Dynamin, a membrane-remodelling GTPase

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Abstract | Dynamin, the founding member of a family of dynamin-like proteins (DLPs) implicated in membrane remodelling, has a critical role in endocytic membrane fission events. The use of complementary approaches, including live-cell imaging, cell-free studies, X-ray crystallography and genetic studies in mice, has greatly advanced our understanding of the mechanisms by which dynamin acts, its essential roles in cell physiology and the specific function of different dynamin isoforms. In addition, several connections between dynamin and human disease have also emerged, highlighting specific contributions of this GTPase to the physiology of different tissues.

Synaptic vesicles
Small (~40 nm in diameter) vesicles within neuronal presynaptic terminals that store and release neurotransmitter.

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Endocytosis, the process through which cells internalize portions of their plasma membrane along with extracellular material, is fundamentally important in cell physiology. Endocytosis counterbalances the continuous delivery of new membrane to the cell surface by exocytosis, regulates the abundance of proteins in the plasma membrane, controls the signalling output of receptors, mediates cellular uptake of nutrients and is also exploited by pathogens to enter cells. To support these various functions, multiple molecularly and morphologically distinct forms of endocytosis, both constitutive and regulated, operate in all cells[1–4].

Generation of an endocytic vesicle requires the recruitment of various proteins from the cytosol that orchestrate the bending inward of the plasma membrane to form a deeply invaginated bud and subsequently promote its fission. One such protein that directly participates in the fission reaction is the GTPase dynamin, the founding member of a family of GTPases that have diverse roles in membrane-remodelling events throughout the cell (FIG. 1). The role of dynamin in endocytosis has now been investigated for more than 20 years[5–8]. A considerable amount of evidence indicates that dynamin assembles into helical polymers at the necks of budding vesicles and that its GTP hydrolysis-dependent conformational change promotes fission of the underlying tubular membrane to generate a free endocytic vesicle (FIG. 2). However, several aspects of its function, its precise mechanisms of action and the specific roles of multiple dynamin isoforms in mammalian cells have remained elusive. Furthermore, although multiple dynamin-dependent and dynamin-independent endocytic pathways have been described, we have a good understanding only of how dynamin is recruited and then acts at fission sites for clathrin-mediated endocytosis. In this Review, we discuss how complementary experimental approaches have greatly increased our understanding of dynamin's action. We also highlight how this information has been further enhanced by new insights into the function and mechanistic action of dynamin-like proteins (DLPs).

The discovery of dynamin

Dynamin was originally identified as a GTPase that co-purified with brain microtubules[6,9]. Its role in endocytosis was revealed only subsequently, when the mutations responsible for the temperature-sensitive paralytic phenotype of *Drosophila melanogaster* shibire mutants were mapped to the dynamin gene[7,8]. In these mutants, paralysis results from the neuronal activity-dependent depletion of synaptic vesicles, which is accompanied by the accumulation of arrested 'collared' endocytic pits at the presynaptic plasma membrane[5]. Subsequent studies revealed that similar collared pits formed at mammalian presynaptic plasma membranes upon exposure to GTP-γS, a slowly hydrolysable GTP analogue, and that they contained oligomeric assemblies of dynamin[10]. The assembly of helical dynamin polymers either in solution[11] or on membrane templates was additionally reconstituted with the purified protein[11] (FIG. 2e). Further investigations in non-neuronal cells showed that dynamin is a general component of clathrin-coated endocytic pits and that its GTPase activity is important for endocytosis: dynamin mutants with impaired GTP binding (such as the commonly used Lys44Ala mutant) and/or hydrolysis cycles have dominant-negative effects on endocytosis[12–15].

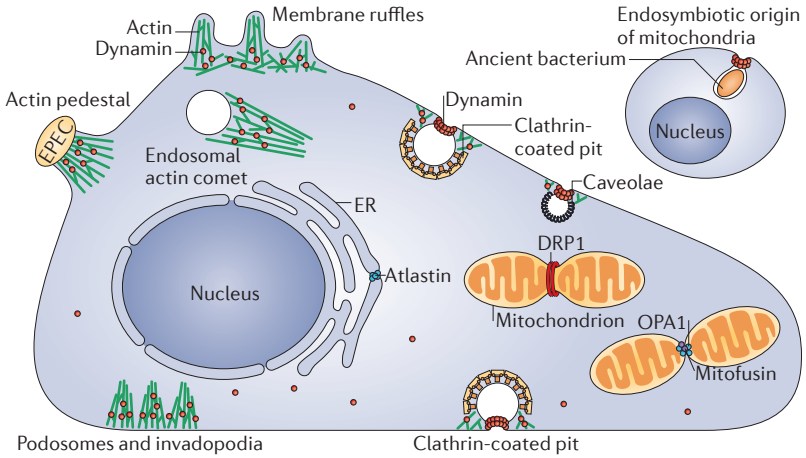

Figure 1 | **Sites of action of dynamin and DLPs in a mammalian cell.** Dynamin is localized at sites of endocytosis. It is also found at actin meshworks nucleated by the actin-related protein 2/3 (ARP2/3) complex, such as membrane ruffles, podosomes and invadopodia, and at actin pedestals induced by pathogenic bacteria (such as enteropathogenic *Escherichia coli* (EPEC)). Other dynamin-like proteins (DLPs), including atlastin, dynamin-related protein 1 (DRP1), optic atrophy 1 homologue (OPA1) and mitofusin, localize to sites of intracellular membrane fission and fusion in the endoplasmic reticulum (ER), mitochondria and peroxisomes (not shown). The possible endosymbiotic origin of mitochondria and peroxisomes (that is, their formation by the endocytosis of an ancient bacterium) may explain the role of a DLP in the fission of these organelles.

Three dynamin isoforms in mammals

Mammalian genomes contain three dynamin genes[16]. The proteins encoded by these genes share the same domain organization and 80% overall homology, but they have distinct expression patterns. Dynamin 1 is selectively expressed at high levels in neurons and is generally not present in non-neuronal tissues[17,18], although it can be detected in many cultured cell lines[19,20]. Dynamin 2 is expressed ubiquitously[17,21]. Dynamin 3 is found predominantly in the brain (at much lower levels than dynamin 1) and testis, and at lower levels in some tissues, such as the lung[16,17,22]. Dynamin diversity is compounded by the existence of multiple splice variants for each of the three dynamins[16].

Invertebrates, such as *Caenorhabditis elegans* and *D. melanogaster*, possess only a single dynamin gene[7,23]. So, the existence of three dynamin genes in mammals could in principle reflect differences between the 'housekeeping' role of dynamin in clathrin-mediated endocytosis, which is mediated by dynamin 2, and the specialized forms of endocytosis in cells that additionally express dynamin 1 and/or dynamin 3. In addition, this triplication of the dynamin gene during evolution may be partly explained by a need to fine-tune overall dynamin levels in specific tissues. Different dynamin isoforms have some unique protein–protein interactions[24–27]. However, most of the differences between isoforms are quantitative rather than qualitative; these include affinities for SRC homology 3 domain (SH3 domain)-containing proteins[22], rates of GTPase activity, oligomerization efficiency and lipid-binding properties[28]. Nonetheless, a recent report stating that differences in the lipid-binding characteristics of dynamin 1 and dynamin 2 cause robust differences in their membrane-fission activity[28] indicates that this remains an area of active research and that additional studies are required to fully address the issue of redundancy between dynamin isoforms.

A family of DLPs

The dynamins are the founding members of a family of GTPases known as the DLPs, the members of which participate in membrane remodelling (FIG. 1; TABLE 1) and share basic features of domain organization (BOX 1). They also have similar catalytic mechanisms and modes of action that differ from those of the RAS superfamily of regulatory GTPases. The G domains of DLPs undergo nucleotide-dependent dimerization and dimer-dependent GTP hydrolysis[29–32]. G domain dimerization reciprocally induces structural rearrangements in the catalytic centre of the partner DLP, which stimulate GTP hydrolysis[33]. In contrast to small regulatory GTPases of the RAS family, DLP family GTPases have low affinity for nucleotides and do not require separate GAPs and GEFs (GTPase-activating proteins and guanine nucleotide exchange factors), as they load spontaneously with GTP and have intrinsic mechanisms for stabilizing the transition state during GTP hydrolysis[29]. In the case of dynamin, GTP hydrolysis is thought to be supported by a sodium or potassium ion within the active site[30]. In other DLPs, divergent mechanisms are used to stabilize the transition state. However, the lack of a requirement for additional GAP proteins, and the importance of G domain dimerization, is conserved within the DLP family.

