THE DYNAMIN SUPERFAMILY: UNIVERSAL MEMBRANE TUBULATION AND FISSION MOLECULES?

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Dynamins are large GTPases that belong to a protein superfamily that, in eukaryotic cells, includes classical dynamins, dynamin-like proteins, OPA1, Mx proteins, mitofusins and guanylate-binding proteins/atlastins. They are involved in many processes including budding of transport vesicles, division of organelles, cytokinesis and pathogen resistance. With sequenced genomes from *Homo sapiens*, *Drosophila melanogaster*, *Caenorhabditis elegans*, yeast species and *Arabidopsis thaliana*, we now have a complete picture of the members of the dynamin superfamily from different organisms. Here, we review the superfamily of dynamins and their related proteins, and propose that a common mechanism leading to membrane tubulation and/or fission could encompass their many varied functions.

SCISSION
Cleavage of the vesicle from the parent membrane — as in the use of scissors to sever.

SNARE
(soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein (SNAP) receptor). SNARE proteins are a family of membrane-tethered coiled-coil proteins that regulate fusion reactions and target specificity in vesicle trafficking. They can be divided into v-SNAREs and t-SNAREs on the basis of their localization.

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Membrane transport between compartments in eukaryotic cells requires proteins that allow the budding and SCISSION of nascent cargo vesicles from one compartment and their targeting and fusion with another. Just as SNARES are proposed to be essential for all vesicle-fusion reactions, so CLASSICAL DYNAMINS and DYNAMIN-RELATED PROTEINS have a similar appeal as the essential vesicle-scission molecules. They are involved in the scission of a wide range of vesicles and organelles, including CLATHRIN-COATED VESICLES (CCVs), CAVEOLAE, phagosomes and mitochondria (FIG. 1, TABLE 1). Although DYNAMINS are not found in all budding reactions, the more places that are investigated, the more they will probably be found. The importance of dynamin was first discovered with the identification of temperature-sensitive mutants in *Drosophila melanogaster*[1,2] that gave rise to a paralytic phenotype. The locus was called *shibire* after the Japanese word for 'paralysed' (BOX 1). The *shibire* gene was then discovered to encode dynamin[3,4]. Dynamin had previously been characterized as a GTPase that can associate with microtubules *in vitro*[5,6], and as a phosphoprotein in nerve terminals[7]. Since then, mutations that abolish the GTPase activity of dynamin have been widely used to characterize its functions.

Dynamins are generally classified as 'large GTPases'. This is to distinguish them from the small Ras-like and other regulatory GTPases, such as the well studied α-subunits of heterotrimeric G-proteins and the translation factors of protein biosynthesis. In addition to having a larger GTPase domain, dynamin and dynamin-related proteins are distinguished from other GTPases by their oligomerization-dependent GTPase activation, their low GTP-binding affinities and the ability of many members of the dynamin family to interact with lipid membranes. There are many large GTPases in the database — even in prokaryotes — that have homology to dynamin only in the GTPase domains, and do not have the additional domains that allow for self-oligomerization, so we do not count these as dynamin-related proteins.

Subdividing the dynamin superfamily

Overall architecture. The minimal distinguishing architectural features that are common to all dynamins and are distinct from other GTPases are the structure of the large GTPase domain (~300 amino acids) and the presence of two additional domains; the middle domain and the GTPase effector domain (GED),

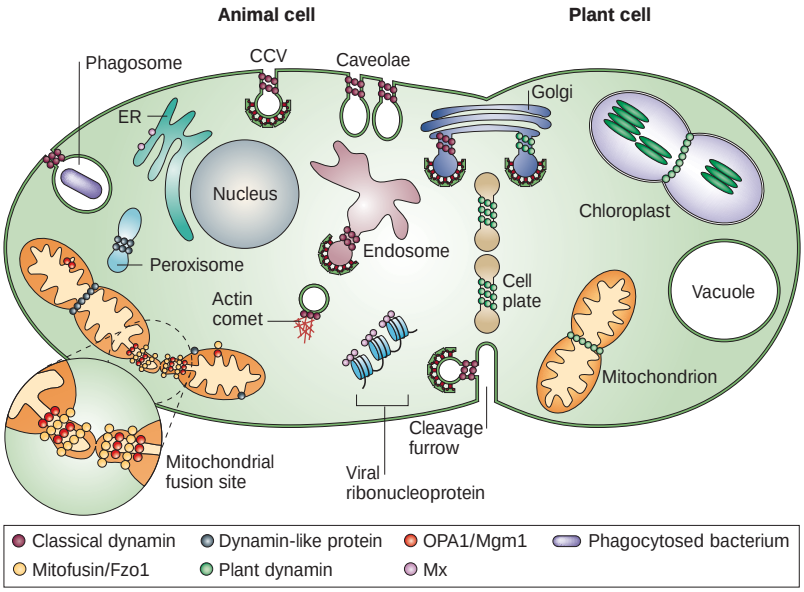


Figure 1 | **Dynamin-superfamily members in animals and plants.** We have subdivided the dynamin superfamily into 'classical dynamins' and 'dynamin-related proteins'. Classical dynamins (maroon) are typified by the dynamin proteins that function in the budding of clathrin-coated vesicles (CCVs) at the plasma membrane, cleavage furrow, Golgi and endosome, but also in non-clathrin-mediated budding events at caveolae and phagosomes. Dynamin-related proteins cover a much wider range of dynamin homologues. Dynamin-like proteins (Dlps, grey) are involved in division of organelles such as mitochondria and peroxisomes. The OPA1 (optic atrophy 1, red) and mitofusin (yellow) families are also involved in reorganization of mitochondria, but probably in mitochondrial fusion, and therefore antagonize the function of Dlp. The Mx family (pink) are induced by interferons and confer resistance against RNA viruses. Human MxA localizes to the smooth endoplasmic reticulum and also interacts with viral ribonucleoproteins. Plants contain many different dynamin proteins (light green). Some of them have similar functions to those in animals (for example, budding of CCVs), but others have functions that are unique to plants, such as formation of the cell plate or chloroplast division (see main text). Mgm, mitochondrial genome maintenance 1.

which are involved in oligomerization and regulation of the GTPase activity (FIG. 2). The GTPase domain contains the GTP-binding motifs (G1–G4) that are needed for guanine-nucleotide binding and hydrolysis (FIG. 3). The conservation of these motifs is absolute except for the G4 motif in guanylate-binding proteins (GBPs; FIG. 3). The GTPase catalytic activity can be stimulated by oligomerization of the protein, which is mediated by interactions between the GTPase domain, the middle domain and the GED. In many of the dynamin-superfamily members, this basic set of domains is supplemented by targeting domains, such as: PLECKSTRIN-HOMOLOGY (PH) DOMAINS; proline-rich domains (PRDs) that bind to SRC-HOMOLOGY-3 (SH3) DOMAINS; or by sequences that target dynamins to specific organelles, such as mitochondria and chloroplasts. It should also be noted that alternative targeting can arise as a result of alternative splicing[8].

It is easy to find dynamin nomenclature confusing because the various homologues and their domains have accumulated various names, which, in some cases, overlap. In BOX 2 we highlight some of the studies on plant dynamin homologues, whereas in TABLE 2, we group dynamin and dynamin homologues across species on the basis of domain organizations. This allows us to classify proteins that have similar targeting domains together, even if the sequence homology is not close. So, Shibire, the dynamin-like protein in *D. melanogaster*, and *Arabidopsis thaliana* dynamin-like protein 6 (ADL6) are classical dynamins because they have the same domain structure as mammalian dynamin 1. It is clear that there might be further subdivisions when more targeting domains are recognized. Our classification is not based on function, and dynamins from different families and with alternative targeting domains might, therefore, have similar functions.

Overview of superfamily members

Classical dynamins. Mammalian dynamins 1, 2 and 3 are the founder members of the dynamin family. In our nomenclature we call them 'classical dynamins' along with other large GTPases that have the following five identifiable domains: GTPase domain, middle domain, PH domain, GED and PRD (FIG. 2). Mammalian dynamin 1 is brain-enriched, where it is concentrated in the presynapse; dynamin 2 is ubiquitous (including the brain); and dynamin 3 is found in the testis, but is also brain-enriched and is found postsynaptically[9–11]. Web pages have been written to complement this review, giving more details on domains and mutants of dynamin 1 (see Dynamin homepage in Online links).

The single isoforms of classical dynamin in *D. melanogaster* and *Caenorhabditis elegans* are assumed to cover the functions of the multiple isoforms in mammals. Mammalian dynamins 1 and 2 have been shown to have a role in scission of CCVs. In this process, the membrane invaginates to engulf cargo into CLATHRIN-COATED PITS (CCPs), which are eventually detached from the parent membrane with the aid of dynamin. However, dynamin also functions in the budding of caveoli, in phagocytosis, in the formation of podosomes, and during actin rearrangements and cytokinesis. These functions have been reviewed elsewhere[12–17], and so will not be discussed further in this review. *In vitro*, dynamin assembles on microtubules, but there is no convincing confirmation of this interaction *in vivo*. The potential of the long PRD, which is absent in the dynamin-related proteins and in GBPs/atlastins, to interact with many SH3 domains (BOX 3) might give this protein its versatility in working with disparate vesicle-budding pathways.

