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A Dynamin Mutant Defines a Superconstricted Prefission State

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SUMMARY

Dynamin is a 100 kDa GTPase that organizes into helical assemblies at the base of nascent clathrin-coated vesicles. Formation of these oligomers stimulates the intrinsic GTPase activity of dynamin, which is necessary for efficient membrane fission during endocytosis. Recent evidence suggests that the transition state of dynamin's GTP hydrolysis reaction serves as a key determinant of productive fission. Here, we present the structure of a transition-state-defective dynamin mutant K44A trapped in a prefission state at 12.5 Å resolution. This structure constricts to 3.7 nm, reaching the theoretical limit required for spontaneous membrane fission. Computational docking indicates that the ground-state conformation of the dynamin polymer is sufficient to achieve this superconstricted prefission state and reveals how a two-start helical symmetry promotes the most efficient packing of dynamin tetramers around the membrane neck. These data suggest a model for the assembly and regulation of the minimal dynamin fission machine.

INTRODUCTION

Dynamin is a mechanochemical GTPase that assembles around the necks of invaginated clathrin-coated pits to catalyze membrane fission during the final stages of clathrin-mediated endocytosis (CME) (Ferguson and De Camilli, 2012; Schmid and Frolov, 2011; van der Bliek and Payne, 2010). Dynamin consists of a catalytic G domain connected to a membrane-binding pleckstrin homology (PH) domain through a helical stalk (middle domain and GTPase effector domain [GED]) (Figures 1A and 1B) (Faelber et al., 2011; Ford et al., 2011). At the C terminus, a proline- and arginine-rich domain (PRD) is present through which dynamin partners interact. In this arrangement, the GED's C terminus (C_{GED}) associates with the N and C termini of the G domain (N_{GTPase} and C_{GTPase}, respectively) to form the bundle signaling element (BSE) (Chappie et al., 2009) (Figures 1A and 1B, cyan). Structural analysis of a minimal G domain-GED fusion protein (GG) revealed that G domain dimerization optimally positions dynamin's catalytic machinery, leading to enhanced catalytic turnover (Chappie et al., 2010). In addition, the BSE undergoes a dramatic hydrolysis-dependent conformational change that may function as a dynamin powerstroke (Chappie et al., 2011). G domain dimerization is an intermolecular interaction and only occurs between tetramers in adjacent rungs of the helical assembly (Chappie et al., 2011; Faelber et al., 2011; Ford et al., 2011). Thus, the architecture of the dynamin polymer ensures that assembly and stimulated turnover are tightly coupled.

It remains unclear how the GTP hydrolysis cycle of dynamin translates to structural changes to promote membrane fission. Dynamin generates high membrane curvature and imposes localized strain on the inner monolayer of the membrane when assembled (Bashkirov et al., 2008; Roux et al., 2010; Schmid and Frolov, 2011). This asymmetric distribution of membrane stress has been predicted to promote a hemifission intermediate if the inner luminal diameter of the neck approaches the bilayer thickness (~4 nm) (Kozlovsky and Kozlov, 2003). Therefore, in the absence of physical constraints, an inner lumen of 4 nm could lead to spontaneous membrane fission (Bashkirov et al., 2008; Kozlovsky and Kozlov, 2003). Previous studies have visualized intermediates along the fission pathway. Wild-type dynamin (^{WT}Dyn) in the absence of nucleotide and truncated dynamin in the presence of β-γ-methyleneguanosine 5′-triphosphate (GMPPCP) ($^{\Delta PRD}Dyn_{GMPPCP}$) form protein-lipid tubes with inner luminal diameters of 20 nm and 7 nm, respectively (Chappie et al., 2011; Sweitzer and Hinshaw, 1998). The structure of the final prefission state where the inner lumen reaches 4 nm and the conformational changes necessary to achieve it remain a mystery.