Furthermore, whereas the GTP binding and hydrolysis cycle of regulatory GTPases mediates the recruitment and release of effector proteins, the cellular function of DLPs is tightly coupled to the dimerization of their G domains and, at least for some of them, to their polymerization[29,31,32,34,35]. The conformational changes produced by GTP binding and hydrolysis in the G domain of DLPs are transduced into a movement relative to adjacent domains[36–38] that, when propagated along the polymer, is expected to produce a force on membranes[31,32]. Thus, for dynamin, the cycle of GTP loading and hydrolysis is intimately coupled to its membrane-fission activity (FIG. 2d). This explains why mutations in dynamin that are designed to lock it into a GTP-bound state do not result in the constitutive activation of endocytosis[15], in contrast to the persistent activity exhibited by regulatory GTPases that harbour equivalent mutations.

Domain organization of dynamin

On the basis of its primary sequence, dynamin, a cytosolic protein, has typically been described as comprising: an amino-terminal G domain; a 'middle' or 'stalk' region; a pleckstrin homology domain (PH domain); a GTPase effector domain (GED), so called because of its interactions with the G domain[39]; and a Pro-rich carboxy-terminal region, typically referred to as the Pro-rich domain (PRD)[16] (FIG. 2a). More recently, information about the structures of dynamin and DLPs[30,34,36,37], including the crystal structure of dimers of nearly full-length dynamin (lacking only the PRD)[31,32] have allowed for a modified definition of dynamin domains that better reflects the predicted three-dimensional hairpin-like folding of the full-length protein (FIG. 2a,b).

SRC homology 3 domain
(SH3 domain). A domain that mediates protein–protein interactions and binds Pro-containing short amino acid motifs. This domain is frequently found in proteins involved in signalling, endocytosis and actin regulation.

GAPs and GEFs
(GTPase-activating proteins and guanine nucleotide exchange factors). GAPs promote GTP hydrolysis by GTPases, whereas GEFs displace the GDP generated by the reaction, thus allowing the next cycle of GTP binding and hydrolysis to proceed.

Pleckstrin homology domain
(PH domain). These domains often contain binding sites for phosphoinositides (most typically phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate) and thus help target proteins to specific membranes. However, they can also function in protein–protein interactions.

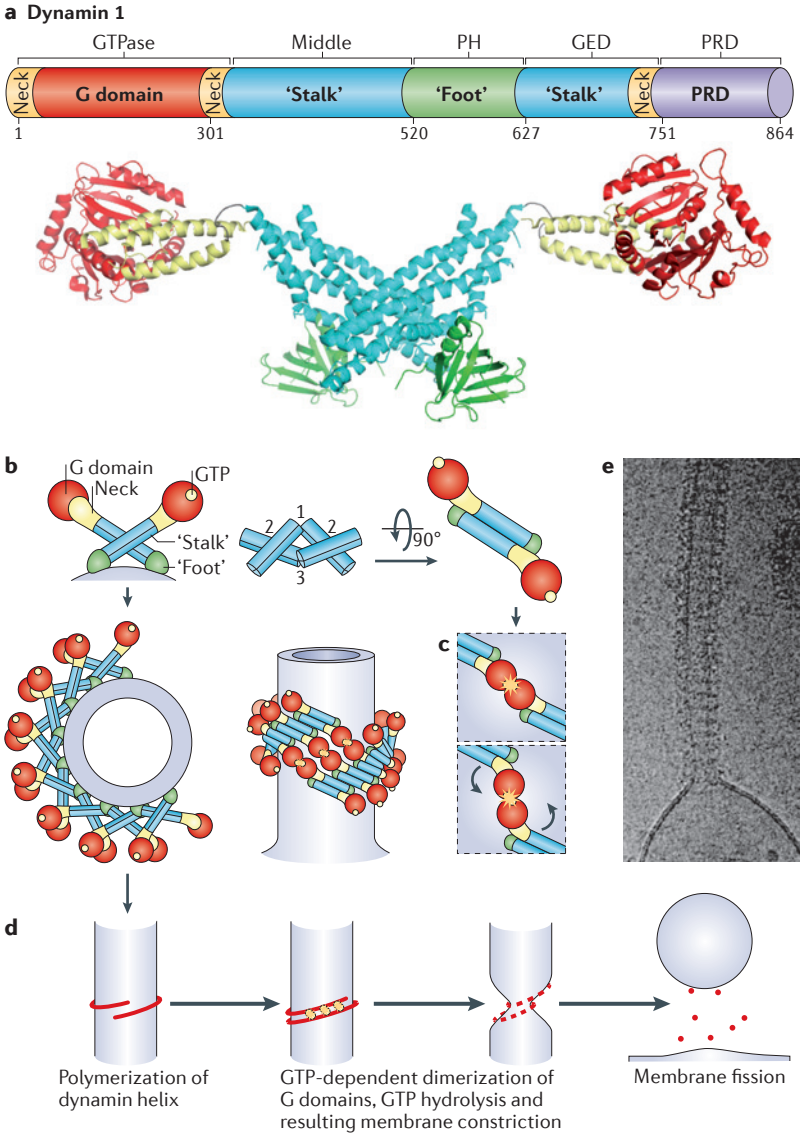

Figure 2 | **Structure of dynamin and putative mechanism of dynamin-mediated membrane fission. a** | Linear representation of the domain organization of dynamin based on its three-dimensional structure, as revealed by crystallographic studies (numbers indicate amino acid position within the primary sequence of the human dynamin 1 xa splice variant). Regions that belong to the same folded module are shown in the same colour. The crystal structure of a dynamin dimer is shown below (minus the PRDs, which are thought to be unfolded), and is colour-coded to match the linear representation (created with PyMOL (Schrödinger); Protein Data Bank code 3SNH[31]). **b** | Schematic representation of dynamin dimers and of helical dynamin polymers around a tubular template in two different orientations (with 90° rotation between them). The colour-coding of the domains matches the colours in part **a** (minus the PRDs, which are thought to project out of the polymerized helix). The approximate location of the bound nucleotide is highlighted in yellow (small circles). Dynamin polymerization occurs as a result of interactions between the 'stalks' of dynamin monomers (interface 2) and between stalk dimers (interfaces 1 and 3). The GTP-dependent dimerization of G domains between adjacent rungs of the dynamin helix (highlighted by yellow stars in the longitudinal view of the helix), is thought to promote assembly-stimulated GTPase activity, resulting in membrane constriction and ultimately fission. **c** | Proposed GTP hydrolysis-dependent lever-like movement of dynamin's neck (the bundle signalling element), relative to the G domain. **d** | Schematic view of these key steps leading to dynamin-mediated membrane fission. **e** | Cryo-electron microscopy image showing a helical polymer of purified dynamin that has driven the formation of a tubule from a liposome. GED, GTPase effector domain; PH, pleckstrin homology; PRD, Pro-rich domain. Image in part **e** courtesy of A. Frost, University of Utah, USA, and V. Unger, Northwestern University, USA.

The G domain and BSE. The G domain sits on a helical bundle, known as the bundle signalling element (BSE)[30] or neck[40], which is formed by three helices derived from sequences at the N-terminal and C-terminal sides of the G domain and from the C-terminal region of the GED, respectively (FIG. 2). Consistent with the idea that this region of the GED is in close physical proximity with, and is functionally linked to, the G domain, a screen for suppressor mutations of a mutation in the G domain of *D. melanogaster* dynamin identified a mutation within the GED C terminus[41]. The BSE is followed by a stalk, which is composed of helices from the middle domain and the N-terminal region of the GED[30–33], and a PH domain[42], which forms the vertex or 'foot' of the stalk hairpin and binds membranes. The PRD, which is expected to be unfolded, emerges at the boundary between the BSE and the G domain, most likely projecting away from the membrane, where it might interact with other proteins.

Dimerization through the stalk. The stalk of dynamin dimerizes in a cross-like fashion (FIG. 2a,b) to yield a dynamin dimer in which the two G domains are oriented in opposite directions[30–33] (FIG. 2b). This dimer is the basic dynamin unit and is different from the additional dimerization interface that is generated by the interaction of two G domains (FIG. 2b,c), which is discussed below.

Phospholipid association through the PH domain. The PH domain binds acidic phospholipids in the cytosolic leaflet of the plasma membrane, and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) in particular, via a positively charged surface at the foot of the dynamin hairpin[42,43]. PH-domain mutants that impair phosphoinositide binding exert dominant-negative effects on clathrin-mediated endocytosis[44,45]. The binding between the isolated dynamin PH domain and phosphoinositides is of very modest affinity (>1 mM), but this membrane interaction is strengthened by charge-dependent association with other negatively charged phospholipids and by avidity afforded by dynamin polymerization[43–46]. A hydrophobic loop emerging from the PH domain may promote membrane interactions and may also have curvature-generating or -sensing properties[28,47].

