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Dynamin architecture — from monomer to polymer

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Abstract

Dynamins form a family of eukaryotic and prokaryotic proteins involved in membrane fission, fusion and restructuring. They have complex mechanisms of self-assembly, which are coupled to the tubulation and destabilization of lipid bilayers. Recent structural data has revolutionized our understanding and is now yielding detailed insights into dynamin structure, from monomer through to polymer. Traditional division of the dynamin subunit into GTPase domain, middle domain and GTPase effector domain based on sequence alignments and biochemistry is not supported by recent structural data. A unified model of dynamin architecture is presented here, based on observation that the basic dynamin fold is conserved across evolutionary kingdoms.

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Introduction

Dynamins form a large family of proteins of great cellular importance with roles in endocytosis, plastid biogenesis, animal and plant cytokinesis, viral resistance and many others. The classical fission dynamins are large multi-domain proteins (~100 kDa) that constitute an N-terminal GTPase domain, a middle domain, a pleckstrin homology (PH) domain, a GTPase effector domain (GED), and a C-terminal proline-rich domain (PRD) [1]. Dynamin-like proteins (DLPs) have a similar conserved domain arrangement although the PH domain is replaced by variable lipid binding motifs, and the PRD is absent.

Although membrane fission and fusion are opposing processes, at least in eukaryotic cells both are often dependent on basic dynamin-like properties such as lipid binding and polymerization, in which membrane is forced, under extreme curvature, to form a highly unstable tubular conformation. How this tubulation is then coupled to membrane fission or fusion is a poorly understood process and represents one of the great questions remaining in the field.

The paucity of structural data over the last two decades has hindered understanding on how dynamin domains are arranged and related to each other, how individual subunits associate during self-assembly, and how the complex nucleotide catalysis cycle is controlled and to what function it is coupled. Just how structurally and mechanistically diverged are the different members of the dynamin family? And what are the implications of their evolutionary heredity? These are the kinds of questions that this review focuses upon and, due to a recent surge in progress, are now beginning to be resolved.

Structural comparison of DLP and dynamin monomers

The first full-length structure of a DLP solved was the human guanylate-binding protein 1 (GBP1) [2] which revealed the N-terminal GTPase domain to be, as in all dynamin family members (DFMs), a modified form of the canonical Ras [3] (Figure 1a). The GTPase domain was found to be conjoined with an extended helical C-terminus whose fold, whilst retaining many dynamin-like features, is now known to be quite diverged and not particularly representative of other dynamin family members. For example, lipid binding in GBP1 is dependent upon farnesylation of the CaaX box located at the very C-terminus of the molecule [4].

The observation that many bacteria have predicted genes with dynamin-like architecture [5], albeit with extremely low homology (~20% sequence identity), culminated in the characterization of the bacterial dynamin-like protein (BDLP) from the cyanobacterium *Nostoc punctiforme* [6•]. BDLP readily tubulates *Escherichia coli* liposomes in the presence of GMPPNP, forming coated tubes that are reminiscent of those formed by eukaryotic dynamin 1 in the presence of phosphatidylserine liposomes [7]. In both, an elongated molecule forms a T-shaped repeat that represents the basic assembly unit of a helical filament, as shown by negative stain electron microscopy (EM). The BDLP apo and GDP bound crystal structures [6•] reveal a surprisingly compact molecule comprising an extended GTPase domain with the middle domain [8] and GED [9] forming four-helix bundles that run the length of the molecule and do not form discrete domains as was predicted (Figure 1b). Interestingly, the DLP Mgm1, which

