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The crystal structure of dynamin

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Abstract

Dynamin-related proteins (DRPs) are multi-domain GTPases that function via oligomerization and GTP-dependent conformational changes to play central roles in regulating membrane structure across phylogenetic kingdoms. How DRPs harness self-assembly and GTP-dependent conformational changes to remodel membranes is not understood. Here we present the crystal structure of an assembly-deficient mammalian endocytic DRP, dynamin 1, lacking the proline-rich domain, in its nucleotide-free state. The dynamin 1 monomer is an extended structure with the GTPase domain and bundle signalling element positioned on top of a long helical stalk with the pleckstrin homology domain flexibly attached on its opposing end. Dynamin 1 dimer and higher order dimer multimers form via interfaces located in the stalk. Analysis of these interfaces provides insight into DRP family member specificity and regulation and provides a framework for understanding the biogenesis of higher order DRP structures and the mechanism of DRP-mediated membrane scission events.

Dynamin-related proteins (DRPs) belong to a highly conserved (Supplementary Fig. 1) GTPase superfamily that catalyses diverse membrane remodelling events^{1,2}. Membrane scission DRPs include dynamin 1, which catalyses clathrin-coated vesicle scission at the plasma membrane and Drp1/Dnm1, which divide mitochondria. Despite their functional diversity, all DRPs undergo GTP cycle-dependent conformational changes to regulate self-assembly and disassembly^{3–8}. DRP architecture includes an amino-terminal GTPase domain, a bundle signalling element (BSE), a middle domain (MD) and a GTPase effector domain (GED)^{9,10}. Many DRPs also have a variable region between the MD and GED; in dynamin 1, this is a pleckstrin homology domain (PH) that binds to

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Author Contributions M.G.J.F. purified, biochemically characterized and crystallized the Dyn1 G397D PRD. M.G.J.F. collected X-ray diffraction data and M.G.J.F. and S.J. solved the structure. M.G.J.F. and J.N. designed experiments and M.G.J.F., S.J. and J.N. interpreted data and prepared the manuscript.

Author Information Atomic coordinates and structure factors for Dyn1 G397D PRD have been deposited with the Protein Data Bank under accession code 3ZVR.

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phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P₂)-containing membranes to facilitate targeting and possibly membrane remodelling via membrane insertion^{11–14}.

Assembled DRPs can form helical structures *in vitro*^{15,16}. Within these structures, GTP cycle-driven conformational changes result in membrane remodelling^{4,5,7,17}. The structural basis for DRP self-assembly and GTP cycle-dependent conformational changes are not fully understood. The mechanism of assembly has been informed by the structure of the ‘stalk’ of MxA, a distantly related DRP, in which the MD and part of the GED form an extended helical bundle that mediates self-assembly via conserved interfaces¹⁸. Several structures have also provided insight into DRP GTP cycle conformational changes (Supplementary Fig. 2)^{19–24}. Cryo-electron microscopic structures of assembled dynamin in guanosine-5'-[(β,γ)-methylene]triphosphate (GMPPCP)-bound and nucleotide-free states have provided models for the assembled oligomers and the location of the GTPase and PH domains within the helical structures^{17,25}. However, to understand the basis of DRP self-assembly and mechanism, the architecture of DRP domains within a single molecule must be elucidated. Here, we report the crystal structure of an assembly-deficient dynamin 1 in the nucleotide-free state that lacks only the unstructured carboxy-terminal proline-rich domain (PRD).

G397D, an assembly-deficient dynamin 1 mutant

The propensity of DRPs to assemble has been an obstacle to obtaining crystals suitable for diffraction experiments. We identified the mutation G436D, an invariant MD residue, in the *Saccharomyces cerevisiae* mitochondrial division DRP Dnm1, by screening for mutations that possessed the same phenotype as assembly-deficient Dnm1 G385D, specifically mutations that shifted Dnm1–green fluorescent protein (GFP) fusion from a punctate to a more diffuse localization pattern in yeast cells¹⁶. We expressed and purified (Supplementary Fig. 3a) the orthologous rat dynamin 1 mutant protein lacking the PRD (Dyn1 G397D

PRD) and examined its ability to self assemble using light scattering (Fig. 1a). Addition of GMPPCP caused an increase in scattering in Dyn1 PRD samples, but caused no change in scattering in Dyn1 G397D PRD samples. Using a combination of size-exclusion chromatography and sucrose gradient centrifugation²⁶, Dyn1 PRD and Dyn1 G397D

