bound dimeric form of the G-G construct can be docked into the cryo-EM structures, the GDP–AlF_4^--bound state cannot. This suggests that the lipid and GMPPCP-bound constricted dynamin filament features the open BSE conformation.

Finally, a super-constricted state of dynamin has been described which has only 11 dimers per helix turn (Sundborger *et al*, 2014). However, in this super-constricted state, dynamin forms a two-start helix, for example, there are two parallel helices wrapping around the membrane tubule, resulting in a double rise per helix turn. The relevance of the two-start helix and its relation to dynamin's constriction mechanism is currently under debate.

Also in this model, the number of interacting GTPase domains is critical for force generation. It was shown by two groups (Cocucci *et al*, 2014; Grassart *et al*, 2014) that *in vivo*, efficient fission could be mediated by at least 26–28 monomers of dynamin, which corresponds to 13–14 dimers. This number corresponds to a single helical turn of dynamin in the non-constricted state, or to 1.5 turns in the super-constricted state. It thus suggests that efficient fission can be mediated by 1 to 5 G-G interactions. Is this sufficient to generate enough force to constrict the tube? Can it generate the enormous torque (approx. 1nN.nm) measured *in vitro* on long dynamin scaffolds (Morlot *et al*, 2012)?

Another problem is the coupling between formation of G-G links and constriction: To allow for sliding of the turns, the G-G domains have to be transiently disrupted. If a given proportion of G-G interacting domains is inactive while others are active (moving), there would be no sliding. This issue may be solved by carefully considering the coupling between GTPase and mechanical cycles of dynamin (see discussion in the next section).

Also, in this model, the constriction force of dynamin probably opposes two resistances: the membrane elasticity, which would tend to widen the tube, and most probably the rigidity of the dynamin coat itself, which counteracts constriction. As shown experimentally, the constriction force is dependent on GTP concentration (Morlot *et al*, 2012), which means that in this model the maximal constriction depends on force and thus on GTP concentration, which may be inconsistent with the well-defined super-constricted state. In this case, dynamin would burn GTP to apply a constant force to hold the tube at its maximal radius of constriction until it spontaneously breaks.

Finally, in this model, how disassembly occurs is less clear. Two hypotheses have been suggested: First, once fission has occurred, dynamin could disassemble because the membrane template is gone. Second, as discussed above, the stresses appearing in the dynamin coat under constriction could cause it to break apart. In those two cases, disassembly would be a consequence of fission and/or constriction, and GTP hydrolysis energy would primarily be spent in generating constriction force (mechanical work).

Thus, the field is left with the challenge of discriminating between two apparently opposing models, one in which most of the constriction is achieved during assembly, and GTP hydrolysis destabilizes G-G dimers to loosen the dynamin scaffold, and a second model where the energy of GTP hydrolysis is spent in mechanical work of interacting GTPase domains, allowing for one turn of the helix to walk on the adjacent one. Of course, the two models may not be mutually exclusive, yet a mechanism consistent with all data has to be found. Clearly, more information is needed regarding how dynamin's GTPase cycle is coupled to its activities (assembly/disassembly, membrane interactions, G domain dimerization, conformational changes, etc.) that lead to fission.

What we need to know: what is GTP energy good for and what is the GTPase cycle of dynamin

The long debate regarding the contributions of dynamin assembly, constriction, and disassembly to fission appears to be close to resolution. The two models discussed above could be discriminated or reconciled by obtaining two important pieces of information: How the GTP hydrolysis energy is spent and whether the super-constricted state is reached through assembly, or through active constriction of the polymer. In the disassembly model, most of the energy of hydrolysis is used to destabilize the polymer, as for tubulin or actin, and the super-constricted state/hemi-fission is reached through assembly in a more curved helix because dimers are in the transition state. In the constrictase model, most of the energy is used to provide mechanical work to slide helical turns and constrict, as for myosin, and the super-constricted state is reached through multiple rounds of GTP hydrolysis. In the following, we discuss recent findings trying to address this point.

How constricted is the GTP-loaded state of dynamin?