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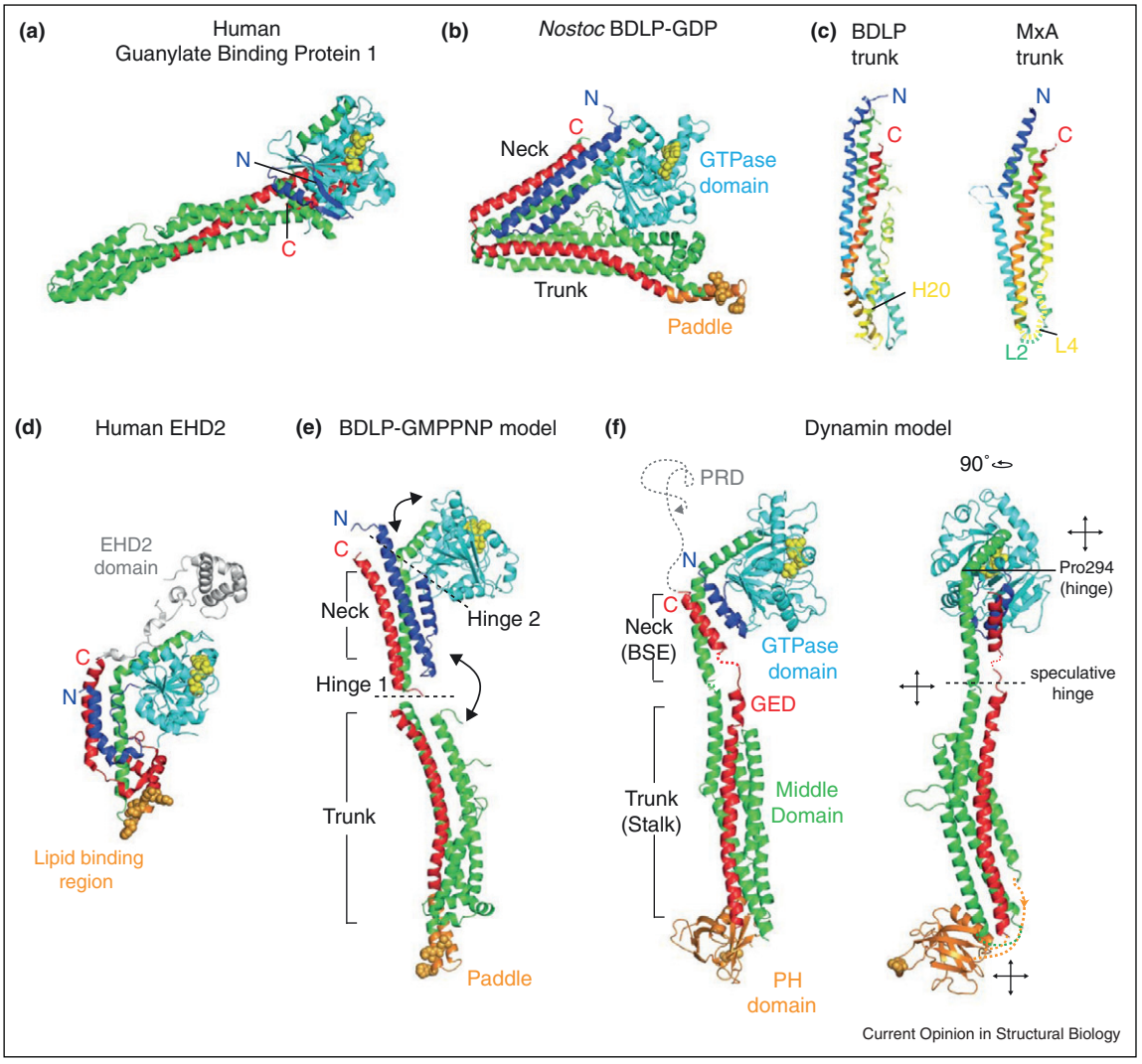
Figure 1



Comparison of dynamin and dynamin-like (DLP) monomers. By structure, the canonical dynamin family member (DFM) divides into discrete motifs: the GTPase, neck and trunk domains. The trunk tip is specialized for lipid binding or supplemented with a PH domain. Residues known to be involved in lipid binding are represented as spheres. **(a)** Human guanylate binding protein 1 (GBP1) is diverged from other DFMs; and binds lipid through farnesylation of its C-terminus [4]. **(b)** The crystal structure of a bacterial dynamin-like protein (BDLP) from *Nostoc punctiforme* reveals a compact fold that radically extends upon lipid and GTP binding, as shown in e [6•]. **(c)** The BDLP and MxA trunks [6•,13•] are homologous despite sharing less than 20% sequence identity. This general fold is expected to be conserved in all true DFMs. **(d)** Human EHD2 exemplifies the modular design of DFMs — the trunk has been lost by annealing the lipid binding region directly to the neck base [19•]. **(e)** Derived from cryo-EM data [11•], the model of a BDLP subunit when GMPPNP associated and polymerized upon a lipid tube. Hinges 1 and 2, and the trunk tip represent regions of high flexibility. **(f)** A speculative model of a classical dynamin monomer based on human dynamin 1 GTPase, neck and PH domain, and the MxA trunk. The general dynamin fold is well conserved from bacteria through to humans. Note the actual orientation of both the neck and PH domain relative to the trunk is unknown. The PH domain is shown as fitted in [13•].

mediates mitochondrial inner membrane fusion, is also thought to form a compact folded molecule in the absence of nucleotide [10]. In the presence of GMPPNP and lipid, the BDLP molecule undergoes substantial reorganization to form an extended conformation that is polymerization competent (Figure 1e) [11•].