PRD were estimated to be dimeric (Supplementary Fig. 3b and Supplementary Table 1) under non-assembly conditions, similar to other assembly-deficient DRP mutants^{16,27}. Under assembly conditions, we observed an increase in the sedimentation coefficient of Dyn1 PRD, but not for Dyn1 G397D PRD (Supplementary Table 1). Dyn1 G397D PRD was defective for assembly-stimulated GTP hydrolysis (Fig. 1b and Supplementary Table 2) and failed to assemble into helical structures on phosphatidylinositol 4-phosphate (PtdIns-4P)-containing lipid nanotubes, in contrast to Dyn1 PRD (Fig. 1c). Dyn1 G397D

PRD was also soluble at higher concentrations than Dyn1 PRD. Together, these data indicate that the G397D mutation severely hampers dynamin self assembly and substantiates the critical role of the MD in intermolecular interactions^{16,18,27–29}. Given these characteristics, it presented an attractive target for crystallization.

Crystallization and structure of Dyn1 G397D PRD

We obtained orthorhombic crystals of Dyn1 G397D PRD that diffracted to a minimum Bragg spacing of 3.1 Å (Methods and Supplementary Table 3). The structure was solved by molecular replacement, using the nucleotide-free rat dynamin 1 GTPase domain, the human dynamin 1 PH and a portion of the human MxA stalk, as sequential search models (Methods)^{11,13,18,30}. We traced the complete model, except for some disordered loops, and assigned amino acids after refinement of the molecular replacement solution. The model was refined to R/R_{free} values of 0.21/0.27. A representative example of the B-sharpened likelihood-weighted $2mF_{\text{obs}} - DF_{\text{calc}}$ electron density map is shown in Fig. 2c.

Dyn1 G397D PRD forms an extended structure with the GTPase and PH domains separated by a stalk consisting of an anti-parallel helical bundle of the MD and a helix from the GED (Fig. 2a, b). By contrast, the distantly related bacterial dynamin-like protein (BDLP) is compactly folded in its nucleotide-free and GDP-bound states (Supplementary Fig. 2a), with its ‘paddle’, in an analogous region to the PH domain of dynamin, in close proximity to the GTPase domain^{19,20}. Thus, unlike BDLP, GTP binding in dynamin is not harnessed to form an extended structure, consistent with nucleotide-independent assembly of dynamin on liposomes^{4,8,31,32}. Linkers connecting the MD to the PH and the PH to the GED are disordered, indicating a flexibly tethered PH. Thus, any of three crystallographic symmetry-related PH domains could connect to the remainder of the structure (Fig. 2d). The probable PH partner, based on the fit with the envelope of an assembly-deficient dynamin dimer determined by small-angle X-ray scattering³³ (SAXS), is shown in Fig. 2b. The structure of the PH is similar to those previously determined^{11,13}, with expected differences concentrated in the variable loops.

The structure of the GTPase domain is similar to that of the previously determined nucleotide-free dynamin GTPase domain structure³⁰. There are minor expected changes in the poorly resolved switch 2 region, the dynamin-specific loop and in the loop connecting the N_{GTPase} to the GTPase domain. The C_{GTPase} helix is kinked at the conserved proline 294 and, together with the N_{GTPase} helix and a helix at the C terminus of the GED (C_{GED}), forms the three-helix BSE⁹. C_{GED} covers a groove of 937 Å² between the N_{GTPase} and C_{GTPase} helices. In the nucleotide-free dynamin 1 GTPase and the GDP-bound *Dictyostelium discoideum* dynamin A GTPase structures, a myosin peptide substitutes for C_{GED} , indicating the importance of this interface for GTPase domain stability^{30,34}.

A linker with elevated crystallographic temperature factors connects the C_{GTPase} to the dynamin stalk (Fig. 2a, b) and contains two prolines (319 and 322), whose equivalents in the distantly related DRP atlastin 1 connect the GTPase and MD (Supplementary Fig. 2b). Although the dynamin stalk sequence shares limited identity to MxA, its overall structure is similar and the MxA nomenclature is retained¹⁸ (Supplementary Fig. 4a). As for MxA, helix $\alpha 1$ is split by a disordered loop, L1. The remainder of $\alpha 1$ diverges from MxA and is split into two helices, termed $\alpha 1^{\text{C1}}$ and $\alpha 1^{\text{C2}}$. Helix $\alpha 1^{\text{C2}}$ connects to helix $\alpha 2$ via a short disordered loop, L2. Helices $\alpha 2$ and $\alpha 3$ run the length of the stalk and are joined by a short loop, L3. The stalk is completed by GED-derived helix $\alpha 4$ that spans the stalk and connects

to C_{GED} via a linker. Following helix $\alpha 3$ is a coil that folds across $\alpha 4$ that is strongly conserved in dynamins, Drp1s and Dnm1, but absent in MxA.