Jenny Hinshaw and her group have tried to answer this question by studying the constriction of the dynamin helix depending on its nucleotide load. The recent finding that dynamin K44A constricted tubes with GTP, but also dynamin wild-type tubes with GTP, are in the super-constricted state suggests that at least at some point in the GTPase cycle assembled dynamin is already in the super-constricted state (Sundborger *et al*, 2014). Moreover, in these cryo-EM structures, dynamin helices are in fact two-start helices (which means two helices intertwined together), and it thus seems rather impossible to constrict a one-start helix into a two-start helix. Thus, if assembled dynamin in the presence of GTP is super-constricted, the energy of GTP hydrolysis must be used for something other than constriction, probably disassembly.

However, because this super-constricted state is only seen in the presence of GTP (no other analogs trigger this state) and because dynamin K44A still has a minimal GTPase and fission activity, it cannot be ruled out that this super-constricted state is not the result of multiple cycles of GTP hydrolysis, inducing constriction by torsion as proposed by the constriction/ratchet model. An important note is that a two-start helix would constrict similarly to a one-start helix. In this case, it would mean that dynamin assembles as a two-start helix in the presence of GTP, which could be mediated by the formation of TRANS-tetramer as a nucleus for two-start helices (see Fig 1A), and then, hydrolysis would trigger constriction by torsion.

The mechano-chemical cycle of dynamin

In an attempt to determine how the energy from GTP hydrolysis is used, Tom Pollard compared the mechano-chemical cycles of myosin and dynamin (see Fig 4). Pre-steady state kinetic experiments established the mechanism of myosin by measuring the rate and equilibrium constants for each step in the cycle of interaction with ATP and actin filaments. Much less is known about dynamin, but the two enzymatic cycles seem to have much in common (see Fig 4), likely arising from the two enzymes having a common ancestor and sharing structural features.

Sliding of filaments in a muscle sarcomere depends on coupling the ATPase cycle to conformational changes. As illustrated in the

lower row of reactions in Fig 4A, myosin binds and hydrolyzes ATP rapidly. Hydrolysis is rapidly reversible, and most of the energy from ATP binding and hydrolysis is stored in conformational changes indicated by M* and M**. Myosin releases the γ-phosphate slowly and then releases ADP quickly, to restart the cycle. Two of the chemical states (nucleotide-free myosin and myosin-ADP) bind strongly to actin filaments (slow dissociation indicated by small downward arrows between the two rows), while myosin-ATP and myosin-ADP-P_i dissociate very fast from actin filaments (large arrows pointing down). A large free energy change associated with phosphate dissociation from the actin-myosin-ADP-P_i intermediate is coupled to a conformational change that produces force on the actin filament. In muscle, these power strokes are uncoordinated and the force-producing intermediates have short lifetimes, so most myosins (95%) are dissociated from actin and do not interfere with sliding by the active heads. If the myosin heads were coordinated, the filaments would only slide 5–10 nm per ATPase cycle and the filaments would slide backwards during the times that no heads were attached to actin filaments.

Although dynamin has a GTPase cycle parallel to that of the myosin ATPase cycles (see Fig 4B), it superficially appears to differ from myosin and other motor proteins, because it does not act upon a separate filament. Rather, dynamin seems to act upon itself through interactions between GTPase domains on adjacent turns of the polymer, with forces transmitted to the dynamin polymer composed of the stalks and then, ultimately to the underlying membrane.

Figure 4B shows what is known about the dynamin mechanochemical cycle. The mechanism involves the GTPase cycles of monomers (bottom row) and dimers (top row) and the formation and dissociation of dimers of each chemical intermediate (vertical arrows, comparable to myosin binding to actin). The analysis is limited by lack of information about some of the parameters, but enough is known to propose general features. Six of the 16 rate constants have been measured [numerical values indicated (Binns *et al*, 2000)], and the values of six more can be estimated from equilibrium constants. The sizes of the blue arrows indicate estimated rates under physiological conditions. Black arrows indicate parameters that have never been measured.

Dynamin monomers bind GTP rapidly, but dissociate GTP faster than motor proteins dissociate ATP (Song & Schmid, 2003). Dynamin monomers (G) hydrolyze GTP at ~0.01/s (Binns *et al*, 2000; Song & Schmid, 2003), much slower than motor proteins. Nothing is known about dissociation of the γ-phosphate, which is unfortunate, since this reaction is crucial in motor ATPases and other GTPases. However, GDP dissociates rapidly, so it can be assumed that P_i does as well. Given physiological concentrations of GTP and GDP, most of monomeric dynamin would have bound GTP in the GT or G*T states.