Remarkably, the general fold observed in BDLP is well conserved amongst eukaryotic dynamins despite very high sequence divergence, especially in the helical parts. This phenomenon of conserved molecular architecture coupled with low comparative sequence identity has been previously observed between for example, eukaryotic actin and tubulin and their bacterial counterparts MreB and FtsZ, respectively [12].

The recent structure of the human MxA stalk [13•] comprises the dynamin middle domain and GED N-

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terminus, and this will likely be representative of all known dynamin family members, excluding the Eps15 homology domain-like (EHD) proteins and GBPs. The MxA stalk forms a four-helix bundle homologous to that previously observed in the BDLP trunk with these domains clearly sharing a common ancestor (Figure 1c). Here on in, this four-helix bundle will be termed the trunk for clarity and in keeping with terminology used when this motif was first reported [6•].

The GTPase domains of DFMs are connected to the trunk by another conserved fold consisting of a four-helix bundle in BDLP termed the neck [6•,11•], and a three-helix plus coil bundle in eukaryotic dynamins termed the bundle signalling element (BSE) [14•,15,16]. The neck and BSE both comprise the GTPase domain N-termini and C-termini, and the GED C-terminus. Here on in, this homologous four-strand bundle will be termed the neck in keeping with terminology used when this motif was first reported [6•,11•]. As is now clear, the role of the GED, acting as an integral component of the neck, is to mediate protein–protein contacts during polymerization [8,11•,17]. Assembly stimulated nucleotide turnover is understood to be a discrete consequence of GTPase domain homodimerization [14•,18•], which the GED, combined with the other neck components, affects indirectly through the promotion of self-assembly.

In summary, the repertoire of dynamin family structures now available has markedly changed our understanding. The emergent theme is that DLPs have three discrete structural motifs — the GTPase, neck and trunk domains, with machinery specialized for lipid binding located at the trunk tip. Interestingly, human EHD2 has a dynamin-like GTPase domain and neck but has lost most of its trunk by annealing the lipid-binding motif directly to the neck base (Figure 1d) [19•]. Classical dynamins have supplemented their trunk with a PH domain inserted into the equivalent MxA L4 region or BDLP paddle, thereby providing lipid head-group specificity. On the basis of these similarities and variations, we have produced a speculative model of a classical fission dynamin by arranging structures of the human dynamin 1 GTPase and neck domain, the MxA trunk, and the human dynamin 1 PH domain, all according to the BDLP structure [20] (Figure 1f). The interfaces between the different domains likely mark regions of conformational flexibility as observed in BDLP [11•], and in human dynamin 1 [14•]. Note that the real orientation of the neck relative to the trunk is the key unknown here with a bend or twist likely. The critical structural difference between BDLP and known eukaryotic fission dynamins is the orientation of the neck relative to the GTPase domain [11•,14•,16,21], which has significant ramification for filament packing as shall be discussed later.

Both the EHD proteins and classical dynamins have an additional C-terminal domain conjoined to the neck, namely the EH domain and PRD, respectively. The EH domain is essential for assembly and subsequent stimulated nucleotide hydrolysis, which is likely due to it stabilizing GTPase domain association within the polymer [19•]. The PRD also has a known regulatory effect on dynamin catalysis and assembly [9,22] and may function in a similar way to the EH domain by either directly stabilizing the polymer or through the indirect recruitment of SH3-domain containing proteins such as amphiphysin or sorting nexin 9 [23–26].