Recent findings suggest that structural rearrangements in the dynamin polymer associated with the transition state may serve as a key determinant of productive fission (Schmid and Frolov, 2011; Chappie and Dyda, 2013). To examine these structural changes and understand how they contribute to membrane fission, we solved the structure of a transition-state-defective human dynamin 1 mutant (^{K44A}Dyn). This structure displays a two-start helical symmetry and is tightly constricted with an inner luminal diameter of 3.7 nm, reaching the theoretical limit for spontaneous fission. Computational docking reveals that a ground-state conformation of the hydrolysis reaction is sufficient to achieve this final "superconstricted" state and shows how the

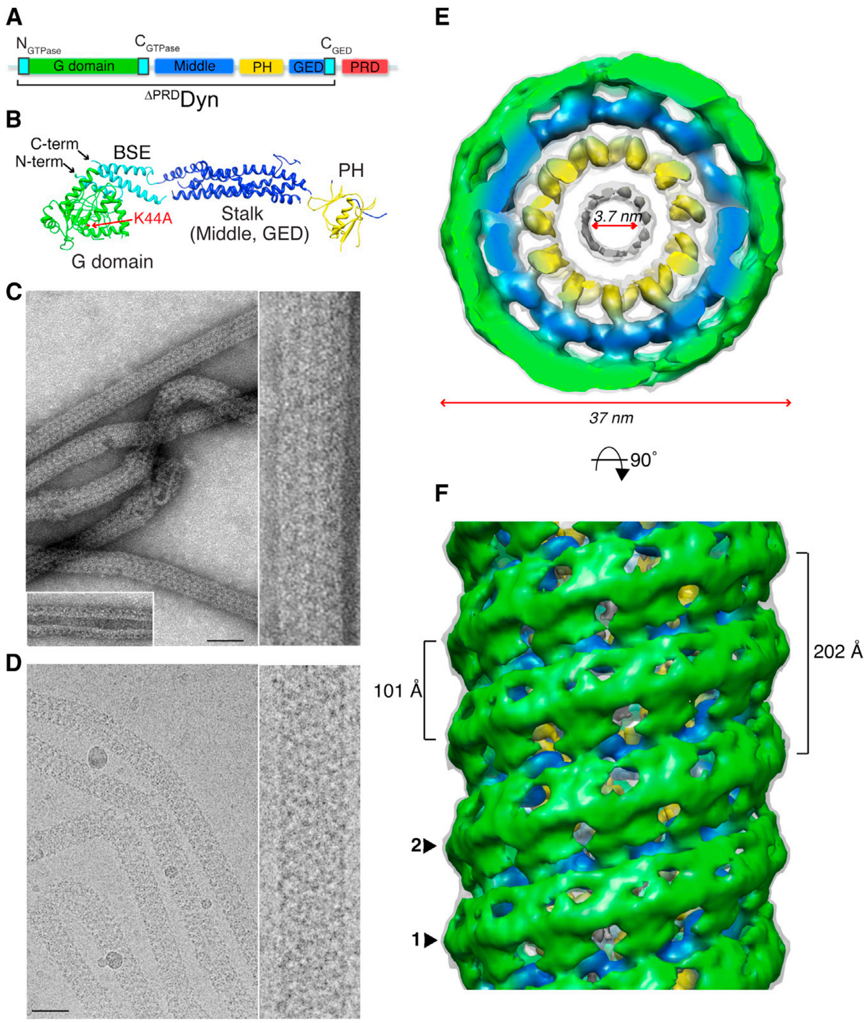

Figure 1. 3D Reconstruction of Superconstricted ^{K44A}Dyn$_{GTP}$ Lipid Tubes

(A) Domain structure of dynamin, illustrating the conserved domains: G domain (green), middle domain (blue), pleckstrin homology domain (PH, yellow), GTPase effector domain (GED, blue), and the proline- and arginine-rich domain (PRD, red). The G domain N and C termini (N_{GTPase}, C_{GTPase}) and GED C terminus (C_{GED}) assemble into the bundle-signaling element (BSE) (cyan).