Dynamin dimers must use a GTPase cycle (top row of Fig 4B) parallel to dynamin monomers. Fortunately, we know the most crucial rate constant, the hydrolysis of GTP, which is 100 times faster for dimers than for monomers. The other rate constants have not been measured.

Only the intermediate shown to form intermolecular dimers in solution had bound GDP•AlF_4^- (a stable mimic of GDP•P_i), but the affinity of this GGD•P dimer is low with a K_d of 8.4 μM (Chappie *et al*, 2011). Depending on the association rate constant, this affinity corresponds to a dissociation rate of 10–100/s. GTPase domains in other intermediate states have such lower affinities for each other (K_d > 30 μM) (J. Chappie and F. Dyda, personal communication) that no dimers are detected in solution (Chappie *et al*, 2010). Thus, such dimers will dissociate rapidly. Although the high local concentration of assembled dynamin favors association of the GTPase domains, only the GGD•P dimers are expected to be stable enough to support motion.

Given these reaction rates, the pathway through the dynamin GTPase cycle probably goes from nucleotide-free monomeric GTPase domain (G) to its form associated with GTP (GT), which then hydrolyzes GTP to be in the transition state associated with GDP+Pi (GD•P). In this state, the GTPase domain can dimerize (GGD•P) and perform the powerstroke. The dissociation of dimers to monomers could be either in the GGD•P state or after release of the phosphate (GGD) (see Fig 4). Note an important difference from myosin; the GTPase with bound GDP and P_i has the highest affinity for itself, whereas the myosin-ATP and -ADP-P_i intermediates have the lowest affinity for actin filaments.

Structural studies indicate that the energy from GTP binding and hydrolysis is most likely used to produce motion during the transition from GGT (GTPase dimer with bound GTP) to GGD•P (GTPase dimer with bound GDP and P_i) when a large conformational change swings the BSE almost 70° (Chappie *et al*, 2011). This event will occur when two GT intermediates are transiently bound together in a GGT dimer. GTP hydrolysis appears to drive both the lever arm motion and stabilize the dimer. This is comparable to the motion of the myosin lever arm composed of the light chain domain, which occurs when the weakly bound A-MDP intermediate dissociates P_i. An important parallel with muscle myosin is that the force-producing intermediates, the strongly bound GG dimers between adjacent turns of the dynamin helix, are transient while the other chemical intermediates are dissociated into monomers that do not interfere with the sliding motion. Verifying this hypothesis should be relatively easy by measuring the missing parameters with pre-steady state kinetics. Steady state kinetics analyzed with Michaelis–Menten assumptions are unlikely to reveal mechanistic details.

The stochasticity of the fission reaction (Merrifield *et al*, 2005; Bashkirov *et al*, 2008; Taylor *et al*, 2011; Morlot *et al*, 2012; Cocucci *et al*, 2014; Grassart *et al*, 2014) and the measured Hill coefficient toward GTP concentration [value of one (Tuma & Collins, 1994)] agrees with the fact that the GTP hydrolysis of GTPase domains in the polymer are not coordinated. The available kinetic data (Fig 4B) show that dynamin is not processive, so multiple uncoordinated dynamins must work together to produce force with only a minority producing force at any point in time. This explains why GTP hydrolysis of ~5 dimers is required for membrane fission (Liu *et al*, 2013).

More information is also required about the assembly/disassembly cycle, in particular the role of GTP in assembly of a one-start versus two-start helix. One possibility is that the interactions between GTPase domains in the GTP bound form allows for the formation of G-G-mediated tetramers (TRANS-tetramer see Fig 1A), corresponding to the nucleus of a two-start helix. However, *in vivo*, the majority of units added to the dynamin polymer at the clathrin-coated pit are dimers (Cocucci *et al*, 2014), even if some tetramers can be seen.