DLP and dynamin self-assembly

DFMs belong to the class of G proteins activated by nucleotide-dependent dimerizations (GADs) [27], in which the GTPase domain homodimerizes across the nucleotide binding pockets upon nucleotide binding. Such dimerization was first described in GBP1 [18•], and subsequently in BDLP [6•,11•], EHD2 (predicted) [19•], human dynamin 3 [Yang S *et al.*, Crystal structure of the dynamin 3 GTPase domain bound with GDP. PDB database 2010, unpublished data], and human dynamin 1 [14•], and will no doubt extend across the superfamily (Figure 2a and b). The detailed catalytic mechanism and rate of nucleotide turnover may vary and appear tuned to function. However, common to all is the nucleotide binding pocket that is self-contained and requires no additional outside contribution to the catalytic machinery. The effect of dimerization is to orient [18•] or stabilize [14•] *in trans* key catalytic components required for efficient nucleotide hydrolysis. With the exception of GBP1, with its ability to hydrolyse GTP to GMP, evidence suggests that DFMs homodimerize upon GTP binding and the dimer persists in the GDP state [Yang S *et al.*, Crystal structure of the dynamin 3 GTPase domain bound with GDP. PDB database 2010, unpublished data] [6•], but breaks apart upon nucleotide release [11•,28•,29•]. The effect of nucleotide binding is not limited to GTPase domain homodimerization but also promotes other contacts. In GBP1, nucleotide induced local rearrangements around the binding pocket are transmitted, through displacement of helix α4′, to the C-terminal helices α12/13, which then undergo conformational change that promotes tetramerisation [30,31]. Similarly, in BDLP, GTP binding shifts the position of H4 located in proximity to the binding pocket, which induces lateral self-association between GTPase domains [11•].

Superposition of the GTPase domains of human dynamin 1 bound to GDP.AlF_4 and human dynamin 3 bound to GDP shows highly complementary main chain positioning (rmsd = 0.62 Å) (Figure 2c). The principle change in the nucleotide binding pocket is that the switch 2 loop becomes disordered when GDP is bound. The orientation of the neck relative to the GTPase domain has been shown to be flexible in the GDP.AlF_4 human dynamin 1 GTPase domain homodimer, pivoting around conserved proline 294 [14•]. Indeed, superposition of the

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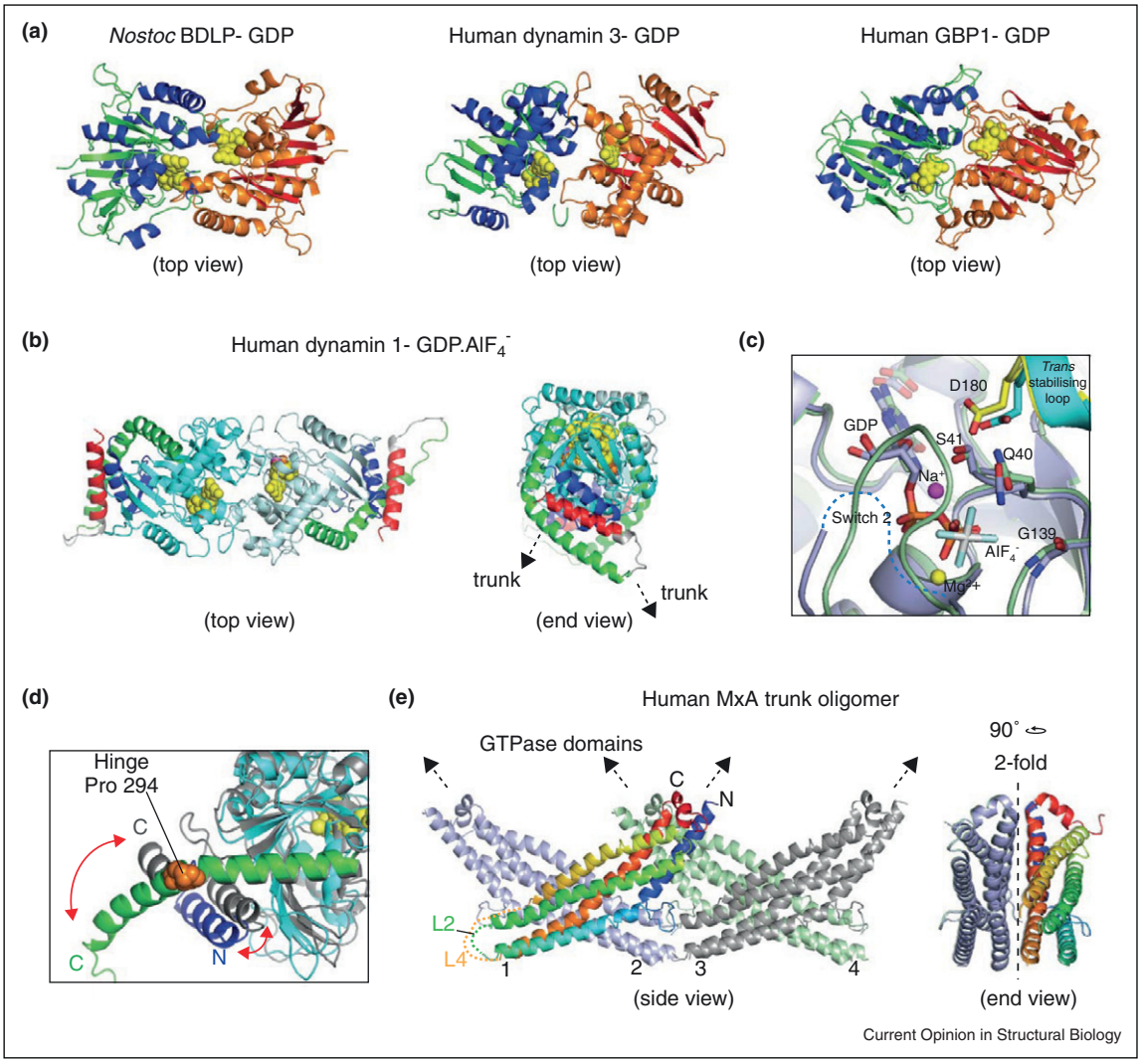
Figure 2