Coordinating dynamin function through the PRD. The PRD contains an array of PXXP amino acid motifs, which interact with many SH3 domain-containing proteins (see Supplementary information S1 (table)) to localize dynamin at endocytic sites and coordinate dynamin's function with these other factors during endocytosis[48–51]. Accordingly, dynamin lacking the PRD cannot rescue endocytic defects in dynamin-knockout fibroblasts[19]. The PRD–SH3 interactions are typically of moderate affinity, but the presence of multiple SH3-binding motifs in the PRD and multiple SH3 domain-containing proteins at endocytic sites, as well as the polymeric state of these proteins, results in a significant avidity effect, which enhances the ability of such interactions to concentrate dynamin. At least some interactions of the dynamin PRD are regulated by phosphorylation[48].

Table 1 | **Diverse roles for DLPs at membrane interfaces**

DLPs	Organisms	Subcellular localization and sites of action	Functions	Refs
Classical dynamins	Animals	Endocytic sites in the plasma membrane; ARP2/3-containing actin meshworks	Endocytic membrane fission; regulation of ARP2/3-dependent actin dynamics	–
DRP1	Animals	Mitochondrial outer membrane; peroxisomes	Mitochondrial fission; peroxisome division	159–162
Mitofusin	Animals	Mitochondrial outer membrane	Mitochondrial fusion	159–162
OPA1	Animals	Mitochondrial inner membrane	Mitochondrial fusion	159–162, 188
Atlastin	Animals	ER	Fusion of ER membranes	167,168, 189
Myxovirus resistance proteins	Animals	ER	Antiviral	169,190
Guanylate-binding proteins	Animals	Intracellular vesicles	Defence against viral and bacterial pathogens	170,191,192
Phragmoplastin	Plants	Cell plate	Cell division	171
ARC5, ADL	Plants	Chloroplast	Chloroplast fission	193,194
Vps1	Fungi	Endosomes; plasma membrane?	Membrane fission	98,195
Bacterial DLP	Cyanobacteria	Integral membrane protein	Membrane fusion?	40

ARC5, ACCUMULATION AND REPLICATION OF CHLOROPLASTS 5; ARP2/3, actin-related protein 2/3; DLPs, dynamin-like proteins; DRP1, dynamin-related protein 1; ER, endoplasmic reticulum; OPA1, optic atrophy 1 homologue; Vps1, vacuolar protein sorting-associated 1.

Coordinating polymerization and activity

Purified dynamin spontaneously polymerizes into rings and helices when incubated in solutions with low ionic strength[11] or in the presence of narrow negatively charged tubular templates (such as membrane tubules, microtubules or actin bundles)[6,52–54]. It can also tubulate membrane bilayers under appropriate conditions by forming a continuous membrane coat around them[10,55–57]. Dynamin polymerization resulting from the apposition of dimers via stalk–tip interactions (FIG. 2b; interfaces 1 and 3) occurs an angle that determines the diameter of the ring[31,32]. The tetrameric form of dynamin, which can be abundant in solution[58], may be an intermediate in higher-order assembly[34,38]. Thus, the stalks form the core of the ring, whereas the BSE and G domains of each dimer project towards the adjacent rungs of the dynamin helix[31,32] (FIG. 2b). It follows that G domain dimerization, which is critical for GTP hydrolysis and for dynamin function, can only occur across adjacent rungs (FIG. 2b), which explains the robust stimulation of dynamin's GTPase activity upon polymerization[54]. A similar overall domain organization and mode of polymerization is predicted to be shared by all DLPs implicated in membrane fission[59]. However, the module that binds the membrane in these other proteins is divergent from dynamin, and the PRD is replaced by sequences with different binding properties. Such divergence probably reflects the different mechanisms that recruit different DLPs to membranes and may speak against a key role of the PRD and the PH domain in the mechanics of fission.

Membrane fission by the dynamin helix

The mechanisms by which dynamin drives membrane fission have been the subject of intense debate and have been analysed in living cells[12,15,44,54], broken-cell preparations[10,60] and minimal systems based on purified dynamin and artificial lipid bilayers[55,56,61–63]. Models derived from these experiments have suggested that, when dynamin has polymerized around the neck of an endocytic bud (or a tubular membrane template *in vitro*), its GTP hydrolysis-dependent structural reorganization triggers constriction (in the 'twistase' and/or 'constrictase' models) or stretching (in the 'poppase' model) to promote membrane fission (FIG. 2b). Importantly, cell-free studies have shown that purified dynamin alone can cut synthetic lipid tubules in the presence of GTP[55,56,61,63]. Thus, although other factors may assist the action of dynamin *in vivo*, dynamin is sufficient to mediate membrane fission.

Recent crystallographic and cryo-electron microscopy (cryo-EM) studies of dynamin and DLPs have offered important new clues about how dynamin works. First, the demonstration that dynamin belongs to the superfamily of G proteins activated by nucleotide-dependent dimerization (GADs)[29] strongly argues against the possibility that GTP-bound dynamin may function by recruiting another effector[39]. Second, as G domain dimerization is critical for GTPase activity[29,30,59,61,64] and requires interactions between adjacent rungs of the dynamin helix[30] (FIG. 2b), dynamin's action requires, at a minimum, a polymer that wraps around a membrane template to connect to the next rung. Third, studies of dynamin and of the DLP atlastin suggest that GTP hydrolysis by a G domain dimer leads to a prominent lever-like movement of the adjacent domain, the BSE in the case of dynamin[36–38] (FIG. 2c). Such a movement could constrict the dynamin helix when propagated along its subunits. As this movement is triggered by the interaction of G domains on adjacent rungs of a helix, it could produce a coordinated rotational sliding of one rung on the next, which is consistent with

Box 1 | **A family of DLPs**

Dynamins are the founding members of a family of GTPases known as the dynamin-like proteins (DLPs), the members of which share basic features of domain organization and roles in membrane remodelling (FIG. 1). Within this family, some proteins act similarly to dynamin in mediating membrane fission. For example, dynamin-related protein 1 (DRP1) is important for the fission of mitochondria and peroxisomes[159–162]. Others are more distant members and mediate homotypic membrane fusion[159–162]. These include mitofusin, which controls the fusion of mitochondrial outer membranes, optic atrophy 1 homologue (OPA1), which is important for inner mitochondrial membrane fusion, and atlastin, which mediates the fusion of endoplasmic reticulum membranes[159–162,167,168]. Also included in this family, on the basis of their structure and assembly properties, are the antiviral, interferon-inducible myxovirus resistance proteins[9,169] and guanylate-binding proteins[170], whose precise functions remain unknown. Plant DLPs control membrane traffic during cell plate formation in cytokinesis[171] and mediate chloroplast division[172,173]. A bacterial DLP implicated in membrane remodelling has also been identified and structurally characterized[40,172]. Additionally, although differing from the DLPs in that they are ATPases rather than GTPases, the EPS15 homology domain-containing (EHD) proteins share structural similarities and are also implicated in membrane remodelling[174].

Mutations in DLPs other than dynamin, and more specifically in DLPs that affect mitochondrial and endoplasmic reticulum dynamics, have been identified as causes of inherited diseases. These include mutations in OPA1 (inducing optic atrophy type 1)[175], mitofusin 2 (leading to Charcot–Marie–Tooth disease type 2A)[176], atlastin (resulting in hereditary spastic paraplegia)[177] and DRP1 (generating lethal perinatal defects in mitochondria and peroxisome fission)[178].

the reported twisting of dynamin-coated tubules during GTP hydrolysis[55]. However, in principle, such movement could also simply constrict the tubule by producing a structural change in the ring without any rotation[32].

Surprisingly, however, if a dynamin helix has been assembled in the absence of hydrolysable GTP, subsequent GTP loading and hydrolysis does not necessarily trigger fission, possibly because a long dynamin coat can function as a stabilizing scaffold[55,61,62]. In cell-free systems, efficient fission of lipid tubules occurs optimally under conditions in which dynamin polymerization occurs in the presence of GTP, allowing rapid coupling between dynamin assembly, GTP hydrolysis and polymer disassembly[56,61,63]. Under these conditions, fission is proposed to result from the membrane destabilization that is predicted to occur when the constricted ring disassembles (FIG. 2d). A long, continuous dynamin helix may be less efficient in promoting fission because it lacks a focal point for constriction or has less efficient subunit dissociation. In fact, taking into consideration bulk cytoplasmic GTP concentrations of ~2 mM versus the binding affinity of dynamin for GTP (K_d = ~1 μM)[65], dynamin oligomerizing into helices should be in a predominantly GTP-bound state and be poised to hydrolyse GTP and disassemble quickly when the G domains of one turn can dimerize with the G domains of the next turn. Under some physiological conditions, local regulation of GTP levels probably contributes to proper dynamin function, as mutations in the nucleoside diphosphate kinase gene, which helps maintain such levels, make synapses more sensitive to dynamin mutations[66].