The dynamin–dimer interface

The crystal lattice contains linear filaments of dynamin assembled via three stalk interfaces, similar to MxA¹⁸, resulting in layers of interacting stalks separated by GTPase and PH domains (Fig. 3a, b). Interface 2, the largest with a buried surface of 1,339 Å², has two-fold symmetry and is formed by residues from stalk helices $\alpha 4$ and $\alpha 3$ (Fig. 3c), with an additional residue from $\alpha 1^{C1}$ (H367). Each protomer in the interface contributes seven direct hydrogen bonds and eight hydrophobic residues line the site of contact between $\alpha 3$ and $\alpha 4$ (Fig. 3c).

Interface 2 sequence conservation indicates a mechanism for dimer specificity within the DRP superfamily. Phylogenetic analysis of the hydrogen bonding partners within this region in dynamins, Drp1s and Dnm1, and Mx proteins allowed us to categorize three classes of residues: conserved in dynamins, Drp1/Dnm1, and Mx proteins (in blue), conserved only within dynamins and Drp1/Dnm1 (in yellow), and conserved only in the dynamins (in red) (Fig. 3d). Plotting these classes onto a surface representation of the dynamin monomer (Fig. 3e) indicates that dimerization specificity is controlled by a spatial combinatorial code. As predicted from this code, hetero-oligomers consisting of dynamin 1 and dynamin 2 are observed³⁵. Several residues contributing to the apparent specificity are localized in the strongly conserved coil of dynamin, Drp1 and Dnm1 that follows $\alpha 3$.

Dynamin stalk interfaces drive self assembly

Interfaces 1 and 3 are also stalk-localized and mediate higher order assembly of dynamin dimers (Fig. 3a). Interface 1 is at the tips of interacting stalks, proximal to the GTPase domain and BSE (Fig. 3f) and is formed through interactions between protomer helices $\alpha 4$ and $\alpha 1^N$. The interface is capped by a stacking interaction between opposing and flexible Y706 residues, conserved in dynamins and Drp1s. Interface 1 includes four hydrogen bonds (Fig. 3f, dashed green lines), with the remainder consisting of hydrophobic residues. The buried surface area of interface 1 is relatively small (624 Å²) and is likely to be dynamic to tolerate protomer interactions in a range of orientations.

Interface 3 is at the distal end of the stalk, where L2 loops from symmetry-related molecules are in close proximity (Supplementary Fig. 4b). The G397D mutation is within L2, which could not be traced due to poor density. The N-terminal region of L2 is also in close proximity to a symmetry-related L1, which features a highly conserved glycine, G346, that when mutated in Dnm1 (G385D) blocks assembly beyond the dimer¹⁶. We therefore predict that G397 and G346 are near one another in assembled dynamin. In addition, mutations at two dynamin arginine residues, R399 (in L2) and R361 (in $\alpha 1^{C1}$, following L1), and the corresponding interface 3 mutations in MxA also inhibit assembly and stabilize dimeric forms^{18,27}. In contrast, disruption of MxA interface 1 yields a mixture of dimers and tetramers¹⁸. Thus, our data indicate that interface 3 is critical at an early step in the dynamin assembly pathway as its disruption stabilizes dimeric dynamin and allows linear filament

formation. In contrast, interface 1 interactions probably function only to stabilize oligomerization.

From our structure, we propose that the dynamin dimer interface 2 is constitutive and relatively rigid. As compared to linear arrangement in the crystal, in helical assemblies we propose that the necessary rotational and translational shifts occur between adjacent dimers at interfaces 1 and 3 (Fig. 4a). Indeed, stalk dimers fit into the GMPPCP-bound helical electron microscopic reconstruction of dynamin²⁵ (Fig. 4b) possess a more tightly packed interface 3 and interface 1. These differences can be attributed to the disordered interface 3 in the crystal that is probably due to steric hindrance from the G397D mutation, and the dynamic nature of the hydrophobic interface 1.