Future directions of the dynamin research

The role of PRD-binding dynamin partners, in particular the BAR domain proteins

One of the unknowns about dynamin is the precise role in its function played by the proteins that bind to its PRD. Many such binding partners have been identified, most of which bind the PRD via SH3 domains (Ferguson *et al*, 2009). These proteins define the context in which dynamin must act by functioning as adaptors to facilitate its membrane recruitment or by coordinating its action with that of other proteins. For example, some SH3 domain-containing proteins that bind dynamin also bind actin regulatory proteins, signaling proteins, or phosphoinositide metabolizing enzymes. The property of some such membrane adaptor proteins (most prominently endophilin) to recruit both dynamin and synaptojanin (Ringstad *et al*, 1997, 1999; Milosevic *et al*, 2011) is of special interest as it helps coordinate the fission reaction of endocytosis with $PI(4,5)P_2$ dephosphorylation. Such reaction could help dissociation of dynamin and other endocytic factors from the membrane after fission.

Several SH3 domain-containing dynamin interactors also contain a BAR family domain, a membrane binding, and, in some cases, a membrane remodeling module (Takei *et al*, 1999; Farsad *et al*, 2001; Peter *et al*, 2004; Itoh *et al*, 2005; Frost *et al*, 2008; Mim *et al*, 2012). A function of proteins with these modules is to help recruit dynamin and facilitate its polymerization. However, a key open question is whether these proteins also participate directly in the fission reaction either via an effect on the curvature of bilayer or via their interactions with dynamin. Data on this topic are conflicting, as based on *in vitro* studies involving purified proteins and liposomes, both inhibitory and facilitating effects on GTPase activity and on dynamin-mediated fission have been observed (Farsad *et al*, 2001; Peter *et al*, 2004; Yoshida *et al*, 2004; Meinecke *et al*, 2013; Neumann & Schmid, 2013). The positive effects may be explained by the property of these proteins to facilitate dynamin assembly because of their dimeric nature (Peter *et al*, 2004) and ability to polymerize. The negative effect is likely explained by two mechanism: First, the crescent shape of the BAR domain may block further constriction by dynamin by forming a rigid scaffold of fixed curvature on the membrane (Boucrot *et al*, 2012). Second, BAR domain rungs could intercalate between opposing GTPase modules in the dynamin spiral, as shown by unpublished cryo-EM images by Adam Frost, which explain the reported increase of the dynamin pitch in the presence of BAR proteins (Takei *et al*, 1999; Farsad *et al*, 2001; Itoh *et al*, 2005; Sundborger *et al*, 2011). This insertion explains reduced GTPase activity as it disrupts the G domain–G domain interaction necessary for GTP hydrolysis. At high BAR/dynamin ratio, all tested BAR domain proteins have a blocking action on dynamin-mediated fission (A. Roux, P. De Camilli and A. Frost, unpublished results).

A more precise elucidation of how these BAR domain proteins regulate dynamin fission activity is critically needed. Most of the available data were derived from different assays, with variable stoichiometric ratios between dynamin and BAR domain proteins and most important under cell-free conditions with purified proteins. These conditions may not faithfully replicate events occurring in living cells.

A functional link between dynamin and actin

An important open question is the functional relation of dynamin to actin. In addition to being detected at endocytic clathrin-coated pits, dynamin is also detected at a variety of sites, primarily involving the Arp2/3 complex–actin network, such as macropinocytosis, cell ruffles, podosomes, invadopodia, and actin comet tails (Ochoa *et al*, 2000; McNiven *et al*, 2004; Bruzzaniti *et al*, 2005). Arp2/3 complex and one of its nucleation-promoting factors, N-WASP, are also frequently observed at endocytic clathrin-coated pits, where they colocalize with dynamin spatially and temporally (Merrifield *et al*, 2002, 2004, 2005; Taylor *et al*, 2011) and dynamin clearly controls actin polymerization at sites of endocytosis, at least in some cell types (Taylor *et al*, 2012; Grassart *et al*, 2014). Colocalization of dynamin with these proteins is mediated at least in part by the dynamin-binding SH3 domain-containing proteins, which also bind N-WASP (Cip4/Fbp17/Toca1 family members) (Frost *et al*, 2009), the WAVE complex (Ochoa *et al*, 2000), and cortactin (McNiven *et al*, 2000; Cao *et al*, 2005).