The exact function of classical dynamins will be the subject of more discussion below, but they have been described in vesicle budding as being responsible for the constriction of the lipid neck, the FISSION of the lipids and the regulation of the scission reaction. Whatever the exact mechanism, dynamin certainly has the potential to put energy into a thermodynamically unfavourable reaction.

Dynamin-like proteins. Compared to our five-domain definition for classical dynamins, Dlps are missing the PRD. From yeast to humans, there is one Dlp homologue per organism, and this is involved in mitochondrial division[18–25]. There is strong evidence that Dlps oligomerize into multimers and are likely to form rings[26–29]. Sequence homology does not allow us to define the region between the middle domain and the

CLASSICAL DYNAMINS
Dynamins that show sequence homology to the protein described in 1989 as 'dynamin', a microtubule-binding protein. For these classical proteins, the homology extends over the complete length of the protein and they have five distinct domains.

DYNAMIN-RELATED PROTEINS
Dynamins that lack one or more domains or have additional domains that are not present in classical dynamins. Some of these proteins might be functionally indistinguishable from classical dynamins.

CLATHRIN-COATED VESICLE
(CCV). Transport vesicles that bud with the aid of a coat protein known as clathrin.

CAVEOLAE
Flask-shaped invaginations of the plasma membrane that are coated with the protein caveolin. Caveolae are endocytosed in a clathrin-independent manner.

Table 1 | Functions of dynamin-superfamily members

Organism	Dynamin homologue	Localization	Function
Animals and yeast	Classical dynamins	Plasma membrane, *trans*-Golgi network and endosomes	Vesicle scission
	Dlp/Dnm1	Mitochondrial outer membrane, peroxisomes	Organelle division
	OPA1/Mgm1	Mitochondrial inner membrane	Mitochondrial fusion and division
	Mitofusin/Fzo1	Mitochondrial outer membrane	Mitochondrial fusion and division
	Yeast Vps1 (a classical dynamin probably performs this function in animal cells)	*trans*-Golgi network	Vesicle scission
	MxA	Smooth endoplasmic reticulum	Viral resistance
	GBPs	Partially membrane associated	Viral resistance and antiproliferative
	Atlastin1	Golgi of pyramidal neurons	Neural integrity, vesical trafficking?
Plants	ADL1A	Cell-plate tubular network	Cytokinesis
	ADL1C	Cell plate: possibly clathrin-coated-vesicle budding	Cytokinesis
	ADL2A	Plastids	Chloroplast division
	ADL2B	Mitochondria	Mitochondrial division
	Phragmoplastin (ADL4,5)	Cell plate	Cytokinesis
	ADL6	*trans*-Golgi network	Vesicle scission
	ARC5	Chloroplasts	Chloroplast division

Some of the functions and localizations of classical dynamins and dynamin-related proteins are listed. ADL, *Arabidopsis thaliana* dynamin-like protein; ARC5, accumulation and replication of chloroplasts mutant 5; Dlp, dynamin-like protein; Fzo1, fuzzy onions 1; Mgm1, mitochondrial genome maintenance 1; OPA1, optic atrophy 1; Vps1, vacuolar protein sorting 1.

DYNAMINS
Members of the dynamin superfamily, which include the dynamin-related proteins, Mx proteins and GBP/atlastin.

PLECKSTRIN-HOMOLOGY (PH) DOMAIN
A protein module of ~100 amino acids that is present in a range of proteins. Different PH domains interact with various phospholipids and are therefore involved in the targeting of the proteins.

SRC-HOMOLOGY-3 (SH3) DOMAIN
A protein module of ~80 amino acids that is present in a range of proteins and was first identified in the protein kinase Src. SH3 domains interact with proline-rich sequences that usually contain a PxxxPxR motif.

CLATHRIN-COATED PIT (CCP). The initial site of invagination of a clathrin-coated vesicle.

FISSION
The breakage of one object into parts — for example, fission of the lipid membrane.

PHRAGMOPLASTIN
Dynamin-like protein found at the phragmoplast, which is the microtubular network in dividing plant cells that transports Golgi-derived vesicles to the cell plate.

CELL PLATE
A flat, membrane-bound incipient cell wall at the division plane of a plant cell. The cell plate is formed by fusion and tubulation of Golgi-derived vesicles, which results in the outward expansion, and finally fusion, of the vesicles with the side walls.

GED of Dlps as a PH domain, but this region in *A. thaliana* ADL2 binds specifically to phosphatidylinositol-4-phosphate (PtdIns4P)[30] and might, in fact, be a PH domain. Lipid binding does not exclude the possibility that this domain is also involved in protein–protein interactions. Given the absence of a PRD, recruitment to mitochondria probably occurs through this domain.

Dictyostelium discoideum does not have a classical dynamin or a Dlp, but we have included Dynamin A (DymA) in the Dlp-family. It lacks the PRD of classical dynamins and also lacks a PH domain, but instead, it has a glutamine-rich domain that is typical for proteins from *D. discoideum*. Deletion of DymA has pleiotropic effects, including defects in cytokinesis, organelle morphology and, to a lesser extent, in endocytosis. Therefore DymA, like other non-classical dynamins, can function in vesicle budding in addition to other membrane-scission events.

Vps1-like proteins. Proteins within this family lack the PRD, and the region between the middle domain and GED is unlikely to accommodate a PH domain. Yeast has no classical dynamin that works in plasma membrane endocytosis, but Vps1 (vacuolar protein sorting 1) is involved in vesicle trafficking from the Golgi[31,32] and probably functions as a classical dynamin homologue without the necessity for multiple targeting sequences because of its limited location. In *A. thaliana*, the proteins that have a similar domain architecture are homologues of soybean PHRAGMOPLASTIN, which is involved in CELL-PLATE formation.

Mx-like proteins. Proteins within this family are missing the PRD and the PH domain. Expression of the human MxA and MxB proteins is induced by type I interferons and MxA gives strong protection against viral infection[33,34]. Fish have many interferon-induced Mx homologues and so it can be assumed that they must live in a sea of viruses. There is no Mx equivalent in *D. melanogaster* or *C. elegans*, presumably because they have not developed this mechanism to protect against infections. However, *A. thaliana* has an Mx-like protein. All the proteins in the Vps1 and Mx families have good conservation of the GED and GTPase domains and so these proteins show oligomerization-dependent GTPase activity (see below)[34,35].

ARC5-like proteins. The *A. thaliana* accumulation and replication of chloroplasts mutant 5 (ARC5)-like proteins also lack the PRD and the PH domain. In plants, members of this family are involved in chloroplast division[36,37]. We have only found homologues in plants and in *D. discoideum*.

OPA1/Mgm1 proteins. This family of proteins, which is present from yeast to humans, has the same domain architecture as the Dlps, including a predicted PH-like domain, but has an additional amino-terminal mitochondrial import sequence that is followed by a predicted transmembrane and coiled-coil sequence. These proteins are found between the inner and outer mitochondrial membranes and are involved in mitochondrial fusion[38,39].

ACTIVE ZONE
A specialized area of the presynaptic plasma membrane where synaptic vesicles fuse and release their neurotransmitter content.

Mutations in human *OPA1* (optic atrophy 1) give rise to dominant optic atrophy (DOA; BOX 4). The yeast homologues *Mgm1* (*mitochondrial genome maintenance 1*) from *Saccharomyces cerevisiae* and *Msp1* from *Schizosaccharomyces pombe* are clearly important in mitochondrial fusion. Mutations of Mgm1/Msp1 lead to mitochondrial fragmentation and this phenotype is suppressed by mutations in the *Dlp* gene of these yeast species[40,41].

Mitofusin/Fzo1 proteins. The mitofusin/fuzzy onions 1 (Fzo1) family of proteins is present from yeast to humans[42–45], and has a predicted transmembrane domain in place of the PH domain. The carboxy-terminal domain is predicted to be α-helical but, other than this, its homology to the GED of dynamin is weak. This is the third dynamin-related GTPase to be involved in mitochondrial dynamics and it localizes to the cytoplasmic side of the outer mitochondrial membrane. Apparently, the coordinated fission and fusion of organelles with several membranes is a complex event and so mitochondria are provided with three GTPases (Dlp, OPA1 and mitofusin) to coordinate fission and fusion without losing the membrane potential. However, this undoubtedly complicates the analysis of mutations in these individual GTPases.