Regulation of DRPs

The dynamin PH is essential for endocytosis and interacts with inositol phospholipids with low affinity^{12,36}. Centronuclear myopathy (CNM) disease mutations cluster at the C terminus of the PH α -helix (Supplementary Fig. 5a), underscoring its importance. They cause an increase in basal GTPase activity, without altering interactions with inositol phospholipids³³. In addition, SAXS analysis of an assembly-deficient dynamin indicates that the CNM mutants have a different conformation compared to wild type^{33,37}. In our lattice, three PH domains related by crystallographic symmetry lie close to interface 3 and the L1 loop (Fig. 2d), suggesting an interaction. Thus, the PH may serve to regulate access to this key multimerization interface to couple dynamin membrane interactions to dynamin assembly. Phosphoinositide binding by the PH variable loops and penetration of the membrane by variable loop 1¹⁴ could help to expose dynamin interface 3 and/or L1 and thus promote multimerization.

Alignment of the dynamin PH and corresponding sequences from Drp1s shows conservation of key residues (Supplementary Figs 1 and 5b), indicating that lipids may similarly regulate mammalian mitochondrial division. However, mammalian Drp1s lack most of the C-terminal PH α -helix, including residues mutated in CNM. The details of Drp1 interface 3 regulation may therefore be different. Consistently, Drp1 splice variants have deletions in this region, pointing to a potential regulatory role. By contrast, *Danio rerio* Drp1 and fungal Dnm1 have an insert B of unknown structure in this region. Mitochondrial division DRPs can therefore be subdivided on this basis, which correlates with the divergence of their corresponding effectors, yeast Mdv1 and mammalian Mff^{38,39}.

Discussion

Our structure provides insight into the mechanism and regulation of dynamin assembly and into how the dynamin GTPase cycle is harnessed for function. Several observations point to a key role of the dynamin BSE in the formation of the GTPase–GTPase dimer interface observed in the GTPase–C_{GED} GDP•AlF₄[–] crystal structure¹⁰. This interface is likely to form from adjacent rungs of a dynamin helix and is critical to dynamin function as it mediates assembly-stimulated GTP hydrolysis. Comparison of the GTPase–GTPase dimer with our nucleotide-free structure indicates that in addition to expected differences, the BSE

is flexible. Dynamin genetic data also support a role for the BSE in the regulation of GTPase–GTPase interface formation. Specifically, the dynamin switch 2 *shibire* ts2 mutation, G146S, which causes endocytic intermediates with ‘collared’ dynamin necks to accumulate⁴⁰, is suppressed by the *sushi* mutation A738T^{41,42}, located in the C_{GED} peptide, facing the hydrophobic groove (Fig. 5a). Insight into how dynamin undergoes conformational changes also comes from distantly related DRP structures (Supplementary Fig. 2). GTP-dependent GTPase domain dimerization is also observed for guanylate binding protein, indicating that this may be a common feature of the DRP superfamily²² (Supplementary Fig. 2c). In addition, two recent structures of the atlastin 1 cytosolic domain, thought to represent pre- and post-endoplasmic reticulum membrane fusion conformations^{23,24}, indicate that large changes occur in the position of the 3-helix bundle ‘middle domain’ relative to the GTPase domain (Supplementary Fig. 2b).

We propose that GTP binding and self-assembly promote dynamin GTPase–GTPase dimer formation via an opening of the BSE relative to the GTPase domain. In support, a modified version of our structure can be fit into the cryo-electron microscopic reconstruction of GMPPCP-bound dynamin with the BSE in a substantially more open conformation (Fig. 4b). We obtained a good fit of the GTPase–GTPase dimer domains and stalk interface 1 using constraints dictated by the helical fit of the oligomeric stalk and the strong GTPase domain-derived density (Fig. 4b). The BSE α -helices fit into the density stretching from the GTPase domain to the oligomeric stalk, indicating that a BSE flipping-out motion occurs at two hinge regions: the α -helical kink at P294 and in the loop connecting the N_{GTPase} and the GTPase domain, at P32 (Fig. 4c). Consistently, superposition of GDP•AlF₄[−] GTPase–C_{GED} fusion GTPase–GTPase dimer partners indicates a smaller yet directionally equivalent opening of the BSE in one protomer¹⁰. This proposed conformational change is feasible as there are relatively few contacts that hold the N_{GTPase} helix to the body of the GTPase domain in our structure. In addition, the BSE links the GTPase domain to the stalk and interface 1 via a short turn, making it well placed to transmit conformational alterations. As previously noted, comparison of the nucleotide-free and GMPPCP-bound electron microscopic reconstructions indicates that the GMPPCP-bound helix has a relatively smaller diameter¹⁷. In addition, the stalk density is more ‘kinked’ in the GMPPCP-bound form¹⁷. When we dock our structure, we observed that the bases of the stalks do not fit the density of the GMPPCP-bound helix. However, where the fit becomes poor there are strongly conserved prolines in α 2 and α 4 and a partially conserved proline in α 1^{C1} clustered in the stalk. We propose that these proline residues facilitate the formation of a kink at the stalk base in the GMPPCP-bound helix, which might allow interface 1 to form more fully in the assembled helix.