There is evidence suggesting that the colocalization of actin and dynamin at endocytic sites reflects the need for actin-based force (via actin polymerization or myosin motors) to facilitate dynamin-dependent fission (Itoh *et al*, 2005; Boulant *et al*, 2011; Morlot *et al*, 2012; Messa *et al*, 2014). This is consistent with the fact that reduced membrane tension delays fission *in vivo* (Boulant *et al*, 2011; Morlot *et al*, 2012). This effect could be achieved locally by direct interaction of the dynamin coat with the actomyosin network through PRD-binding proteins, as the actin cortex is the main membrane tension regulator in the cell. However, the localization of dynamin at other actin rich sites remains without a clear explanation and calls for further studies. For example, recent findings from the Chen laboratory strongly support a role of dynamin in organizing the asymmetrical, actin-based protrusions that myoblasts use to fuse with myotubes (E. Chen, unpublished). Similar observations have been made for osteoclasts fusion (Shin *et al*, 2014).

The dynamin family—similarities and differences

Other members of the dynamin family, such as OPA1 and mitofusin, function in membrane fusion and tubulation rather than membrane fission. The challenge remains to understand how insights into dynamin's membrane scission mechanism can be applied to other members of the family to explain fusion and membrane tubulation, in addition to scission.

Extensive crystallographic analyses of GTPase-BSE constructs in a variety of nucleotide states from Drp1 *Arabidopsis thaliana* AtDRP1A (Chen *et al*, 2012), human MxA (Rennie *et al*, 2014), and Dnm1 (Kishida & Sugio, 2013; Wenger *et al*, 2013) support that all of these members share a mechanism of GTP hydrolysis with dynamin—namely, dimerization of the GTPase domains and, likewise, a nucleotide-dependent conformational change of the BSE. However, further studies will confirm how general this mechanism is.

Comparative analyses of stalk interaction interfaces of close dynamin relatives suggest similar assembly principles but different helical geometries (e.g. helices of increased diameter in the case of Dnm1) that may be adapted for particular cellular functions, such as tubulation of the endosome or mitochondrial constriction. Other family members, such as the mitochondrial fusion dynamins, have

predicted all-helical stalk regions though in general molecular insight into assembly mechanisms and architecture of such assembled structures remains sparse. There is limited understanding of how stimulated nucleotide hydrolysis is converted into a membrane remodeling event for most of the members. It may be expected that appropriate alterations in rates of assembly, hydrolysis, and disassembly can convert a scission dynamin into a longer lived tubulating or fusion dynamin.

Dynamin has unique features compared to all other members of its family: most prominently, its PH domain and PRD. Absence of these would require an alternative mechanism for recruitment, association/interaction with target membranes, and, potentially, participation in scission (see above). Drp1 (higher eukaryotes)/Dnm1 (fungi) have, in place of a PH domain, an "Insert B" region of low sequence complexity. In these cases, membrane recruitment is therefore outsourced to accessory factors. Drp1 can be independently recruited by MFF and, separately, by the closely related proteins MiD49, MiD51 although the functional consequences of recruitment by either pathway may differ (Gandre-Babbe & van der Bliek, 2008; Koirala *et al*, 2013; Palmer *et al*, 2013; Liu & Chan, 2015; Loson *et al*, 2015). In yeast, Dnm1 is recruited by Fis1 and the adaptors Mdv1 and Caf4 (Lackner *et al*, 2009; Guo *et al*, 2012). BDLP has a "paddle" where dynamin has a PH domain. The paddle has a number of hydrophobic residues that are required for membrane interaction (Low *et al*, 2009). An alternative solution is exhibited by the mitofusins (mammals), Fzo1 (fungi), atlastins, and some forms of OPA1 and Mgm1 (mammals/fungi), which are membrane-anchored via transmembrane segments.

Conclusions

This review emphasizes large areas of consensus, but also the remaining issues to solve for a complete understanding of dynamin mechanism. We also propose approaches that need to be taken to resolve these issues. Yet, the synthesis of 30 years of work on dynamin allows us to be optimistic, and already, we can state that many aspects of dynamin-mediated membrane fission have been understood. As the prototypic member of a large family of related GTPases that catalyzes both fission and fusion, we hope that the current and future knowledge acquired on the mechanism of dynamin-catalyzed fission will aid our understanding of multiple cellular fission and fusion reactions.

Conflict of interest

The authors declare that they have no conflict of interest.

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