Although there have been important advances in our understanding of the structure of dynamin, we have much to learn about how conformational changes produced by the GTP hydrolysis cycle of dynamin relate to dynamin-mediated membrane fission. For example, the relative contributions of G domain dimerization versus subsequent GTP hydrolysis in the constriction and fission of membranes remain undefined. Furthermore, GTP hydrolysis may not only produce a power stroke but also contribute to membrane destabilization by inducing helix disassembly.

Additional factors cooperating in fission. Although dynamin alone can constrict and cut lipid tubules, the constriction of the dynamin ring *in vivo* is likely to be assisted or regulated by other factors to achieve fission (FIG. 3). These include: membrane tension generated by actin, either via its polymerization or via myosin motors[55,61,67,68]; a heterogeneous distribution of lipids on the two sides of the constriction, contributing to line tension[69]; enzymatic degradation of PtdIns(4,5)P_2, leading to catastrophic dissociation of dynamin and other endocytic factors after GTP hydrolysis[70]; and the partial destabilization of the bilayer by neighbouring lipid-binding proteins, such as proteins containing Bin–amphiphysin–Rvs (BAR) domains[71]. The potential role of BAR domain-containing proteins has acquired interest with the realization that the GTPase activity of dynamin involves interactions between dynamin rungs because the intercalation of BAR domains between rungs could hinder such an interaction[71–73] and thus potentially inhibit dynamin's action. Cryo-EM studies aimed at elucidating the precise organizational relationship between dynamin rings and BAR domain proteins should help to determine the contribution that this makes.

A key component of clathrin-coated pits

Dynamin may contribute to multiple forms of endocytosis, but its action is best understood in the context of clathrin-mediated endocytosis[12–14,17,19,20,22,67,74] (FIG. 3). During the formation of endocytic buds (FIG. 3), a subset of scaffold proteins (such as FCH domain only 1 (FCHO1), epidermal growth factor receptor substrate 15 (EPS15) and intersectin)[67,75] and clathrin adaptors (for example, the adaptor protein 2 (AP2) complex, AP180 and its homologue clathrin assembly lymphoid myeloid leukaemia (CALM; also known as PICALM) and epsin) are first recruited to the PtdIns(4,5)P_2-rich plasma membrane, coincident with the binding of some of these proteins to endocytic sorting motifs of integral membrane proteins[1,76]. Such components cluster cargo, induce membrane curvature and also have actin-nucleating properties[1,76]. The coat subsequently grows through the assembly of the clathrin lattice, which, through positive feedback, recruits additional cargo adaptors and endocytic factors[1,76,77]. Dynamin also slowly accumulates around the growing pit[67]. Deep invagination of the bud and formation of a narrow neck, which is often assisted by a burst of actin polymerization[19,78], involves the recruitment of BAR domain-containing proteins, several of which bind dynamin as well as the PtdIns(4,5)P_2 phosphatase synaptojanin[19,72,73,79] (BOX 2; FIG. 3; see Supplementary information S1 (table)). It is at this point that dynamin rapidly accumulates at bud necks to mediate fission[67,80] (FIG. 3).

Line tension
A force that acts to minimize the length of the energetically unfavourable interface between adjacent membrane domains of different composition.

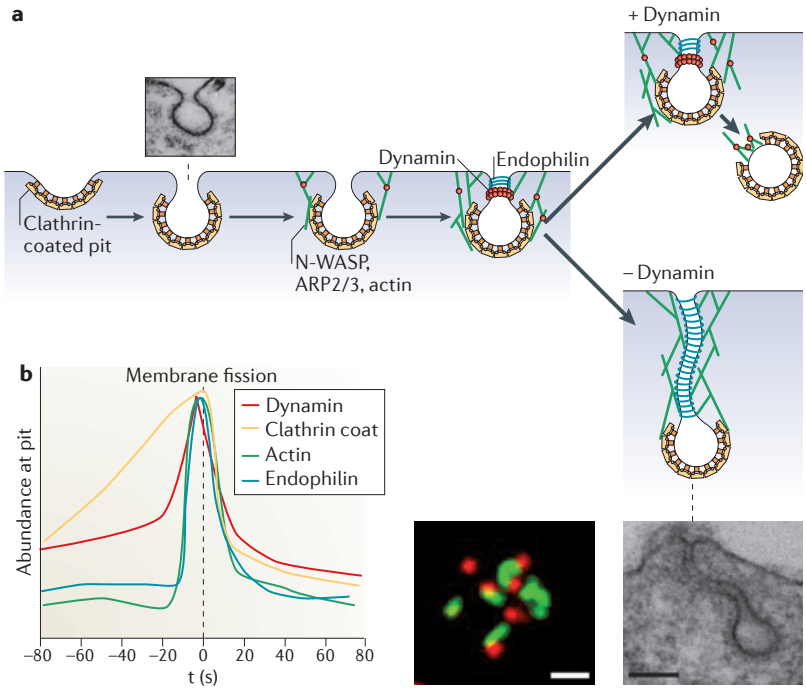

Figure 3 | **Dynamin and clathrin-mediated endocytosis. a** | The putative sequence of events in the action of clathrin adaptors, actin, Bin–amphiphysin–Rvs (BAR) domain-containing proteins and dynamin at clathrin-coated endocytic pits, as revealed by studies of cells that lack dynamin (dynamin 1 and dynamin 2 double-knockout fibroblasts)[19]. Lack of dynamin results in an arrest of the endocytic reaction at the stage of deeply invaginated pits and in the actin-dependent elongation of their BAR domain-containing protein-coated tubular necks (lower-right electron microscopy (EM) micrograph). When actin is depolymerized (for example, through latrunculin B treatment), clathrin-coated buds collapse to Ω-shaped pits with short, wide necks (upper-left EM micrograph). The fluorescence micrograph shows tubulated endocytic clathrin-coated pits in dynamin 1 and dynamin 2 double-knockout fibroblasts, as revealed by live-cell imaging of the BAR domain-containing protein endophilin 2, which was labelled with green fluorescent protein (tubular neck), and of clathrin light chain, which was labelled with red fluorescent protein. **b** | Schematic timeline showing the accumulation of endocytic proteins at endocytic pits. The zero time represents the fission reaction, as determined by the loss of accessibility of the bud lumen to the extracellular medium (based on data from REF. 67). Note that the role of actin may be non-essential under some conditions[78,100]. ARP2/3, actin-related protein 2/3; N-WASP, neural Wiskott–Aldrich syndrome protein. EM and fluorescence micrographs in part **a** are reproduced, with permission, from REF. 19 © (2009) Elsevier.

The enrichment of dynamin at clathrin-coated endocytic pits has been extensively demonstrated by fluorescence microscopy[12,51,67,80], including super-resolution methods[60] and EM[10,81]. Furthermore, live imaging of cells expressing fluorescent dynamin shows that dynamin levels at the pits are low during early stages of clathrin-coated pit maturation (FIG. 3), most likely reflecting low-affinity interactions with other endocytic factors, and rise markedly during later stages — reflecting polymerization at the bud neck — to reach a peak that coincides with membrane fission[67,80,82] (FIG. 3b). Factors that cooperatively, but redundantly, regulate dynamin assembly here include: PtdIns(4,5)P_2 and other acidic phospholipids present in the plasma membrane[83,84]; the tubular membrane template at the bud neck[53]; proteins containing dynamin-binding SH3 domains (primarily BAR domain-containing proteins[72,73], whose polymerization forms a powerful high-avidity affinity matrix[71]; see Supplementary information S1 (table)); and actin, which can directly bind dynamin[85] (FIG. 3). The coinciding accumulation of BAR proteins, actin and dynamin at late stages of endocytosis[67,80], which is consistent with feed-forward mechanisms, is particularly striking (FIG. 3b).