As predicted by our model, GTP hydrolysis would induce closure of the BSE via a transition state represented by the structure of the GTPase domain–BSE fusion in the presence of GDP•AlF₄[−], towards the conformation observed in our nucleotide-free structure (Fig. 5b). As GTP binding to dynamin is not rate limiting and GTP hydrolysis is stimulated by the formation of the GTPase–GTPase interface between adjacent rungs, it is likely that the BSE-dependent conformational change occurs in the context of short dynamin helical assemblies rather than within a helix consisting of many rungs^{8,32}. Short assemblies could also result in

an approximate temporally coordinated conformational change (Fig. 5c–e). The conformational changes we propose would cause disruption to the assembled helix and the underlying membrane via local rung shifts. Interface 1 in our structure, which buries a relatively small surface area in the assembled stalk lattice, will be especially susceptible to change by inter-rung GTPase–GTPase dimer formation and its subsequent disruption by GTP hydrolysis, and these changes will be transmitted to interface 3 and the PH. The combined effects of curvature stress imposed by a short dynamin helical assembly coupled with PH insertion into the membrane is likely to destabilize the membrane and result in membrane fission¹⁴. Given the strong similarities between dynamins and other DRP family members, the structure of dynamin and our proposed model will serve to guide studies on the mechanisms of action of DRPs in diverse cellular functions.

METHODS SUMMARY

The conserved Dyn1 PRD assembly-defective mutant G397D was identified by a cytological assay using the yeast mitochondrial fission DRP Dnm1–GFP. Dyn1 PRD and Dyn1 G397D PRD were expressed in *E. coli* and were purified as described in Methods. Light scattering, sucrose density gradient centrifugation, mass determination, continuous GTPase assays and electron microscopy were performed as described in Methods. Crystals were grown by microbatch from 3.2 µl droplets containing 52.5mM Tris/Cl pH 7.7, 175mM NaCl, 32.5mM NaNO₃, 20% v/v PEG 400, 0.97mM β-mercaptoethanol and 31.9 µM Dyn1 G397D PRD and cryo-protected with ParetoN. Reflection data were collected at 100K at Beamline 8.3.1 at the Advanced Light Source (Berkeley, California, USA) at a wavelength of 0.9488Å. Data collection and processing are described in Methods. The structure was determined by molecular replacement, using known structures of the nucleotide-free rat dynamin 1 GTPase domain (PDB accession no. 2AKA chain B), the human dynamin 1 PH domain (PDB accession no. 2DYN chain B) and a truncated form of the human MxA stalk (PDB accession no. 3LJB chain B) as sequential search models. Structure refinement is described in Methods. The stalk interface 2 dimer was fit into the previously described cryo-electron microscopic reconstruction of GMPPCP-bound dynamin by applying tilt and twist to the sequential dimers from the linear filaments observed in the crystal to match the helical parameters described for the reconstruction. The previously described GTPase domain dimer formed in the presence of GDP•AlF₄[–] was subsequently fit into the density. Connection of the GTPase domain to the stalk required a conformational rearrangement of the BSE, which was independently fit into the visible density. The fit was subjected to rigid body refinement as described in Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The G397D mutation in Dyn1 PRD blocks self-assembly

a, 90° light scattering. Dyn1 PRD (blue, 1 μ M) and Dyn1 G397D PRD (red, 1 μ M) were monitored after addition of 0.5mM GMPPCP and, in the case of Dyn1 PRD, 1mMGTP (blue arrows). Red arrow indicates opening of sample port. AU, arbitrary units. **b**, Steady-state GTP hydrolysis kinetics of Dyn1 PRD and Dyn1 G397D PRD in the absence (light blue and red) and presence (dark blue and orange) of 0.1mgml⁻¹ liposomes containing 10% PtdIns-4P, monitored by a NADH-dependent coupled assay as described⁴³. A representative trace is shown with 1mM GTP. **c**, Transmission electron microscopy of negative-stained 0.25 mgml⁻¹ 10% PtdIns-4P lipid nanotubes with Dyn1 PRD (middle) and Dyn1 G397D PRD (right) and no added protein (left) and 0.5mM GMPPCP. Scale bars, 200 nm.