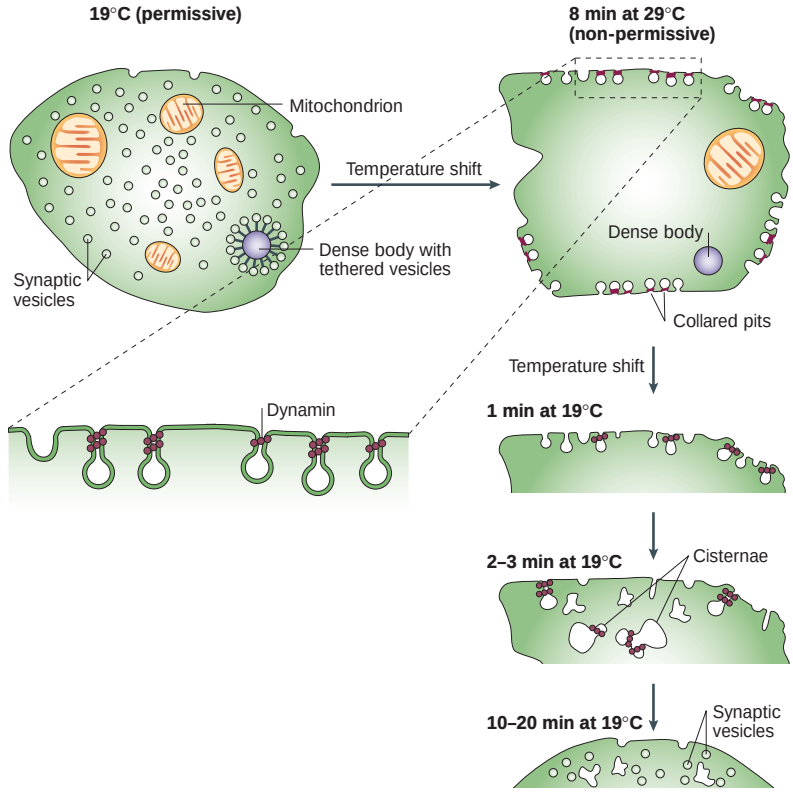
Guanylate-binding proteins/atlastins. GBPs/atlastins might fall outside the definition for dynamin-related proteins because the GTPase domain is the only significant region of sequence conservation, but the group has been included here because the proteins have probably arisen from a common ancestor (see phylogenetic analysis in FIG. 2). The crystal structure of GBP[46,47] shows intramolecular interactions that are similar to those that have been predicted for dynamins and so this structure might be a preliminary model for the GTPase, middle and GED domains of dynamin (for movies of structures see DymA/GBP structures in Online links). The expression of GBPs is induced by type II interferon and these proteins have a role in resistance against intracellular pathogens, which is similar to, but less efficient than, that of the Mx proteins[48,49]. GBPs are not found in *D. melanogaster* or *C. elegans*, but there is a weak homologue in *A. thaliana*. Unlike dynamin, GBP can hydrolyse GTP not only to GDP, but also to GMP[50].

Atlastins are homologous to GBP and mutations in atlastin1 have been identified in patients suffering from hereditary spastic paraplegia (HSP/Strümpell–Lorrain syndrome), a neurodegenerative disorder of the motor neuron system that causes progressive spasticity and weakness of the lower limbs[51]. The protein is associated with the *cis*-Golgi[52]. Another group of interferon-induced GBPs (p47 GBPs, which include IIGP1, see FIG.3) shows biochemical and biological similarities to the dynamin superfamily[53]. However, with a size of 47 kDa, they are too small to contain all three domains and therefore were not included in our definition of the dynamin-superfamily members.

Biochemical activities of dynamins

For lipid fusion, the SNAREs are the universal fusion molecules that are found on many compartments in cells[54]. In a similar manner, we propose that dynamins are universal lipid-stretching/fission molecules that

Box 1 | Shibire



In *Drosophila melanogaster*, temperature-sensitive (*ts*) mutants of the dynamin 1 homologue result in the reversible accumulation of endocytic profiles and a paralytic phenotype at the non-permissive temperature[114] (see figure). Within 1 minute of returning to the permissive temperature, these endocytic profiles enlarge and later detach to form cisternae. Synaptic vesicles then reform from these cisternal membranes. This phenotype shows a clear role for dynamin in synaptic-vesicle retrieval in nerve terminals and led to the idea that dynamin has a mechanochemical function in vesicle scission. However, the observations are equally consistent with dynamin being a regulator of the scission reaction.

No clathrin coats are visible by electron microscopy on the endocytic profiles in *shibire* nerve terminals[114]. This might be because uncoating of endocytic profiles occurs independently of scission. Indeed, the uptake of transferrin in COS cells is clathrin-dependent and mutants of dynamin that slow the scission reaction give rise to trapped vesicles that no longer have a clathrin coat[87]. Alternatively, the *shibire* phenotype might be evidence of a clathrin-independent 'kiss-and-run' mechanism of vesicle recycling, in which the synaptic vesicle does not fully collapse into the plasma membrane. Dynamin would be responsible for the 'run' stage — that is, the fast closure of the pore that separates the vesicle from the membrane.

A number of *ts* mutations occur in the GTPase domain and probably inactivate dynamin at the non-permissive temperature (see Shibire web site in Online links). There are also endocytic defects in other cell types and in *ts*1 (G273D), the resulting paralysis of the fly occurs with amazing speed, even before there is a great depletion of the vesicles[115]. The speed of paralysis in *shibire* has led to suggestions that dynamin might also be involved in vesicle exocytosis, but this effect is more likely to be the result of the failure of endocytosis, which would disrupt the ACTIVE ZONE.

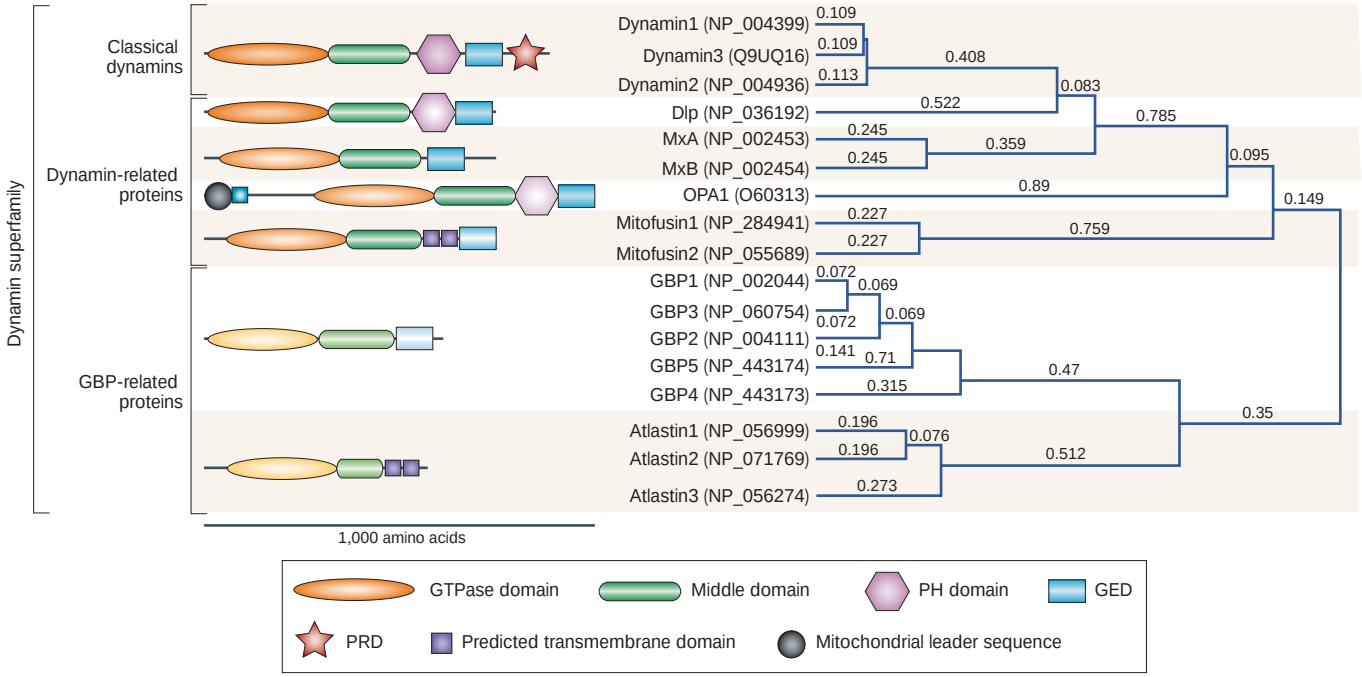


Figure 2 | **Domain structure of the human dynamin superfamily.** Domains are represented by different symbols and the decreasing intensities of the colours correspond to decreasing sequence homology. All dynamins contain a GTPase domain that binds and hydrolyses GTP, a middle domain and a GTPase effector domain (GED) that are involved in oligomerization and stimulation of GTPase activity. Additionally, most dynamins contain a domain for interactions with lipid membranes. This can be a pleckstrin-homology (PH) domain, a transmembrane domain or a sequence for lipid attachment. Classical dynamins contain a proline-rich domain (PRD) at the carboxyl terminus that interacts with Src-homology-3 (SH3) domains. Human dynamin-superfamily members have been grouped according to their domain structure and their accession numbers are shown. The family tree was calculated in MacVector using the tree building method with Poisson-corrected distances. DLP1, dynamin-like protein1; GBP1, guanylate-binding protein 1; OPA1, optic atrophy 1.

have been adapted to function at many different compartments in the cell. Before discussing the mechanisms of dynamin action, it is useful to survey the biochemical activities that give dynamins their unique properties.

GTPase activity. The conserved core of all related GTP-binding proteins is the GTPase domain of ~160 amino-acid residues, which consists of a mixed six-stranded β-sheet that is surrounded by five α-helices (see BOX 5 and GTPase domain web site in Online links). The Ras protein, which has 184 amino-acid residues, is considered to be the minimal GTP-binding protein[55]. The biochemical properties and biological functions of many GTP-binding proteins are influenced by insertions in their GTPase domain[56]. In the dynamin family, the GTPase domain is extended to ~300 amino-acid residues, the structure of which has been solved by Niemann and colleagues[57]. Dynamins are characterized by their low affinity for GTP and their even lower affinity for GDP (TABLE 3). Under physiological conditions, the protein is predicted to be constitutively loaded with GTP, but the protein will also be very sensitive to the energy status of the cell[58]. This contrasts with the Ras-like GTPases, which have a high affinity for GTP and GDP and require guanine nucleotide-exchange factors (GEFs) to catalyse nucleotide exchange.