The sequence of events underlying clathrin-mediated endocytosis is evolutionarily conserved, as this process shows mechanistic similarity to endocytosis at actin patches in yeast[86,87]. Surprisingly, however, there are mixed reports as to whether the closest yeast homologue of dynamin, vacuolar protein sorting-associated 1 (Vps1), does[88,89] or does not[86,90] participate in actin patch endocytosis, questioning how essential dynamin is for clathrin-mediated endocytosis. It is possible that unique properties of the yeast plasma membrane, for example tension and/or the presence of a cell wall, explain this difference[91].

Dynamin in other endocytic pathways

Endocytosis can also occur without clathrin, and dynamin has been implicated in some clathrin-independent endocytic pathways (FIG. 1). For example, a role for dynamin in caveolin-dependent internalization has been suggested, and both dynamin and actin were transiently detected at caveolae, coincident with their fission from the plasma membrane[92,93]. However, the dynamics and function of dynamin at caveolae remain largely undefined. Surprisingly, the absence of dynamin is accompanied by a loss of caveolae and of their core component caveolin 1, rather than by the increase in caveolae abundance that might be expected if dynamin were required for their fission[19]. Thus, it is not yet clear whether dynamin has a key role during the endocytic fission of caveolae.

With respect to clathrin- and caveolin-independent endocytic pathways, evidence for a role of dynamin is, in many cases, primarily dependent on approaches using dominant-negative mutants (which may exert indirect effects) or pharmacological inhibition with drugs (which may have off-target or non-physiological effects; see Supplementary information S2 (box)). Thus, definitive answers as to how dynamin affects such pathways will require a combination of methods and should include loss-of-function studies. As robust bulk endocytosis still occurs in fibroblasts lacking dynamin[19] and in the presence of dominant-negative dynamin mutants[12], dynamin cannot have a universal role in endocytic vesicle fission.

Roles in intracellular budding

Vesicle budding, and thus membrane fission, occurs at multiple points throughout the secretory and endocytic pathways. Although dynamin does not affect most of these events, several experimental approaches have suggested that it might contribute to the fission of clathrin-coated vesicles that bud from the *trans*-Golgi network (TGN)[94], as well as of other vesicles that bud from either the Golgi complex or endosomes[20,95–97]. The yeast dynamin homologue Vps1 has also been implicated in retrograde traffic between endosomes and the Golgi complex[98]. However, there was no obvious accumulation of clathrin-coated pits at the TGN of fibroblasts lacking dynamin[19], suggesting that dynamin is not universally required for

Caveolae
Small flask-shaped invaginations of the plasma membrane that are involved in the endocytic uptake of various cell surface molecules and some viruses, in signalling and in the regulation of plasma membrane tension.

Box 2 | **Sensing and generating membrane curvature**

Some proteins that function at the membrane interface bind preferentially to curved membranes. This can result from: the intrinsic curvature of the membrane-binding surface of these proteins; their propensity to oligomerize into curved scaffolds; or the presence of amphipathic helices whose partial insertion into the bilayer is facilitated by the loose packing of phospholipids at sites of high positive curvature. The same proteins can also force the membrane to curve and thus induce, propagate or stabilize bilayer curvature[71,179–182]. The ability of these proteins to function primarily as curvature sensors or curvature inducers depends on various factors, such as regulated affinity for the membrane (through changes in the phosphorylation, folding state or surface charge of the membrane) and local concentration[179]. Dynamin has important curvature-sensing and -generating properties[53], owing to its polymerization into rings[11] and to the presence of a hydrophobic loop emerging from its pleckstrin homology (PH) domain[47].

BAR domains. Many proteins that bind dynamin's Pro-rich domain (PRD) via an SRC homology 3 (SH3) domain also contain Bin–amphiphysin–Rvs (BAR) domains, which are protein modules with curvature-generating and -sensing properties[71,79,183] (see Supplementary information S1 (table)). BAR domains comprise amino acid sequences that fold into coiled-coils and dimerize into elongated, curved structures that are optimally suited to bind the negatively charged cytosolic leaflet of the plasma membrane[71,182,184,185]. The presence of amphipathic helices or lipid-binding modules that flank the BAR domains and their propensity to polymerize further enhance the bilayer-moulding properties of these proteins[71]. In BAR domain-containing proteins that bind dynamin, the bilayer-binding surface is optimally suited to bind endocytic intermediates[71] because it is typically concave (with shallow curvature in FCH-BAR (F-BAR) domains, such as syndapin, formin-binding protein 17 (FBP17), transducer of CDC42-dependent actin assembly 1 (TOCA1) and CDC42-interacting protein 4 (CIP4)[71,185,186], and narrow curvature in classic BAR domains, such as endophilin, amphiphysin, sorting nexin 9 (SNX9) and SNX18 (REF. 71)). BAR domain-containing proteins are proposed to coordinate the progressive acquisition of bilayer curvature at the necks of coated pits with the recruitment of factors that drive constriction, fission and immediate post-fission events (FIG. 3). Dynamin binding to the SH3 domain of BAR or F-BAR domain-containing proteins also relieves autoinhibitory intramolecular interactions that limit the membrane-binding activity of the BAR or F-BAR domain[186,187].

clathrin-mediated budding. It is also important to consider that some defects observed at non-endocytic sites when dynamin is inhibited may arise indirectly through clathrin sequestration at the cell surface, perturbation of membrane traffic, effects on signalling pathways and/or cytoskeletal dynamics.

Dynamin and the cytoskeleton

Dynamin interacts both directly and indirectly with the cytoskeleton. The relationship between these interactions and the endocytic function of dynamin is one of the most interesting and poorly understood aspects of dynamin biology.

Intimate links with actin. A major property of dynamin, and one that is likely to be fundamentally important to its action, is its link to the actin cytoskeleton. A link between dynamin and actin at endocytic sites is not surprising and is consistent with the importance of actin for many endocytic events, including at least a subset of clathrin-mediated endocytic processes[19,67,68,99,100]. However, immunofluorescence studies and live-cell analyses show that dynamin also colocalizes prominently with actin meshworks that are nucleated by the actin regulators neural Wiskott–Aldrich syndrome protein (N-WASP)–WAVE and actin-related protein 2/3 (ARP2/3) at several other sites, including lamellipodia[101], membrane ruffles (including circular dorsal ruffles[102] and ruffles that mediate phagocytosis[103]), invadopodia[104], podosomes[105], actin comets[106,107] and the actin pedestals that can be associated with bacterial cell entry[108] (FIG. 1). At these sites, dynamin is not restricted to the membrane interface but is present in the actin meshwork itself (FIG. 1). This colocalization is consistent with the PRD-mediated binding of dynamin to numerous actin-regulating proteins, including proteins that contain CDC42-interacting or -regulating domains (summarized in Supplementary information S1 (table)). Indeed, dynamin can bundle around actin filaments assembled in the presence of its partner cortactin, which can simultaneously bind filamentous actin (F-actin)[52,102]. The stalk region of dynamin itself can also bind F-actin directly[85]. This raises the question of whether dynamin can directly influence actin dynamics and may have a role in actin function independent of endocytosis. However, as membrane remodelling is the shared property of dynamin and other DLPs, and thus is likely to be the most critical function of dynamin, links between dynamin and actin may predominantly reflect its role in membrane remodelling during endocytosis. Studies in yeast emphasize the critical role of actin in endocytosis, further supporting this possibility[86,90,109].

The interaction of dynamin with actin and its regulatory proteins may help position dynamin at endocytic sites (FIGS 1,3). For example, a role of actin in directing the localization of dynamin at endocytic sites is supported by studies of mammalian cells that lack dynamin, which have shown that F-actin acts upstream of dynamin at clathrin coated pits[19] (FIG. 3). Actin may also generate membrane tension at the neck of endocytic buds, thus synergizing with dynamin during fission. Conversely, perturbation of dynamin function in living cells (by the expression of mutant dynamin or by pharmacological inhibition) can perturb actin dynamics[85,101,106,107,110,111]. However, in cells that lack dynamin, stress fibres do not seem to be affected[19]. The most obvious change observed in these cells is the accumulation of ARP2/3-dependent actin meshworks around the necks of arrested endocytic clathrin-coated pits[19] (FIG. 3). There is also evidence of alterations to other ARP2/3-dependent actin-based structures in these cells, such as circular membrane ruffles and invadopodia and/or podosomes[104,105,111] (S.M.F., O. Destaing, R. Baron and P.D.C., unpublished observations). However, it is difficult to discriminate between direct and indirect effects in these experiments, as disrupted endocytosis may alter signalling pathways (see below).