DLP and dynamin self-assembly. **(a)** All dynamin family members (DFMs) dimerize via their GTPase domains. Nucleotide binding induces GTPase domain homodimerization, forming the typical sandwich dimer with the two nucleotides trapped between the protein subunits. Dimerization then mediates polymer formation and assembly stimulated nucleotide hydrolysis [Yang S *et al.*, Crystal structure of the dynamin 3 GTPase domain bound with GDP. PDB database 2010, unpublished data] [6•,18•]. **(b)** The two-fold symmetry between the human dynamin 1 GTPase domain homodimer in the presence of GDP.AlF_4 means the neck domains are angled in opposing directions [14•]. **(c)** Superposition of the nucleotide binding pockets of human dynamin 1-GDP.AlF_4 (green/yellow) [14•] and dynamin 3-GDP (blue/cyan) [Yang S *et al.*, Crystal structure of the dynamin 3 GTPase domain bound with GDP. PDB database 2010, unpublished data]. **(d)** Superposition of human dynamin 1-GDP.AlF_4 [14•] and rat dynamin 1-apo GTPase domains [16]. Differing orientation of the necks may speculatively represent a nucleotide driven conformational change. **(e)** Related by a two-fold symmetry axis, the MxA trunk packs as a criss-cross oligomer within the crystal [13•].

apo rat dynamin 1 GTPase domain [16] with GDP.AlF_4 human dynamin 1 GTPase domain shows a marked shift in orientation and positioning of the neck relative to the GTPase domain, which raises the tantalizing question of whether an important nucleotide driven conformational change is being observed here (Figure 2d). It is known that the binding of nucleotide in BDLP induces substantial conformational change in precisely the homologous region in which the dynamin 1 proline 294 is located [11•]. In *Dictyostelium* dynamin A, the orientation of the neck (minus the GED C-terminal helix) relative to the GTPase domain is the same both in the apo and in the GDP forms suggesting that, as in BDLP, any conformational change in this region is coupled to actual nucleotide binding and later phosphate release [21].

The crystallization of the MxA trunk was particularly informative as it potentially reveals the packing of this domain within the MxA filament, and by homology the dynamin filament as well [13•]. The asymmetric unit