Oligomerization-dependent GTPase activity. Dynamins are different to Ras-like GTPases in that oligomerization stimulates the GTPase activity once a 'critical mass' is reached, and the resulting GTPase activity resembles a chain reaction (there is cooperativity in the GTP hydrolysis). An oligomer normally refers to a complex of more than one monomer, but as the basic dynamin building block is a dimer or tetramer, we are using oligomerization as the ordered assembly of these building blocks into rings or helices[59–63]. Ras-like regulatory GTPases do not oligomerize — their GTPase activity is stimulated by the binding of GTPase-activating proteins (GAPs). For the oligomerized (assembled) form of dynamin, dynamin itself is the GAP. To understand this GAP activity, it is important that we understand the interactions between/within dynamin molecules. Electron microscopy images of dynamin oligomers and biochemical studies have shown that the GED can interact with the GTPase domain, the middle domain and also with itself[64–67]. Further structural information is required to understand this on a molecular level. The Ras-like regulatory GTPases use the kinetic control of the GTP–GDP cycle to function as a switch. In the GTP-bound form they can interact with effectors that they are unable to interact with in the GDP-bound form. There is no protein known to interact with the GTPase domain of dynamin and no PRD-interacting proteins

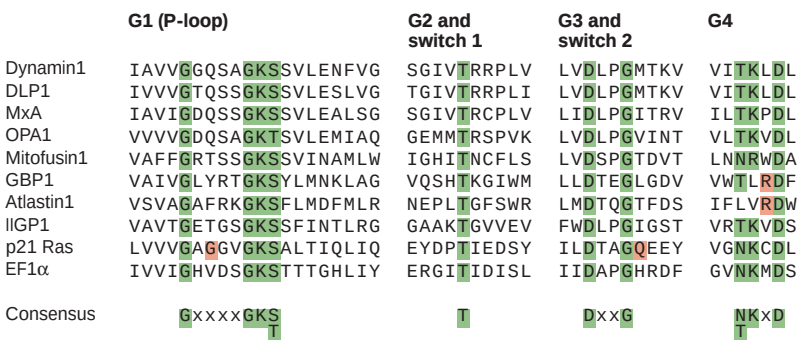


Figure 3 | **GTP-binding motifs.** Only 1 GTP molecule is bound per GTPase domain, but the sequences that contribute to the interactions are spread over the domain. The key residues are shown in green. The G1 motif (in the so-called P-loop) coordinates the phosphates, whereas the threonine in the G2 motif is involved in catalysis and is hard to predict by sequence comparisons but has been confirmed experimentally in some cases. The glycine in the G3 motif forms a hydrogen bond with the γ-phosphate of GTP. The G4 motif is involved in base and ribose coordination. The oncogenic mutations in p21 Ras and the unusually conserved G4 base-coordination motif in GBPs/atlastins are shown in red. Accession numbers for proteins are as in FIG. 2 with the additions: mouse interferon inducible GTPase (IIGP)/p47 (AAF07195), human p21 Ras (P01112), human elongation factor 1α (EF1α) (4503471). (For further details of the importance of these residues see also GTP-binding motifs web site and GTP-binding residues web site in Online links.) All sequences shown are for human proteins, except IIGP1, which is the mouse protein sequence. DLP1, dynamin-like protein1; GBP1, guanylate-binding protein 1; OPA1, optic atrophy 1.

have been found to be specific for a certain nucleotide-bound state so far. Therefore, in this scenario, dynamin would be its own effector. However, GTP-bound dynamin can assemble more efficiently on liposomes and the assembled protein should have a higher avidity for interacting partners that contain several SH3 domains.

The oligomerization of dynamin in low salt conditions and its ability to bind and cause tubulation of negatively charged liposomes[68,69] (FIG. 4) have been used as assembly tests for other members of the dynamin family (see Oligomerization web site in Online links). Dlp is a tetramer in solution and has similar domain interactions to dynamin[26]. Oligomerization in low salt conditions and with GTP analogues has also been shown. Dlp tubulates membranes when it is overexpressed in cultured cells as well as on synthetic liposomes[28]. A more detailed study has been done with *D. discoideum* DymA, which assembles into rings and helical structures even in the absence of lipids[29], and also shows cooperative GTP hydrolysis. The first dynamin-family member to be identified in plants was called phragmoplastin, and this also assembles in low salt conditions[70,71].

Murine Mx1 was the first protein from the dynamin family that was shown to oligomerize[72,73]. In human MxA, a single point mutation (L612K) in the GED blocks the interaction with the middle domain and inhibits oligomerization[74]. This residue is close to the leucine mutation in the GED domain of OPA1 that is found in patients with DOA (BOX 4). MxA also assembles into oligomers in low salt conditions and binds/tubulates liposomes[75].

There is no direct evidence for oligomerization of Vps1, OPA1/Mgm1 or mitofusin/Fzo1, although there is much circumstantial evidence from mutations and overexpression studies. GBP is a monomer in the nucleotide-free state but it can form dimers and tetramers in the presence of GTP analogues[46]. There is also recent evidence that atlastin 1 oligomerizes[52].

Box 2 | Dynamins in plants

In plants such as *Arabidopsis thaliana*, there are more dynamin homologues than there are in mammals, and so there is probably some functional redundancy. For example, there are at least five forms (A–E) of the *A. thaliana* dynamin-like protein 1 (ADL1).

ADL1A and ADL1C are associated with cell-plate formation in dividing cells[116,117]. ADL1A is found on tubular networks at the cell plate[105]. In plants devoid of ADL1A, cytokinesis proceeds normally, but *adl1A adl1E* double mutants show defects[116]. ADL2A (previously called ADL2) is found on plastids, which is consistent with its amino-terminal chloroplast transit peptide[93]. ADL2B is localized to the constriction sites of dividing mitochondria, and expression of a dominant-negative mutant of ADL2B results in the fusion and tubulation of mitochondria, which indicates a potential role in the scission reaction[118]. ADL3 shares a lot of sequence identity with ADL6, but ADL6 is the only member of the dynamin superfamily in *A. thaliana* that has all five distinctive domains, and therefore represents a classical dynamin. Through the proline-rich domain, ADL6 interacts with the Src-homology-3 (SH3) domain of the *A. thaliana* protein AtSH3P3, which is a homologue of mammalian amphiphysin[119]. A dominant-negative mutant of ADL6 (K51E, where K51 is the equivalent of K44 in mammalian dynamin 1) causes an accumulation of lytic-vacuole-targeted cargo within the *trans*-Golgi network, which supports a role in trafficking from the *trans*-Golgi network to the vacuole[120]. ADL4, ADL5, At5g42080 and At2g44590 cannot be definitively sorted into the vacuolar protein sorting 1 or myxovirus-resistance-protein families. However, it is clear from sequence homology that they are the *A. thaliana* homologues of the soybean phragmoplastins, which are also involved in cell-plate formation.

The proteins ARC5 (accumulation and replication of chloroplasts mutant 5) and At1g53140 are more distant dynamin homologues, and ARC5 is found on the outside of chloroplasts and is involved in their division[36]. No clear homologues of the optic atrophy 1 (OPA1)/mitochondrial genome maintenance 1 (Mgm1) and mitofusin/Fzo1 (fuzzy onions 1) families can be found in plants, but this could be the result of low sequence homology.

A simpler set of dynamin-related proteins is present in the primitive red algae *Cyanidioschyzon merolae*, which contains a single chloroplast and a single mitochondrion. The dynamin-related protein *C. merolae* Dnm1 is involved in mitochondrial division and is a close homologue of ADL1 and ADL2. By contrast, *C. merolae* Dnm2 is involved in chloroplast division[37,121] and shows 40% identity to *A. thaliana* ARC5 and 25% identity to *Dictyostelium discoideum* AAO51595.