The recent crystallographic studies of dynamin[30–33] have raised new questions about potential non-endocytic effects of dynamin on actin dynamics. The helical organization of dynamin polymers at the bud neck appears to be optimally suited to allow an interaction in *trans* between dynamin G domains to promote GTP hydrolysis (FIG. 2b). However, purified dynamin can also polymerize into helices around actin bundles and microtubules. It will be important to determine whether such a transient helical arrangement occurs physiologically at non-endocytic sites; these structures would be expected to produce detectable bursts of fluorescence in cells expressing green fluorescent protein-labelled dynamin but have not yet been observed.

Lamellipodia
Broad, thin plasma membrane protrusions that are driven by actin polymerization and are critical for cell motility and for some forms of bulk endocytosis.

Podosomes
Focal sites of dynamic actin polymerization at the plasma membrane that are found in motile cells at sites of cell–matrix interaction, where they promote the local degradation of the matrix. These structures are critical for supporting cell migration through the extracellular matrix and for bone resorption by osteoclasts. Similar to invadopodia, which are found in invasive cells.

Possible ties to microtubule dynamics? Although dynamin interacts with microtubules *in vitro*[6], dynamin only shows strong colocalization with microtubules in cells when its PH domain is mutated[112]. Moreover, disruption of dynamin function or lack of dynamin both result in a robust increase in the level of acetylated tubulin[19,112], a modification associated with stable microtubules. This effect may be related either directly to the ability of dynamin to bind microtubules or to altered microtubule dynamics that result from alterations in the structure and functional state of the cell cortex (for example, the great abundance of endocytic clathrin coated pits and of a robust accumulation of actin around them), where the stability of the plus end of microtubules is regulated.

Control of cytokinesis. Dynamin 2 concentrates at sites of abscission and was implicated in the completion of cytokinesis[113–115]. Given the role of microtubules as well as membrane traffic during abscission[116–118], and the connections that dynamin has to each of these pathways, the precise role of dynamin in cytokinesis remains unclear. Interestingly, dynamin 2 is phosphorylated during mitosis by cyclin-dependent kinase 1 (CDK1), and its dephosphorylation by calcineurin at these sites is required during cytokinesis[114]. This phosphorylation cycle of dynamin 2 is reminiscent of the phosphorylation cycle of dynamin 1 in nerve terminals, which is mediated by CDK5 and calcineurin[119] (FIG. 4). The broad use of this signalling pathway may be indicative of its fundamental importance. Indeed, this extends to CDK1 phosphorylation of dynamin-related protein 1 (DRP1), which promotes mitochondrial fission during mitosis[120].

Endocytosis and signalling

As clathrin-mediated endocytosis is the major pathway of receptor internalization, dynamin regulates the abundance on the plasma membrane, and thus the signalling output, of diverse receptors, including growth factor receptors, G protein-coupled receptors (GPCRs), ionotropic neurotransmitter receptors, ion channels, cell adhesion proteins and signalling proteins, such as Notch[121–124]. This was first emphasized by the major neurogenic developmental effects of the *D. melanogaster* shibire mutation[125], which arise from the inhibition of Notch signalling (a phenotype that is also observed when dynamin is lost[124]). In addition to these indirect effects of dynamin on signalling, dynamin also interacts directly with a large collection of signalling proteins (see Supplementary information S1 (table)). These interactions may help coordinate signalling from receptors internalized by clathrin-coated pits with the dynamics of the pits and resulting vesicles, and they probably reflect an inseparable partnership between endocytosis and signalling. The broader effects of endocytosis on signalling have been extensively studied and reviewed elsewhere[3,121,126].

A full and systematic assessment of how dynamin affects signalling has yet to be performed. However, dynamin loss significantly increases phosphorylation and activation of the Tyr kinase activated CDC42 kinase (ACK), which interacts with clathrin and other factors implicated in clathrin-mediated endocytosis[127]. These findings suggest that dynamin may limit ACK signalling by promoting clathrin-coated pit turnover.

Unique roles for dynamin isoforms

Gene-knockout studies in mice[17,19,22] and the cells derived from them have provided numerous insights into dynamin function and the specific roles of the three dynamin isoforms. Dynamin 2-knockout mice die early in embryonic development, which is consistent with the ubiquitous expression and housekeeping functions of this isoform[19]. Conditional dynamin 2-knockout cells showed transient defects in clathrin-mediated endocytosis. However, the severity of this phenotype was reduced by dynamin 1 upregulation, demonstrating that these two isoforms have at least some overlapping functions[19,20].

A double conditional knockout of dynamin 1 and dynamin 2 in mice[19], and an analysis of fibroblasts derived from these mice, confirmed that dynamin has an essential role in clathrin-mediated endocytosis but also yielded unexpected results. Surprisingly, dynamin 1 and dynamin 2 double-knockout fibroblasts survived for several weeks in culture without proliferation[19], which precluded analysis of the proposed role for dynamin in cytokinesis[128]. Most strikingly, dynamin 1 and dynamin 2 double-knockout cells accumulated endocytic clathrin-coated pits connected to the plasma membrane by long tubular necks[19] (FIG. 3), similarly to the phenotype produced by some dynamin mutants[81] and to that observed in cells recovering from a temperature-induced block in endocytosis[129]. These tubular necks, which were highly dynamic, had an outer diameter of approximately 36 nm (REF. 19), which is consistent with that of tubules coated by BAR domain-containing proteins[71]. Accordingly, fluorescent imaging analysis shows that various BAR domain-containing proteins, such as endophilin, sorting nexin 9 (SNX9), amphiphysin and tuba are present in these tubules[19,127], as are N-WASP, components of the ARP2/3 complex, other actin regulatory proteins and F-actin[19] (FIG. 3a).

Thus, although dynamin may modulate early stages of clathrin-coated pit formation, it is essential only for a late step of clathrin-mediated endocytosis, when membrane fission occurs. Moreover, the key function of dynamin is to terminate the formation of a deeply invaginated pit, which is orchestrated by other factors. These studies have also helped elucidate the sequence of events that occur upstream of dynamin, by allowing otherwise transient intermediates to accumulate (FIG. 3a). They showed that the actin cytoskeleton has a primary role in elongating the tubular necks of the coated pits and that the recruitment of BAR domain-containing proteins on tubules, which is mediated to some extent by their curvature-sensing properties, may help shape, stabilize and elongate the tubules in tight coordination with actin.

Dynamin and the nervous system

Endocytosis has a housekeeping role in neurons, as in other cells. However, endocytosis, and clathrin-mediated endocytosis in particular, also has a specialized role in nerve terminals, mediating the local recycling of synaptic vesicle membranes after exocytosis[130] (FIG. 4a). Consistent

Actin comets
'Tails' of filamentous actin nucleated by endosomes or intracellular pathogens that propel the endosome or pathogen through the cytoplasm through the force produced by actin polymerization.