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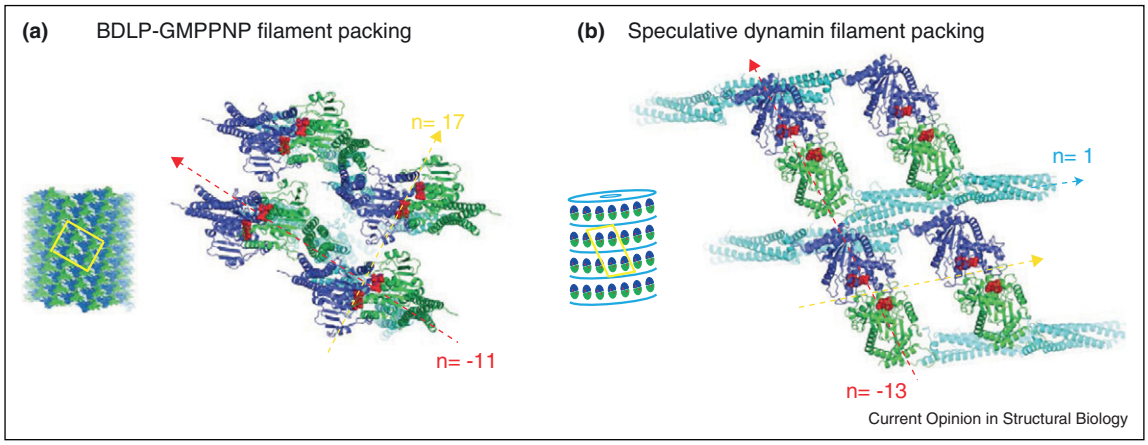
Figure 3



Comparison of a BDLP and classical dynamin lipid tube. Critical to both is two-fold symmetry running orthogonal to the tube long axis **(a)** Surface view of a molecular model of a BDLP lipid tube bound to GMPPNP. The GTPase domains homodimerize and through back-to-back association of neck and trunk domains, that run essentially orthogonal to the trunk axis, form a left-handed helix (Bessel order, $n = -11$). Small lateral contacts are mediated by H4 on the side of the GTPase domain (Bessel order, $n = 17$) [11•]. **(b)** Equivalent surface view as in (a), showing a speculative molecular model of a dynamin lipid tube based on [13•]. The hand of the GTPase domain homodimers is unknown although it is speculated here to be similar to BDLP and to follow the Bessel order, $n = -13$ [33], when GTP is bound. The trunk domains oligomerise in a criss-cross to form the core of the filament wrapping laterally around the lipid tube (Bessel order, $n = 1$). The GTPase domain homodimers bridge neighbouring rungs of the helix meaning their respective neck and trunk domains are separated and run in opposing directions, in contrast to BDLP. The orientation of the neck relative to the trunk is the key unknown. Conformational flexibility is expected between the GTPase and neck domains relative to the intimately packed trunk.

comprises a trunk dimer arranged in a criss-cross fashion that importantly incorporates a twofold symmetry axis (Figure 2e). Membrane binding would be mediated by the L4 region at the trunk base with the neck and GTPase domain attached at the opposing end. Repeat of the asymmetric unit within the crystal generates a linear oligomer (Figure 2e), which if curved provides the basis for a plausible model of the dynamin helix [13•].

Comparison of the BDLP and speculative dynamin filament

Using cryo electron microscopy (EM) and a single particle technique adapted for helical structures [32•], it was possible to generate an 11 Å reconstruction of a BDLP filament with GMPPNP bound and coating a lipid tube [11•]. By fitting the BDLP–GDP crystal structure as three rigid bodies (GTPase, neck and trunk) into the reconstruction a molecular model of the entire filament was generated. The tubulated membrane is observed under extraordinary curvature with the inner leaflet having a diameter of just 10 nm, and the outer leaflet seemingly substantially displaced and disordered. The GTPase domains homodimerize and through back to back interaction along neighbouring neck and trunk domains, a left handed helical filament is formed (Bessel order, $n = -11$) (Figure 3a). Small lateral contacts restricted to H4 on the side of the GTPase domain generate a right-handed Bessel order running along the tube long axis.

As discussed, the human dynamin 1 subunit will look similar to the BDLP subunit but with a PH domain linked to the trunk base. Therefore, variation between BDLP and human dynamin 1 filaments is predominantly due to significant modification in packing of the individual subunits during evolution. On the basis of the MxA trunk oligomer, one of the key differences in dynamin 1 seems to be that the trunk does not run orthogonal to the tube long axis as in BDLP, but instead lies at about 45° relative to the membrane surface [13•] (Figures 2e and 3b), and probably constitutes the short helix (Bessel order, $n = 1$) [33] wrapping around the lipid tube. The membrane bound PH domain may therefore be positioned quite some radial distance from its corresponding GTPase domain, depending on the angular relationship between neck and trunk domains. Another important difference is that in BDLP, individual subunits homodimerized through their GTPase domains (Bessel order, $n = -11$) contribute both trunks to the same rung of the helix. Whilst in dynamin, the trunks appear separated and, angled in opposing directions, are divided between neighbouring rungs with the GTPase domains bridging the gap in between [13•] (Figure 3b). In the presence of GTP, the GTPase domains will homodimerize as observed in the presence of GDP.AlF_4 [14•], and align essentially along the tube axis [13•] (Figure 3b), which speculatively corresponds to the 'long' helices (Bessel order, $n = -13$) derived from the power spectra of dynamin 1 tubes in vitreous ice [33]. An alternative GTPase domain packing has been proposed [34], based on low resolution cryo EM reconstructions of human dynamin 1 lipid tubes [33,35], that does not incorporate the GTPase domain homodimer. For the GMPPCP bound tube