Table 2 | Dynamin-superfamily members in different species*

Protein family	*H. sapiens*	*D. melanogaster*	*C. elegans*	*S. pombe*	*S. cerevisiae*	*A. thaliana*	*D. discoideum*
Classical dynamins	Dynamin1, dynamin2, dynamin3	Shibire/Dlp1 (S17974)	Dynamin/Dyn1 (AAD50438)	None	None	ADL6 (AAF22291)	Not identified
Dynamin-like	Dlp (alternative names: Drp1, DVLP and Dymple)	Dlp2 (AAF51235)	Drp1 (AAL56621)	Dlp (Q09748)	Dnm (AAA99998)	ADL1 (D84514), ADL2 (NP_567931), ADL3 (BAA77516)	DymA (CAA67983)
Vps1-like	None	None	None	Vps1 (Q9URZ5)	Vps1 (NP_012926)	Phragmo -plastin-like‡: ADL4 (CAC19657), ADL5 (CAC19656), At5g42080 (P42697), At2g44590 (NP_850420)	Not identified
Mx-like	Mx	None	None	None	None	Mx-like (AAB71966)	Not identified
ARC5-like	None	None	None	None	None	ARC5 (NP_188606), At1g53140 (NP_175722)	ARC5-like (AAO51595)
OPA1/ Mgm1-like	OPA1	Mgm1 (AAL13595)	Mgm1 (CAA87771)	Msp1 (JE0327)	Mgm1 (NP_014854)	Not identified	DymB (CAB64379)
Mitofusin-like	Mitofusin1, mitofusin2	Mitofusin (fuzzy onions, NP_732840)	Mitofusin (NP_495161)	Fzo1 (NP_595241)	Fzo1 (NP_009738)	Not identified	Not identified
GBP and atlastin-like	GBP1, GBP2, GBP3, GBP4, GBP5, atlastin1, atlastin2, atlastin3	Atlastin (NP_651274)	Atlastin (NP_500253)	None	None	GBP-like (BAB08252)	Not identified

*Using the sequenced databases we tabulate members of the dynamin superfamily in several organisms. The accession numbers for human sequences are as in FIG. 2 and others are shown beneath each protein name. The *Dictyostelium discoideum* genome is incomplete, at present. Note the absolute conservation from yeast to man of the three dynamin-related proteins corresponding to human Dlp, OPA1 and mitofusin that are involved in mitochondrial morphology, and at least one dynamin for vesicle scission corresponding to human classical dynamin or Vps1. ‡The phragmoplastin-like proteins are distinct from Vps1 but have a very similar domain organization. They are, in fact, very similar to the *A. thaliana* Mx-like protein, but as they are involved in vesicle trafficking, we have classified them with the Vps1-like family at present. ADL, *A. thaliana* dynamin-like protein; ARC5, accumulation and replication of chloroplasts mutant 5; Dnm, dynamin; DVLP, Dnm1/Vps1-like protein; Dymple, dynamin family member proline-rich carboxy-terminal-domain less; Dyn, dynamin; GBP, guanylate-binding protein; Mgm1, mitochondrial genome maintenance 1; OPA1, optic atrophy 1; Vps1, vacuolar protein sorting 1.

Membrane binding that precedes oligomerization-dependent GTPase activity. Ras GTPase activity is controlled by GAPs, whereas the GTPase activity of dynamin is controlled by self-oligomerization. Fortunately for the cell, futile cycles of dynamin GTP hydrolysis are prevented, as the oligomerization is regulated by membrane recruitment of dynamin to its sites of action. The PH domain of classical dynamins is responsible for their interaction with negatively charged lipid membranes. The affinity of a single PH domain for head groups is low (about 1 mM for inositol-1,4,5-trisphosphate) compared to other PH domains[76,77] (see PH domain web site in Online links). So the strong binding of dynamin to lipids relies on high avidity caused by the oligomerization of the protein. A single point mutation (K535A) in the PH domain of mammalian dynamin 1 has a dominant-negative effect on endocytosis in cells[78–80]. This low affinity of unassembled dynamin for lipids and its oligomerization after lipid binding ensures that there is tight control of GTPase activation. This feature of 'oligomerization-dependent GTPase activity on membrane binding' can now probably be extended to the dynamin superfamily. Therefore, although Dlps do not contain a bona fide PH domain, they localize to mitochondrial membranes and bind liposomes *in vitro*[27,28]. The region between the middle domain and the GED is probably involved in this. Likewise, human MxA protein is localized to the endoplasmic reticulum (ER)[75]. The OPA1/Mgm1 proteins have a mitochondrial targeting sequence at their amino termini (~100 amino acids), which is followed by a possible membrane insertion domain that is cleaved by a rhomboid-like protease[81]. They also contain a PH-like domain. So OPA1 strongly associates with mitochondrial membranes. Mitofusins/Fzo1 contain a transmembrane domain in place of a PH domain, which results in its constant attachment to the exterior of the outer mitochondrial membrane[44,82]. Most GBPs have a CaaX box (where 'a' is an aliphatic amino acid and X is any amino acid) for

Box 3 | Dynamin-binding proteins

Dynamin functions in the budding of many vesicle types. Given the low affinity and weak specificity of its pleckstrin homology (PH) domain for negatively charged lipids (see affinity measurements in PH domain in Online links) and the widespread use of dynamin in vesicle scission, many binding partners of the proline-rich domain (PRD) might target dynamin to sites of action. The PRD of classical dynamins, in which up to one in every three amino acids is a proline, can potentially interact with many Src-homology-3 (SH3)-domain proteins. (Some of these are documented in the PRD domain web site in Online links.) After vesicle scission has occurred, residual dynamin remains attached to the scission site on the vesicle and at this point it might form a docking site for dynamin-binding proteins that promote polarized actin assembly, as occurs in 'actin comet' formations[17,122,123].

Dynamin interactions found *in vitro*, and SH3–dynamin interactions tested in overexpression studies need to be verified carefully. Amphiphysin has been well studied as a dynamin-PRD-binding protein. As dynamin is a stable oligomer and full-length amphiphysin is a dimer, full-length amphiphysin will have a higher affinity for dynamin than for the monomeric isolated amphiphysin SH3 domain. The same scenario also applies to proteins that contain several dynamin-binding SH3 domains, for example Grb2 and intersectin, where a protein containing tandem SH3 domains will have a higher affinity and probably a better specificity for its target sequences. Overexpression studies can lead to artefactual results because levels of expression are often 10-fold to 100-fold above normal concentrations, so any weak dynamin-binding, monomeric SH3 domain can sequester dynamin away from its normal function *in vivo*. The case might be even more complex because overexpression of a dynamin-targeting protein for phagocytosis would be predicted to inhibit clathrin-mediated endocytosis and so might be called a 'clathrin-mediated endocytic protein'. Future experiments need to carefully characterize the budding events in which dynamin-binding proteins are implicated.

the attachment of an isoprenoid moiety to their carboxyl terminus[83], and mouse Gbp2 has been found to localize to vesicle-like structures in cells[84]. Finally, atlastin1 has a transmembrane domain and is found on the *cis*-Golgi[52].

Main functions of the dynamin superfamily

Vesicle scission. Classical dynamins work in many vesicle scission reactions. They are recruited by protein and lipid interactions through their PRD and PH domains. Overexpression of GTPase mutants has been widely used to investigate the involvement of dynamins in different pathways. It is now appreciated that the functions of dynamins are not limited to CCV scission (FIG. 5a), but that they are associated with many non-clathrin vesicle budding events such as caveolae budding and phagocytosis (FIG. 1)[12–14]. It has also become appreciated over the past few years that the mechanism of dynamin action is not clear.

Dynamin was originally proposed to act as a mechanochemical enzyme by tightening a dynamin collar around the vesicle neck after GTP hydrolysis and thereby constricting it. This was known as 'pinchase' activity, describing the 'pinching off' of vesicles, and was supported by the finding that GTP hydrolysis by dynamin on liposomes caused vesiculation[69]. Later, the mechanochemical 'poppase' model was proposed, in which a helix of dynamin assembles at the neck of a vesicle, and a lengthwise extension of this helix after GTP hydrolysis — caused mainly by an increase in helical pitch — results in the 'popping off' of vesicles from the parent membrane (FIG. 4 and, for movies, see Dynamin web site in Online links).

A fundamentally different role for dynamin in vesicle scission was presented at about the same time. This 'regulatory' model for dynamin function is based on comparisons with small GTPases in which a 'catalytic arginine finger', which is contributed by the activating protein, points into the active site and promotes GTP hydrolysis[85]. For dynamin, it is known that self-oligomerization activates hydrolysis and so the catalytic residue is likely to be on the domain that interacts with the GTPase domain. The GED got its name from the discovery of an essential arginine residue that was shown to contribute to the GTP hydrolysis[62]. When this arginine residue was mutated, mutant dynamin still

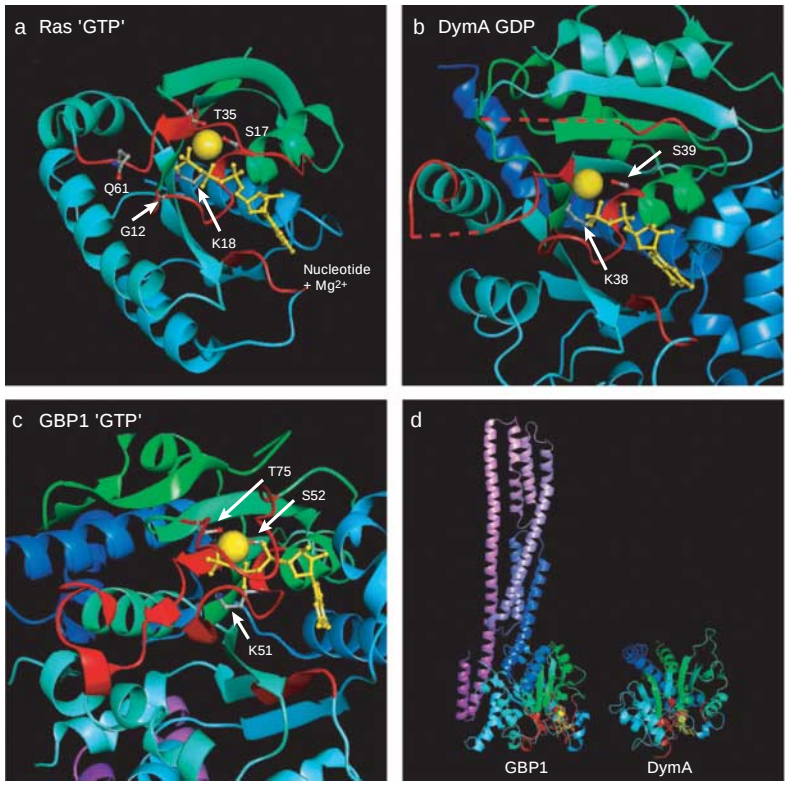
Box 4 | Dominant optic atrophy and *OPA1* mutations

Optic atrophy 1 (OPA1) is the only dynamin-related GTPase in which there are known disease-causing mutations in humans, presumably because mutations in other members of the superfamily are lethal. RNA-interference-mediated knockdown of OPA1 expression in cultured cells results in mitochondrial fragmentation, disruption of cristae structure and a loss of the mitochondrial membrane potential. Eventually, the loss of cytochrome *c* leads to apoptosis[124]. This is consistent with deletions of the yeast protein mitochondrial genome maintenance 1 (Mgm1) (REF. 41) and with OPA1 having a role in mitochondrial fusion (see main text).