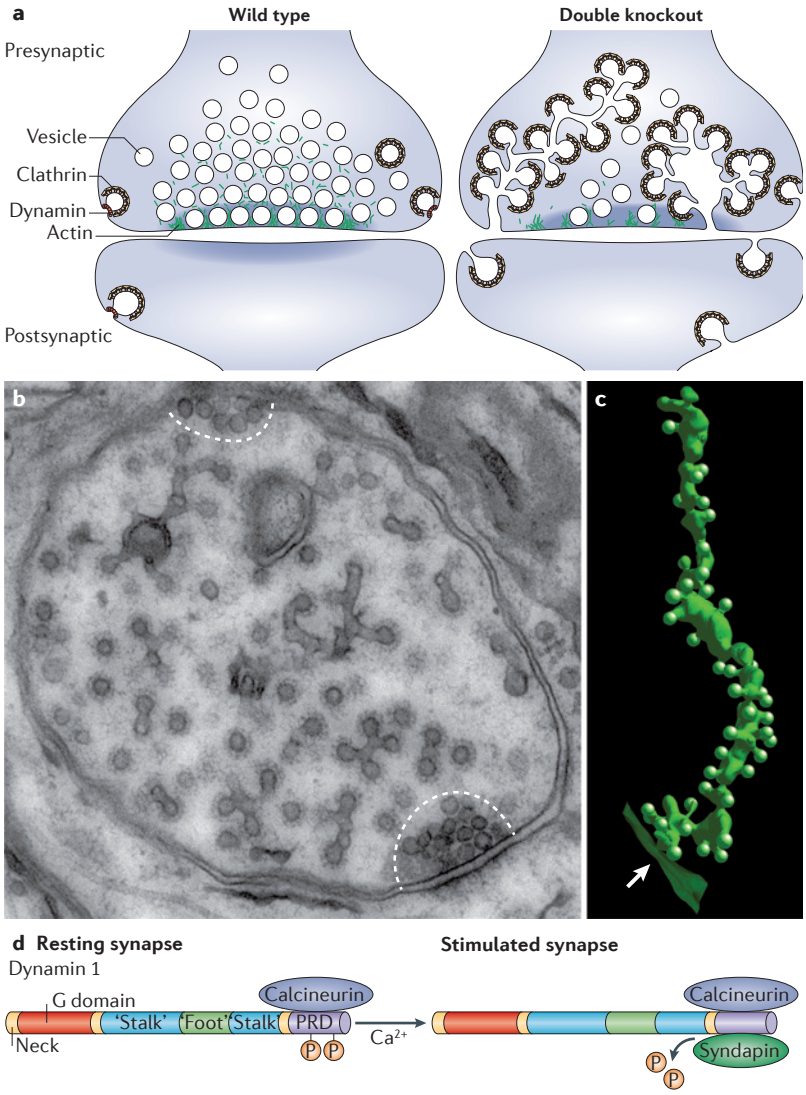

Figure 4 | **Dynamin participates in synaptic vesicle recycling at neuronal synapses. a** | From electron microscopy analysis, wild-type synapses (left) are characterized by an abundance of synaptic vesicles and very few clathrin-coated pits or clathrin-coated vesicles, which is consistent with the transient nature of clathrin-coated structures even during intense synaptic activity. At synapses that lack the great majority of their normal dynamin content (for example, from dynamin 1-knockout neurons or, even more strikingly, from dynamin 1 and dynamin 3 double-knockout neurons; right) there is a depletion of synaptic vesicles and a striking accumulation of uniformly sized clathrin-coated pits, which bud from long invaginations of the plasma membrane[17,22,145]. A small increase in the abundance of clathrin-coated pits is also observed postsynaptically, where pits are more heterogeneous in size. **b** | Representative electron micrograph of a presynaptic terminal from a dynamin 1-knockout neuron. Presynaptic vesicle clusters at two synaptic junctions are very small (regions defined by dashed lines), whereas clathrin-coated vesicular structures are highly abundant and occupy the bulk of the terminal. As shown by electron tomography (see part **c**), all such structures are coated pits, although in this thin section (~60 nm) their tubular 'stalks' can only be seen in few cases, and their connection with the plasma membrane cannot be appreciated[17,145]. **c** | Partial electron tomography reconstruction from dynamin 1 and dynamin 3 double-knockout nerve terminals, showing a long tubular plasma membrane invagination studded with numerous individual clathrin-coated pits. The arrow indicates the opening of this tubular structure at the peripheral presynaptic plasma membrane. **d** | In resting synapses, the Pro-rich domain (PRD) of dynamin 1 is phosphorylated, preventing association with syndapin. Upon stimulation and increased cytosolic calcium concentration, the protein phosphatase calcineurin, which directly binds a dynamin 1 splice variant (xb), dephosphorylates dynamin 1, allowing its interaction with syndapin. This regulated interaction is thought to promote synaptic vesicle endocytosis at times of elevated neuronal activity.

with this, disruption of synaptic transmission is the most dramatic and rapid effect produced by the temperature-sensitive inactivation of dynamin in *D. melanogaster*[5,7,8]. Furthermore, perturbing dynamin impairs synaptic vesicle recycling, and thus neuronal communication, in several experimental models[5,17,22,23,131–136].

The fact that all three dynamins are expressed in neurons, with both dynamin 1 and dynamin 3 being expressed primarily in the nervous system, raises questions about the specific roles that each of the three dynamins has here[17,21]. On the basis of knockout studies, only dynamin 2 is essential for the development of the nervous system[19]. Its conditional knockout using the Cre–*loxP* system in early neuronal progenitors[137], prior to the onset of expression of dynamin 1 and dynamin 3, drastically impairs brain development (S.M.F. and P.D.C., unpublished observations). However, conditional deletion of dynamin 2 in neurons at an early postnatal stage[138] does not affect mouse viability or cause any discernible neurological phenotype (S.M.F. and P.D.C., unpublished observations). This indicates that, when dynamin 1 and/or dynamin 3 are expressed, they can replace the function of dynamin 2.

Dynamin 1 and nervous system function. Dynamin 1 is present at high concentrations in the nervous system, is concentrated at synapses and shows markedly increased levels during synaptogenesis[17,18]. Additionally, dynamin 1 and several other endocytic proteins, collectively called 'dephosphins' (REFS 139–141), are constitutively phosphorylated in resting synapses. This dynamin phosphorylation depends, at least in part, on CDK5 (REF. 119), and, upon nerve stimulation, dynamin is rapidly dephosphorylated by the calcium- and calmodulin-dependent phosphatase calcineurin[139] (FIG. 4d); this is predicted to facilitate dynamin interactions with endocytic proteins to promote endocytosis[48]. The direct interaction of a dynamin 1 splice variant (the 'b' C-terminal variant) with calcineurin, through a PXIXIT-like calcineurin-binding motif (PRITIS), further emphasizes the potential physiological importance of this calcium-dependent dephosphorylation reaction for synaptic function[24,25] (S. Giovedi, S.M.F. and P.D.C., unpublished observations). On the basis of these considerations, dynamin 1 was expected to be essential for synaptic vesicle endocytosis.

Surprisingly, dynamin 1 is dispensable for basic functions of the nervous system[17]. However, dynamin 1-knockout mice rapidly develop a severe neurological phenotype, fail to thrive and die within two weeks of birth[17]. Thus, dynamin 1 is not unique among the three dynamins in its ability to support synaptic vesicle recycling. However, the massive increase in dynamin 1 expression during the first postnatal weeks is critical to sustain the increase in synaptic activity that occurs as the nervous system matures. Consistent with this, direct measurements of endocytic rates at the calyx of Held synapse in the mouse brain stem show that the endocytic rate in dynamin 1-knockout mice is normal after small stimuli but rapidly saturates and fails to scale with exocytosis as stimulus strength increases[136].

Dynamin 3 at the synapse. Dynamin 3 was proposed to have a specific postsynaptic role, forming specialized endocytic sites that locally recycle AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors in dendritic spines[142]. Consistent with this, dynamin 3 binds the ENA–VASP-homology 1 (EVH1) domain of the postsynaptic protein Homer through a PXXF motif that is not present in dynamin 1 and dynamin 2, and this property appeared consistent with the reported strong localization of dynamin 3 to dendritic spines[27]. However, this dendritic enrichment of dynamin 3 was not confirmed by another study, which instead emphasized an overlapping role with dynamin 1 presynaptically[22]. Thus, it is unclear whether any one dynamin isoform shows preferential enrichment or function within the postsynaptic compartment, although strong evidence indicates that the dynamins play an important part in the endocytosis of neurotransmitter receptors[143,144].

Dynamin 3-knockout mice do not show any obvious pathological phenotype[22]. By contrast, dynamin 1 and dynamin 3 double-knockout mice have a more severe phenotype than dynamin 1 single-knockout mice[22]. They develop normally *in utero* but, although they maintain a limited degree of synaptic transmission, they die within several hours after birth. This result supports a partially overlapping function of the two neuronally enriched dynamins in synaptic transmission.

Synaptic transmission in dynamin 1 and dynamin 3 double-knockout mice. Electrophysiological recordings from cultured dynamin 1 single-knockout neurons and dynamin 1 and dynamin 3 double-knockout neurons confirmed that synaptic transmission can still occur in the absence of these neuronal dynamins, although the ability to release neurotransmitter, and to sustain release upon sustained stimulation, is strongly reduced[17,22]. Likewise, these mutants show a delay in compensatory endocytosis in response to a stimulus[17,22]. The main ultrastructural defects observed are a reduced number of synaptic vesicles and a massive accumulation of endocytic clathrin-coated pits located along deep invaginations of the plasma membrane[17,22,145] (FIG. 4a,b). Dynamin 1 has been implicated in the bulk endocytosis that helps to recover synaptic vesicle membrane in response to strong stimuli[146]. However, a form of bulk endocytosis still occurs in response to massive stimulation in both dynamin 1-knockout[136,145] and dynamin 1 and dynamin 3 double-knockout neurons (Y. Wu, S.M.F. and P.D.C., unpublished observations).