(B) Crystal structure of nucleotide-free human dynamin 1 (Faelber et al., 2011) (PDB ID 3SNH) showing the organization of the protein with an alternative PH domain assignment (Chappie and Dyda, 2013). The position of the K44A single mutation in the G domain (red arrow), the assembled BSE (cyan), and the N and C termini are labeled.

(C and D) Negative-stain (C) and cryo-EM (D) images of well-ordered ^{K44A}Dyn$_{GTP}$ tubes at low (left) and high (right) magnifications. Inset in (C) shows ^{K44A}Dyn tubes in the absence of GTP. Scale bars, 50 nm.

(E) End view of the ^{K44A}Dyn$_{GTP}$ 3D density map. The map is subdivided into three radial densities colored green, blue, and yellow. The outer diameter is 37 nm, while the inner lumen is 3.7 nm.

(F) Side view of the ^{K44A}Dyn$_{GTP}$ 3D map shows how ^{K44A}Dyn$_{GTP}$ assembles as a two-start helix labeled 1 and 2 with a helical pitch of 202 Å and an axial distance between neighboring subunits of 101 Å.

two-start helical arrangement generates the most efficient packing of dynamin tetramers around the membrane neck. Together, these data support a model for dynamin-catalyzed membrane fission that requires a transition-state-dependent conformational change to proceed successfully.

RESULTS

K44A Dynamin Tubes Constrict to 3.7 nm in the Presence of GTP

^{K44A}Dyn elicits a strong dominant-negative effect in vivo that blocks CME and impairs the assembly-stimulated GTPase activity of dynamin in vitro (Damke et al., 1994). ^{K44A}Dyn retains the ability to tubulate liposomes (Figure 1C and 1D), making it an ideal candidate for examining nucleotide-dependent conformations of the assembled polymer. Like ^{WT}Dyn, ^{K44A}Dyn generates protein-lipid tubes with an inner luminal diameter of ~20 nm in the absence of nucleotide (Figure 1C, inset). Addition of GTP to ^{K44A}Dyn tubes (^{K44A}Dyn$_{GTP}$) induces a constriction far beyond what has previously been reported for other dynamin variants (Figure 1C and 1D). Sedimentation assays indicate that ^{K44A}Dyn superconstricted tubes are stable in the presence of GTP, whereas ^{WT}Dyn disassociates from the lipid rapidly (Figure S1A) (Warnock et al., 1996). ^{WT}Dyn also forms superconstricted tubes in the presence of GTP, though they are short-lived and less stable (Figures S1B). Tilt series of dynamin tubes (^{K44A}Dyn$_{GTP}$, $^{\Delta PRD}$Dyn, $^{\Delta PRD}$Dyn$_{GMPPCP}$, ^{WT}Dyn$_{GTP}$) revealed them all to be right-handed (Figure S1C; Chappie et al., 2011; Chen et al., 2004; Zhang and Hinshaw, 2001), suggesting this property is inherent to the assembled dynamin oligomer and does not change as a consequence of superconstriction.

The prolonged stability of ^{K44A}Dyn$_{GTP}$ tubes allowed us to solve its structure by cryo-electron microscopy (cryo-EM) (Figure 1D). The 3D map was calculated using the iterative helical real-space method (IHRSR) (Egelman, 2007) to yield a 12.5 Å reconstruction (Figures 1E and 1F) with 11.8 subunits per turn (Table S1). The map was generated from tube segments with outer diameters of 35–36 nm and with an even projection distribution (Figures S1D and S1E). As with previous reconstructions (Chappie et al., 2011; Chen et al., 2004; Zhang and Hinshaw, 2001), the ^{K44A}Dyn$_{GTP}$ polymer contains three radial densities (Figures 1E and 2): an inner density embedded in the outer leaflet of the lipid bilayer (yellow), a middle density that stabilizes the helical packing (blue), and an outer density that provides connectivity between the rungs of the helix (green). However, this map presents two unique features. First, ^{K44A}Dyn assembles