Mutations in, or deletion of, one copy of the *OPA1* gene lead to dominant optic atrophy (DOA), a glaucoma that is caused by atrophy of retinal ganglion cells and the optic nerve, resulting in blindness[125–130]. Given the role of OPA1 in mitochondrial fusion, loss of mitochondrial integrity owing to *OPA1* mutations probably leads to deficient energy metabolism, thereby compromising the highly energy demanding neurons of the optic system. A complete deletion of both copies of the *OPA1* gene has not come to light. This shows that the protein is essential, and a single copy of the wild-type gene is sufficient for normal function in most cell types other than optical neurons (which show haploinsufficiency).

Most missense mutations in *OPA1* change highly conserved residues and cluster in the GTPase domain, whereas mutations in the three residues that cause dominant-negative phenotypes in dynamin (K44 and S45 in the G1 motif, and T65 in the G2 motif) are not found. There is at least one mutation in the GTPase effector domain (GED). In some cases there are two (different) missense mutations on different alleles leading to a stronger phenotype compared to one or the other single mutation, so these individual mutations are not dominant[128]. Intragenic complementation studies on the homologue *Mgm1* in yeast show that the protein oligomerizes, so mutations in the GED domain of one allele can complement mutations in the GTPase domain of another allele[41]. This implies that incorporation of mutant proteins into the oligomer with the wild-type protein is inhibitory. Detailed descriptions of the various mutations can be found at The OMIM OPA1 entry in the Online links.

Box 5 | Crystal structures of GTPases



Our understanding of GTPases has been greatly aided by crystal structures of small GTPases bound to substrates. Here, the overall architecture of the GTPase domain is conserved and allows us to predict the residues that are essential for coordinating nucleotide binding and hydrolysis. In the figure, parts **a**–**c** show close-ups of the nucleotide-binding sites of *Homo sapiens* Ras, the *Dictyostelium discoideum* dynamin-related protein DymA and *H. sapiens* guanylate-binding protein 1 (GBP1). In part **d**, the structure of full-length *H. sapiens* GBP1 is compared to the GTPase domain of DymA. For a better comparison, the ribbon diagrams of the structures are coloured from amino- to carboxyl-terminus (green to purple) so that the corresponding regions of the proteins have the same colour. As a result, the insertions in the GTPase domains of DymA and GBP1, with respect to Ras, are easily detectable. The conserved GTP-binding motifs G1–G4 as described in FIG. 3 are shown in red. The bound nucleotide and critical residues are shown as ball-and-stick models and the catalytic magnesium as a sphere.

Part **a** shows the Ras structure, in which K16 coordinates the β- and γ-phosphate of GTP, and S17 and T35 coordinate magnesium binding and waters involved in hydrolysis. All these residues are conserved and their mutation leads to hydrolysis-defective GTPases. The corresponding mutations have been useful in determining the mechanism of dynamin action (see main text). The oncogenic mutations in Ras (G12V and Q61L) are not conserved in dynamin-related GTPases. Part **b** shows the GDP-bound form of *D. discoideum* DymA, in which parts of the SWITCH REGIONS are disordered and are shown as dotted lines. The additions and insertions in the GTPase domain shield the nucleotide from the solvent and limit the access of a possible catalytic residue from the outside. Part **c** shows the GBP1 structure, in which the insertions are at different positions, reflecting the distant relationship between these proteins. Again, the GTP analogue is shielded. Part **d** shows the DymA structure, in which a long helix extends from the core of the GTPase domain (shown in dark blue), and could act as a lever arm on GTP hydrolysis. However, from the available structures, it is hard to see how movements could be coupled to hydrolysis because, as in GBP1, this helix has no direct contact with the nucleotide-binding site.

oligomerized but was defective in GTP hydrolysis. Endocytosis with this mutant was not inhibited and the authors concluded that the GTP-bound state represented the active form of dynamin that positioned other proteins for the scission reaction, and so GTP hydrolysis was not the driving force behind vesicle scission[62,86].

This regulatory model was challenged by further evidence for dynamin as a mechanochemical enzyme, showing that the hydrolysis of GTP was required for vesicle scission and that the GED contributes to the catalytic activity indirectly through oligomerization[87,88]. Furthermore, using dynamin assembled on nanotubes, coupling of the GTP hydrolysis to the lengthwise extension of the dynamin helix was shown to be necessary for endocytosis[87].

The controversy continued with further evidence for the regulatory model when postulated 'effector molecules' — Hsc70 and auxilin — were identified, and found to interact with dynamin exclusively in the GTP-bound state[89]. It is important to know if these proteins interact with the GTPase domain. Both proteins have known functions in the regulation of clathrin assembly and disassembly and the authors suggested that the main role of dynamin is to recruit these effector proteins to the necks of an invaginating pit and that the formation of the clathrin coat also drives fission. This clathrin-centric model for endocytosis does not, however, take into account the role of dynamins in clathrin-independent scission events such as phagocytosis or caveolae uptake. A crucial point is to test why other GTPase-defective dynamin mutants that are in the GTP bound state (like T65A) do not accelerate endocytosis[87]. So this controversy is still unresolved and no doubt there will be many more interesting papers forthcoming.

We believe that the evidence presented so far for classical dynamins more strongly supports a mechanochemical model. A role for dynamin GTPases as regulators of reactions might be attractive from an evolutionary point of view, as only ATPases were previously known to carry out work. However, the fact that dynamins are the common proteins that are found in many vesicle scission reactions and the absence of a better scission candidate points to dynamins being the mechanochemical molecules.

Organelle division and fusion. Mitochondria have an inner and an outer membrane. The inner membrane is highly invaginated to form CRISTAE and is also electrically insulated to maintain the proton gradient that drives ATP synthesis. Mitochondria undergo a constant process of fission and fusion, which ensures an appropriate distribution of mitochondria within the cell and of mitochondrial DNA in dividing cells, and might also be important in the turnover of integral membrane proteins.

It might be predicted that there are two fission molecules, one for each of the membranes in mitochondria. However, three families of dynamin-related proteins are clearly involved in mitochondrial dynamics: Dlp, OPA1

Table 3 | Comparisons of nucleotide binding and hydrolysis

Measurement	Mammalian dynamin	*D. discoideum* DymA	*H. sapiens* MxA	*H. sapiens* GBP1	*H. sapiens* H-Ras	Mammalian Gα
K_m	8–15 μM	70 μM	260 μM	470 μM	Irrelevant. GDP dissociation is limiting	Irrelevant. GDP dissociation is limiting
k_{cat}	190–260 min^{-1}	26 min^{-1}	*27 min^{-1}	*80 min^{-1}	*Unstimulated: 0.028 min^{-1} GAP stimulated: 1200 min^{-1}	Unstimulated: 2–5 min^{-1} RGS stimulated: 100–250 min^{-1}
K_d	0.2–1 μM (mGTP) 19 μM (mGDP)		1 μM (mGppNHp) 20 μM (mGDP)	1.5 μM (GppNHp) 15 μM (GDP) 3 μM (mGDP) 7.3 μM (GMP)	5 pM (GTP) 21 pM (GDP) 29 μM (GMP)	6–300 nM (bGTPγS)

Values indicated in the table are extracted from the following references: Dynamin[63,87,136], DymA[29], MxA[137], GBP[46,138,139], Ras[140,141] and Gα[142,143]. Fluorescently labelled nucleotides are Mant (m) and Bodipy (b). Please note that most, but not all, studies were carried out at 20°C and, where labelled with a star (*), the hydrolysis data were collected at 37°C. Catalytic rates for the dynamins are maximal figures. The total GTP concentration in a cell is 100 μM to 2 mM, of which 50–150 μM is free GTP[144]. The concentration of GDP is usually 10-fold lower[145]. Bodipy, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; GppNHp, β:γ-Imidoguanosine-5′-triphosphate; GTPγS , guanosine-5′-*O*-(3-thiotriphosphate); Mant, 2′/3′-*O*-(*N*-methylantraniloyl).