Overall, the results of these knockout studies in mice demonstrate that even the combined absence of dynamin 1 and dynamin 3 does not abolish synaptic vesicle recycling. It is most likely that dynamin 2 is sufficient to support synaptic vesicle endocytosis, albeit at a slower rate. This conclusion is supported by the ability of dynamin 2 overexpression to, at least partially, rescue synaptic vesicle recycling defects in dynamin 1-knockout neurons[17]. However, the additional occurrence of dynamin-independent synaptic vesicle recycling cannot be excluded. These findings support a model wherein the occurrence of two neuronally enriched dynamin isoforms (dynamin 1 and dynamin 3) and the extremely high levels of one of them (dynamin 1) are primarily needed not to support a specific form of endocytosis, either presynaptically or postsynaptically, but to allow clathrin-mediated endocytosis to function over a very broad range of neuronal activities. Nonetheless, unique properties of dynamin 1 and dynamin 3 (and their splice variants) may fine-tune their functions in neurons.

Dynamin links to human disease

Roles of abnormal dynamin function in genetic disease have begun to emerge. Whereas mutations in dynamin 2 show links to tissue-specific diseases, mutations in dynamin 1 specifically affect the nervous system.

Dynamin 2 and tissue-specific diseases. Multiple unique missense mutations, or short deletions, within the middle, PH and stalk domains of dynamin 2 have been identified in patients with two autosomal-dominant genetic conditions, intermediate Charcot–Marie–Tooth disease[147] and centronuclear myopathy[148]. Charcot–Marie–Tooth disease is a peripheral neuropathy that is characterized by muscular weakness of the extremities and defects in neuronal axon conduction. Centronuclear myopathy is a group of congenital diseases characterized by muscle weakness. The dominant mode in which these disorders are inherited supports the idea that the mutant dynamin gains a toxic function (see Supplementary information S2 (box)).

How perturbed dynamin 2 function results in these conditions is unclear. There is no clear correlation between the diseases and the mutation sites in the dynamin structure, making mechanistic interpretations difficult. It is interesting to note, however, that mutations in the PH domain of dynamin 2 in patients with Charcot–Marie–Tooth disease are more frequently located in the N-terminal portion of the domain, whereas mutations in patients with centronuclear myopathy are more frequently located in the C-terminal portion of the dynamin 2 PH domain[149]. Structural analysis shows that, although disease-linked mutations in the PH and stalk domains are distant within the primary sequence of dynamin, they actually cluster at an interface between these two domains and so can potentially influence dynamin oligomerization[31]. It additionally remains unknown why altering the function of dynamin 2 affects certain tissues so selectively. Mutations in amphiphysin 2, a major binding partner of dynamin in brain and muscle tissues, can also give rise

Calyx of Held
A large synapse within the mammalian auditory brainstem that is suitable (in mice and rats) for presynaptic measurement of membrane capacitance and thus for the detection of synaptic vesicle exocytosis and endocytosis on a millisecond timescale.

Dendritic spines
Small (~1 μm), spine-like, actin-rich protrusions of neuronal dendrites that function as postsynaptic sites for excitatory neurotransmission. They are closely opposed to presynaptic sites of neurotransmitter release and are enriched in neurotransmitter receptors.

Charcot–Marie–Tooth disease
A peripheral nervous system dysfunction that is characterized by slow action potential conduction owing to defects in either the myelin sheath, which insulates neuronal axons, or the axons themselves.

Centronuclear myopathy
A group of skeletal muscle diseases that result in muscle weakness and which are characterized by the abnormal central location of nuclei within muscle fibres.

to an autosomal-recessive form of centronuclear myopathy[150]. The muscle-specific isoform of amphiphysin 2 is expressed at very high levels and affects muscle function[151], raising the possibility that muscle pathology in these centronuclear myopathies involves an interaction between dynamin and amphiphysin 2.

A role for dynamin 1 in epilepsy? So far, no human disease has been mapped to the dynamin 1 or dynamin 3 genes. Nonetheless, given the prominent expression and functions of these proteins in neurons, mutations that produce neurological phenotypes are likely to occur. Supporting this possibility, a spontaneous mouse mutation — creating the fitful mutant mouse — that causes seizures and hearing impairment maps to the dynamin 1 gene[152]. This missense mutation (Ala408Tyr) occurs in an alternatively spliced region within the middle domain of dynamin 1 and may affect its oligomerization. Electrophysiological recordings from neurons of these mice demonstrated defects in GABA (γ-aminobutyric acid)-ergic neurotransmission in response to prolonged stimulation[152], a defect that is similar to that of dynamin 1-knockout mice[17,145]. Thus, subtle perturbations of dynamin 1 function that are otherwise compatible with life can enhance susceptibility to seizure, and dynamin 1 should be considered as a candidate gene in idiopathic cases of human epilepsy. It is of further interest that knockout mice for mutations in any of three dynamin-binding proteins, amphiphysin[153], syndapin (also known as PACSIN)[154] and endophilin[155], also have seizures. Additionally, mutations in human synapsin 1, another protein that has been implicated in synaptic vesicle recycling, have been identified in patients with epilepsy[156]. It is most likely that impairing synaptic vesicle recycling lowers the threshold for seizure through greater effects on inhibitory neurons that have a high rate of synaptic vesicle turnover and thus a high endocytic demand.

Another spontaneous animal model with impaired dynamin 1 function is exercise-induced collapse, a recessive condition that has been observed in dogs[157]. These otherwise healthy animals display acute and severe muscle weakness resulting in a life-threatening collapse in response to intense exercise or excitement. The underlying missense mutation, Arg256Leu, is located within the dynamin 1 G domain. The functional consequence of this mutation on GTPase activity remains uncharacterized.

Conclusions and future questions

The function of dynamin has been extensively investigated *in vitro* and *in vivo*, and this has provided powerful insights into its cellular roles. Studies of dynamin have also helped identify factors that deform membranes and/or sense membrane curvature and that participate in the control of membrane fission. Cryo-EM and crystallographic studies of dynamin and other members of the dynamin superfamily have provided important new insights into how it mediates membrane fission. However, major questions remain open.

The precise mechanism through which dynamin achieves fission remains to be elucidated. The similarities and evolutionary relationship between DLPs that mediate membrane fission (such as dynamin and DRP1) and those that mediate membrane fusion (such as atlastin, optic atrophy 1 homologue (OPA1) and mitofusin) are intriguing and unexpected because they suggest analogies between the mechanisms that mediate these seemingly opposite membrane-remodelling processes. Elucidating the basis of these similarities will be important.

Likewise, it will be interesting to determine why DLPs are needed for only a subset of membrane-fission reactions. Other GTPases have been implicated in some membrane-budding reactions that do not involve dynamin or other DLPs: for example, the ADP-ribosylation factor 1 (ARF1) GTPase affects coatomer complex I (COPI)-dependent budding from the Golgi complex and the secretion-associated RAS-related 1 (SAR1) GTPase that regulates COPII-dependent budding from the endoplasmic reticulum[158]. However, both ARF1 and SAR1 are classic regulatory GTPases that function by recruiting downstream effectors, indicating that they use a fundamentally different mechanism to that of dynamin. The two intracellular fission reactions besides endocytosis that clearly require DLPs are the fission of mitochondria[159–162] and the fission of chloroplasts (in plants). The endosymbiotic origin of these organelles implies that their outer membrane is ancestrally derived from the plasma membrane, possibly explaining why the fission of their outer membrane requires a dynamin (FIG. 1).

Other exciting areas that need to be explored include possible non-endocytic functions of dynamin, particularly those involving the cytoskeleton (and the actin cytoskeleton in particular). The possibility that dynamin could be a target for cancer therapy, because of its role in either cytokinesis[114] or cell migration and tissue invasion[163], deserves consideration. Interestingly, the invasive activity of pancreatic ductal carcinoma was associated with elevated dynamin 2 expression[163]. Are the potential non-endocytic actions of dynamin mediated by its polymerization in a manner that is analogous to that at the neck of clathrin-coated pits? Structural studies indicate that helical polymerization of dynamin around tubular templates[30–33] is optimally suited to promoting G domain dimerization and thus GTP hydrolysis. However, perhaps other structural configurations are possible.

It can be anticipated that the range of known genetic diseases resulting directly from mutations in dynamin genes will expand. Interestingly, several genes implicated in endocytosis, including *CALM* and genes that encode the dynamin-binding proteins CD2-associated protein (CD2AP) and amphiphysin 2, have been linked to Alzheimer's disease by genome-wide association studies[164,165]. Dynamin 2 also mediates the internalization and infectivity of bacterial[166] and viral pathogens, including HIV[2,26]. In addition to these possibilities, polymorphisms within dynamin genes may contribute to susceptibility to many other specific diseases, as dynamin-dependent endocytosis is implicated in multiple processes that are relevant to disease.

Twenty years after the discovery that dynamin has a critical role in endocytosis, the function and mechanisms by which this protein acts remain a most important and timely field of investigation.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
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