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specifically, such packing is unlikely given the examples of GTPase domain homodimerization [Yang S *et al.*, Crystal structure of the dynamin 3 GTPase domain bound with GDP. PDB database 2010, unpublished data] [6•,11•,14•,18•].

Mechanisms of membrane fission and fusion

Many mechanisms of DFM mediated membrane fission have been proposed which include filament pitch extension [36], filament constriction [7,37], and a mechanism based on torsion between neighbouring rungs of the filament [38•]. These predict that nucleotide hydrolysis coupled to a conformational change within the dynamin filament actively induces membrane breakage. More recently, a model has been proposed in which dynamin induced membrane curvature is sufficient to drive spontaneous fission, and the GTPase cycle is coupled to membrane cycling [28•,29•]. This latter model is in agreement with the proposed 'passive' mechanism for fission and fusion based on BDLP data [11•]. Here, nucleotide driven conformational changes are coupled to membrane binding, polymer formation and membrane release. As a consequence, the membrane forms a highly curved unstable intermediate thought competent for fission or fusion. Whatever the mechanism, conformational freedom in the dynamin subunit will likely be restricted to the interfaces between the different domains, as observed in BDLP [11•]. The PH domain may also be quite mobile given the relatively long linker with which it connects to the trunk. Inherent within both the BDLP and speculative dynamin filament are two 2-fold symmetry axes, between the GTPase domain homodimers, and between the back to back trunks within a filament rung. Such organization suggests that any conformational change that exerts force in one direction will be countered by the symmetry mate exerting force in the opposite one, thereby making a torsion or ratchet mechanism superficially unviable.

Although many dynamin proteins involved in membrane fusion are known, such as Fzo [39] located at the mitochondrial outer membrane and Mgm1 [40] at the inner membrane, the actual fusion mechanism is still a poorly understood process [41]. It has been speculated that since some of the DLPs involved in fusion, such as Mgm1 [10], have relatively low levels of nucleotide hydrolysis in comparison to fission dynamins, fusion may require a much more stable polymer. Therefore, differences in filament packing along with variable rates of catalysis, may represent the fundamentals of how fission and fusion diverge. Mitochondrial fusion has been shown to be dependent on a tethering mechanism mediated by mitofusin complexes acting *in trans*. On the basis of crystal packing, it is thought that the mitofusin heptad repeat region (HR2), or GED equivalent, associates in an anti-parallel fashion thereby bridging apposing membranes [42]. Although anti-parallel association of the HR2 region may prove to be an exception, it is not currently reconcilable with the parallel association of the GED in for example, dynamin 1, EHD2, or BDLP.

Concluding remarks

Recent structures have shown that the traditional modular division of DFMs into separate GTPase, middle and GEDs based on sequence alignment is not representative of the structural arrangement. The canonical DLP actually emerges to consist of an extended GTPase domain connected to a complex interwoven arrangement of parallel helices that divide into discrete motifs termed here the neck and trunk domains. Furthermore, such structural topology is conserved across kingdoms. Nucleotide driven conformational flexibility is likely limited between these domain boundaries (and the lipid binding motif). Assembly stimulated nucleotide hydrolysis has been shown to be driven by GTPase domain homodimerization which is modulated by self-assembly. The GED does not form a discrete domain but contributes to both trunk and neck where it plays an important part in self-assembly. Future work will focus on understanding the precise arrangements of the dynamin (or DLP) subunits when polymerized in each different nucleotide state, and how these arrangements are coupled to membrane restructuring. The recent surge in structural understanding represents the dissolution of over a decade of impasse. There is now a robust platform upon which to design ever more focused experiments, making this a hugely exciting time for the field.

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