SWITCH REGIONS
Regions of nucleotide-binding proteins that have different conformations in the triphosphate- compared to the diphosphate-bound state.

CRISTAE
The inward folds of the inner mitochondrial membrane that increase its surface area.

and mitofusin (TABLES 1,2; FIGS 5b,c). Only one family, the Dlps, has been implicated in fission, whereas the others are implicated in fusion (a function which has also been seen for dynamins in plant cytokinesis; see below). All of these proteins have the GTPase, middle and GED domains, and mutations based on conserved residues in the dynamin superfamily, which have been well characterized in dynamin 1, lead to changes in the dynamic structure of mitochondria[22,24,41,43,90–92].

Dlp is found at sites of constriction on mitochondria, and Dlp GTPase mutants are defective in mitochondrial division. A remarkable demonstration of the importance of Dlp in mitochondrial division came from studies of a GTPase mutant of *C. elegans*. Here, two daughter cells, which migrate after division, were found to be connected by a narrow bridge of mitochondrial membrane owing to the failure of mitochondrial division[24] (FIG. 5b). This is the only mitochondrial GTPase

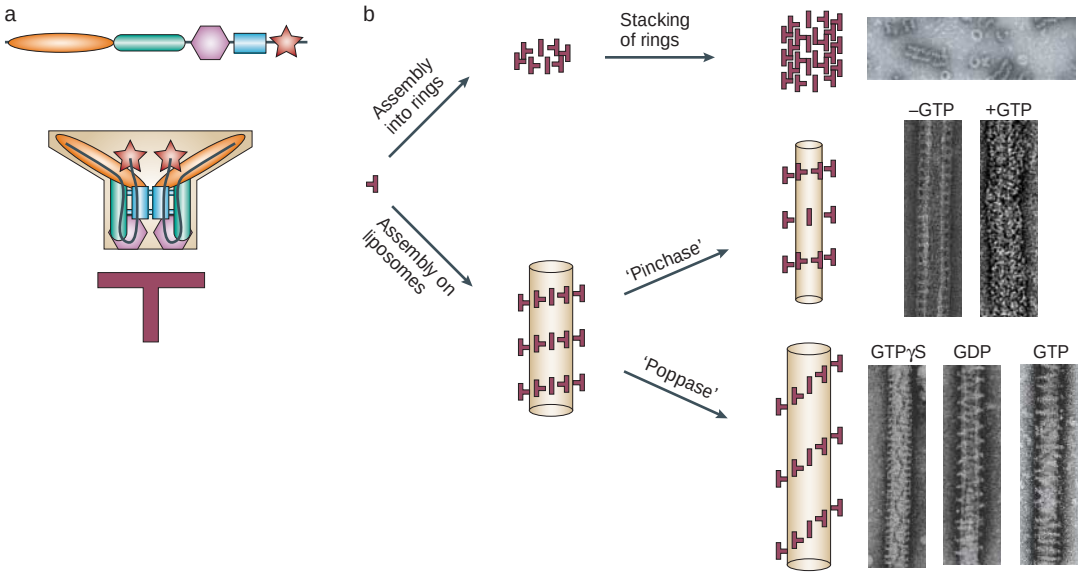


Figure 4 | **Oligomerization of dynamins. a** | Classical dynamins, Dlps and Mx proteins have been reported to assemble into stable dimers/tetramers[26,29,68,72,132–134]. This involves inter- and intra-molecular interactions of the GTPase effector domain (GED), middle and GTPase domains as illustrated in the cartoon. On the basis of electron microscopy, the building blocks for oligomeric dynamin 1 are seen as T-bar-like structures in which the pleckstrin homology domains are at the base of the T-bar, and this structure probably corresponds to the dimeric form of dynamin 1. **b** | Classical dynamin, Dlps and Mx all assemble in low ionic conditions or in the presence of GTP-analogues into rings and stacks of rings. These proteins induce the tubulation of liposomes that are often seen to be decorated with helical oligomers[28,63,69,75]. A comparison of the *in vitro* oligomerization and tubulation of dynamin-family members can be found on the Oligomerization web site in Online links. Nucleotide hydrolysis (in the case of dynamin 1) can lead to an increase in the pitch of the helix or to a decrease in the tubule diameter, and these are the basis for the proposed mechanical actions of dynamin. These pinchase versus poppase activities are illustrated by representative electron micrographs[63,69]. (See also Dynamin assembly in Online links.) The upper pannel of part **b** is reproduced with permission from REF. 132 © (1997) the American Society for Biochemistry and Molecular Biology. The middle panels of part **b** are reproduced with permission from REF. 69 © (1998) Elsevier. The lower panels of part **b** are reproduced with permission from REF. 69 © (1998) Macmillan Magazines Ltd.

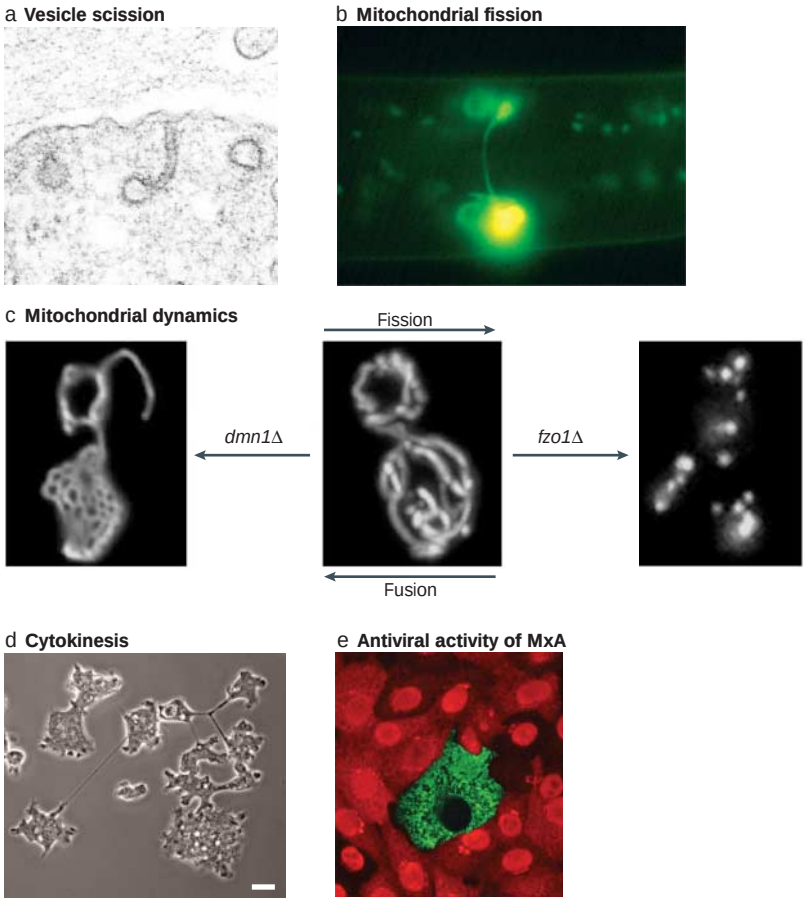


Figure 5 | **Functions of dynamins. a** | Vesicle scission. An electron micrograph of dynamin at the constricted neck of an endocytic vesicle[112]. Reproduced with permission from REF. 112 © (2003) National Academy of Sciences USA. **b** | Mitochondrial fission[24]. Divided cells in *Caenorhabditis elegans* are connected by the mitochondrial outer membrane in cells expressing the mutant Dynamin-like protein DRP1 (K40A). Reproduced with permission from REF. 24 © (1999) Elsevier. **c** | Mitochondrial dynamics. Mutations in dynamin-related protein 1 (Dnm1) and fuzzy onions 1 (Fzo1) show opposite effects on mitochondrial morphology in yeast. Deletion of Dnm1 leads to a network of mitochondria, whereas deletion of Fzo1 leads to fragmentation[135]. Reproduced with permission from REF. 135 © (2000) The Rockefeller University Press. **d** | Cytokinesis. Dynamin (DymA)-deleted *Dictyostelium discoideum* cells fail to complete cell division and stay connected by long, narrow, cytoplasmic bridges[25]. Reproduced with permission from REF. 25 © (1999) the American Society for Cell Biology. **e** | Antiviral activity. The antiviral activity of MxA in cells transfected with MxA compared to the surrounding cells labelled for a viral protein[34]. Reproduced with permission from REF. 34 © (2000) the American Society for Microbiology.

that is implicated in fission and so constriction of the outer mitochondrial membrane must automatically cause the constriction of the inner membrane, which leads to a simultaneous division of both membranes. Chloroplast division also involves a dynamin, and the dynamics of other organelles such as peroxisomes involve similar molecules[37,90,93–97]. All plant PLASTIDS and some mitochondria in primitive unicellular eukaryotes have maintained a homologue of the bacterial cell-division GTPase protein FtsZ that constricts the organelle by pulling the inner membrane. Two such GTPases, which are unrelated to dynamin, are also found in the *D. discoideum* genome, in which sequence searches have uncovered few dynamin homologues. This indicates that the FtsZ homologues might function alongside dynamins in organelle division.