Figure 2. The crystal structure of Dyn1 G397D PRD

a, Schematic of the Dyn1 domain structure. Numbers indicate domain-ending amino acid. Colour scheme used here is retained. **b**, Crystal structure of Dyn1 G397D PRD. Linkers between the N_{GTPase} and GTPase domain and the C_{GTPase} and MD are shown in grey. Loops with no density are represented with dashed lines. Stalk nomenclature is based on that of the MxA stalk structure¹⁸. VL, variable loop. **c**, An example of the refined to B-sharpened $2mF_{obs} - DF_{calc}$ map, contoured at 1σ . The region shown is part of the stalk boxed by purple dotted lines in **(b)**. **d**, Three symmetry-related PH domains in the lattice.

Figure 3. Dyn1 G397D PRD stalk interfaces mediate self-assembly

a, Schematic diagram of four monomers showing interfaces in the crystal lattice. **b**, Surface representation showing the locations of interfaces 1 and 2. **c**, Detail of interface 2. Protomers are shown in lighter and darker hues. Green dotted lines are hydrogen bonds. **d**, Conservation of interface 2 residues involved in hydrogen bonding in dynamins, Drp1/Dnm1 and Mx proteins. Blue, conserved in dynamins, Drp1/Dnm1 and Mx proteins; yellow, conserved in dynamins and Drps; red, conserved in dynamins. Alignment shows a subset of sequences used to determine the conservation. Sequences are identical to Supplementary

Fig. 1, with the addition of *Rattus norvegicus* Mx1 (P18588.1) and *Homo sapiens* Mx1 (P20591.4). Fruitfly, *Drosophila melanogaster*; human, *Homo sapiens*; rat, *Rattus norvegicus*; worm, *Caenorhabditis elegans*; yeast, *Saccharomyces cerevisiae*. **e**, Surface representation of conservation data shown in **(d)**. **f**, Interface 1. Density interpreted as a PEG400 molecule is shown in black.

Figure 4. Oligomerization of dynamin into helical structures

a, Dynamin helices derived from the linear arrangement in our crystal structure. Two stalk dimers (green and magenta) that engage in interface 1 and 3 are related by crystallographic translation. Experimentally determined helical parameters for dynamin assembled into helices in the GMPPCP-bound state²⁵ were matched by applying a small shift and tilt of one stalk dimer with respect to the other. **b**, Placement of oligomerized dynamin model into the electron microscopy density map contoured at 1.2σ. In side view: the fit of the GTPase domain as a GTPase–GTPase dimer with the BSE in open conformation to connect to interface 1 of the stalk helix (solid density is contoured at 3.6σ). **c**, Observed conformational flexibility of the BSE. Model fitted into the helical reconstruction is shown as black superimposed ribbon on the crystal structure of the GTPase–C_{GED} fusion dimer (PDB accession no. 2×2E)¹⁰.

Figure 5. Model for dynamin GTP cycle conformational changes

a, Mapping of dynamin *shibire* and *sushi* mutations. **b**, Nucleotide-dependent dynamin conformations. The GTPase core domains (red) are in the same orientation. Left, GTP-bound state with open BSE conformation of dynamin as fitted into the GMPPCP-bound electron microscopic reconstruction shown in Fig. 4. Right, transition state of dynamin obtained by superposition of the BSE residues 291–312 and 727–743 of our structure on the corresponding residues of the GDP·AlF₄⁻-bound GTPase–CGED fusion dimer (PDB accession no. 2×2E)¹⁰. Transition from open to closed BSE conformation results in

movement of stalk domains. **c**, Model for Dyn1 PRD GTP-bound helix. The BSE is opened to allow GTPase–GTPase dimer formation. **d**, GTP hydrolysis closes the BSE and adopts the conformation of the $\text{GDP}\cdot\text{AlF}_4^-$ -bound transition state. This results in a substantial global constriction of the helical oligomeric assembly causing membrane deformation and scission. **e**, Schematic of how the proposed GTP hydrolysis triggered BSE conformational change is transmitted to oligomerized stalk domains.