As mentioned above, two mitochondrial dynamins are involved in fusion. OPA1 and its homologue in baker's yeast (Mgm1) are processed by the inner-membrane protease that is related to rhomboid, and which cleaves off the predicted transmembrane domain[81,98]. So OPA1 is found associated with the outer surface of the inner membrane and the inner surface of the outer membrane where it might link the two membranes together by oligomerization. RNA-INTERFERENCE-mediated loss of OPA1 causes a disorganization of the cristae structure and mitochondrial fragmentation, and deletion of the yeast protein results in a failure of mitochondria to fuse[39,41,98,99]. The third mitochondrial GTPase is mitofusin and, as its name suggests, it is implicated in mitochondrial fusion. It is found on the outside of the outer mitochondrial membrane and has a membrane insertion domain. In immunoprecipitation experiments, the yeast Fzo1 homologue was found to interact with the yeast OPA1 homologue, and deletions of Fzo1 in yeast also resulted in a mitochondrial fusion defect[43–45,100]. Given the close association of these two mitochondrial fusion proteins, they probably function together to promote the fusion of both the inner and outer membranes. As such fusion has been shown to take place in areas of high membrane curvature, we suggest that the dynamin-related proteins provide this curvature by coordinating the constriction/tubulation of the outer and inner membranes (FIG. 1). It is easy to imagine how mitofusin molecules on opposing mitochondria could be involved in tethering and juxtaposing the fusagenic ends.

Cytokinesis. During cytokinesis in plants, dynamins are involved in the formation of the tubular–vesicular network (which is the precursor to the cell plate) and in the retrieval of excess membrane by vesicle budding from the cell plate as it matures (BOX 2). Cytokinesis has been studied in great detail by electron microscopy. Vesicles from the *trans*-Golgi network fuse with the aid of a syntaxin-like molecule[101], and then form a tubular–vesicular network, in which the tubules are covered in a 'fuzzy' coat and contain callose (which forms the new cell wall)[102]. ADL1 or phragmoplastins are concentrated at the cell plate[103] and are candidates for transforming the vesicles into tubules[104,105,116,117]. This formation of a tubular network allows the generation of a two-dimensional structure, rather than a large vesicle that subsequently has to be flattened to form the membrane of the dividing cell. In the final stages, the tubular network is converted into a planar sheet and the membrane surface area is reduced by ~70%, which involves budding of CCVs[104,105]. This form of cytokinesis might be very specialized, but cell division in other species is equally dependent on dynamin, although it might be necessary for other purposes. In *D. discoideum*, for example, cell division fails in the absence of DymA, and cells remain attached by a narrow membrane bridge (FIG. 5d). In animal cells, dynamin is also involved in cytokinesis where it is important for vesicle budding from the CLEAVAGE FURROW[106,107].

PLASTIDS
Organelles that are found in eukaryotic plant cytoplasm. They all contain DNA and are surrounded by a double membrane.

RNA INTERFERENCE
(RNAi). A form of post-transcriptional gene silencing in which expression or transfection of double-stranded RNA induces degradation — by nucleases — of the homologous endogenous transcripts. This mimics the effect of the reduction, or loss, of gene activity.

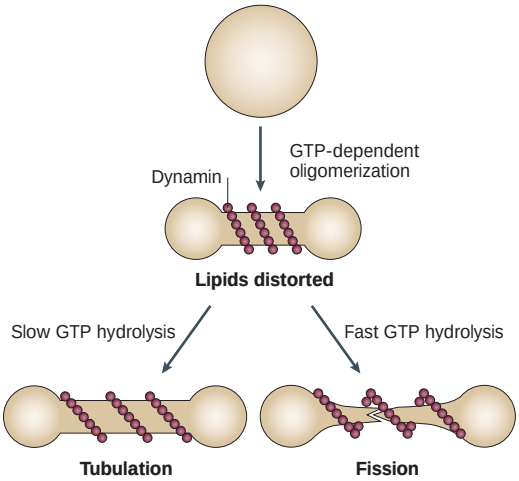


Figure 6 | **GTPase activity and lipid distortion.** A schematic of dynamin binding to a lipid vesicle, which leads to the formation of a helix on a lipid tube. Slow or uncooperative GTPase activity leads to an extension of the helix, which results in stretching and subsequent tubulation, whereas fast and cooperative dynamin GTPase activity leads to a rapid extension of the helix and fission of the tube.

Antiviral activity. Mx proteins were identified in mice owing to their antiviral effect against influenza virus[108]. No activity has been identified for MxB, but the human MxA protein has antiviral activity against a broad spectrum of viruses (FIG. 5e). This is a remarkable activity, the exact mechanism of which is not understood, but it might be directed at several stages of the viral replication pathway and a direct and GTP-dependent interaction of MxA with viral nucleocapsids has been shown[109,110]. It is known that MxA can associate with the smooth ER, through which viral proteins can pass. Furthermore, it has been suggested that the GTPase-inactive, monomeric Mx protein has anti-viral activity, which might break with the tradition that other dynamins require their GTPase activities for function[34]. There have been suggestions that Mx proteins might interfere with dynamin functions, although there is no evidence that they can hetero-oligomerize with any dynamins. An anti-viral effect of human GBP1 has been shown[49] but the inhibitory effect was much weaker than that of human MxA for its target viruses. GBP1 has also been shown to have antiproliferative action in epithelial cells[111].

Conclusion and perspectives

How dynamins work. Dynamins carry out a broad range of functions in cell biology, but there are expected to be some underlying themes. Given the domain structures and biochemical information, it is not unreasonable to assume that oligomerization-stimulated GTPase activity will be common to all members. Does this mean that dynamins are always scission molecules? Dynamins are also found in circumstances in which scission is not required — for example, in tubulation during cytokinesis in plants[104], in mitochondrial fusion reactions[41] and in podosome formation[17]. However, most of these circumstances also involve interactions between dynamin and lipid membranes. It is possible that differences in the GTPase activity of the molecule will determine its ability to tubulate or to cause lipid fission. We already know that classical dynamins can cause lipid tubulation *in vivo* under circumstances in which vesicle scission does not work. In a recent example of this, knockdown of clathrin expression led to overexpression of dynamin 2, which resulted in an accumulation of endocytic profiles (long tubular invaginations from the plasma membrane) that were surrounded by a dynamin helix[112] (FIG. 5a). This is very similar to the recovery of the Shibire phenotype in the study of Kessell *et al.*, in which long tubules appeared when the cells were moved back to the permissive temperature[113]. Moving cells to a low temperature also resulted in the elongation of vesicle necks. Finally, GTPase mutants that undergo slow hydrolysis also give the same phenotype. When hydrolysis is slowed so much that there is a complete failure of vesicle scission, necks continue to grow until the compartments at the ends of these tubules, which are rich in endocytic markers, fuse together forming interconnected tubules throughout the cell[87]. So classical dynamins can tubulate lipids *in vivo* under circumstances in which scission does not take place, and it should be expected that other dynamins also have the ability to tubulate, depending on GTPase activity and context.

Although there might be many similarities, there are also many differences in the dynamin superfamily. We have already mentioned differences in recruitment to membrane compartments. There are also differences in the ability of dynamins to oligomerize — Dlps oligomerize less readily than classical dynamins in a low salt solution. It is fascinating to note how classical dynamins can tubulate liposomes in the absence of GTP hydrolysis, which shows that the energy of lipid binding and oligomerization can also do work. This might not be the case for other dynamins where GTP hydrolysis might be needed to cause deformation of membranes.

The scene is set for testing how the dynamin-superfamily members work in the cell. We envisage that all members will go at least part way along the following continuum. Dynamins are initially recruited and localized to specific sites of action by the lipid and protein interactions of the PRD, PH and PH-like domains. Here, they assemble in a stable helix and constrain the shape of the lipid membrane. GTP hydrolysis might aid this process, making it more efficient, although the primary effect of GTP hydrolysis will be to distort the helix away from its stable GTP-bound conformation. By analogy with ATP hydrolysis in myosins, it seems likely that dynamin also moves a lever arm when GTP is hydrolysed, which results in a conformational change in the oligomeric dynamin helix. For classical dynamin, we believe that this results in a lengthwise extension of the helix. Two possible outcomes can therefore be envisaged, which depend on the speed of extension, the lipid composition and the surrounding proteins (FIG. 6). In the first scenario, the lipids might simply be pulled into a new shape, for example, an extended tubule. In the second scenario, the lipids might be sheared owing to a sudden

CLEAVAGE FURROW
An invagination of the plasma membrane in the division plane of an animal cell that contains a contractile ring, and which leads to scission of the daughter cells.

perturbation that rips the lipid molecules apart. So dynamins might adapt their biochemical properties to cause tubulation and/or fission, depending on the context.

The key to solving how dynamins work will be the structural analysis (preferably of a mammalian classical dynamin) in its various nucleotide states so that we can accurately determine what, if any, conformational changes occur on GTP hydrolysis. If a conformational change does indeed occur, this should be shown to produce a force that is capable of explaining membrane fission. An alternative biochemical approach might be to examine whether the tubulation and vesiculation abilities of dynamin are related to the speed of GTP hydrolysis and/or to the extent of a conformational change. On the other hand, if dynamin is not the scission molecule, then it is important to identify what is. Finally, moving back into cells, we should test the importance of the speed of GTP hydrolysis on the phenotypes of various organelles by making mutants of dynamins that slow GTP hydrolysis.

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Competing interests statement

The authors declare that they have no competing financial interests.

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