Structural insights into membrane fusion at the endoplasmic reticulum

Oliver Daumke[a,b,1] and Gerrit J. K. Praefcke[c,1]

[a]*Kristallographie, Max Delbrück Centrum für Molekulare Medizin, 13125 Berlin, Germany;* [b]*Charité, Institut für Medizinische Physik und Biophysik, 10117 Berlin, Germany; and* [c]*Center for Molecular Medicine Cologne (CMMC), Institute for Genetics, University of Cologne, 50674 Cologne, Germany*

The endoplasmic reticulum (ER) in eukaryotes is composed of an interconnected network of membrane tubules that undergoes permanent membrane fusion and fission. A hallmark of this membrane system is the presence of so-called three-way junctions (Fig. 1). These connections between adjacent membrane tubules are essential for the continuity of the ER luminal space. In 2009, two groups identified the ER resident atlastin GTPases as key players involved in the maintenance of normal ER morphology (1, 2). Whereas down-regulation of atlastin function in *Drosophila* motor neurons resulted in shorter and unconnected ER tubules (1), interference with atlastin function in humans and yeast resulted in long unbranched ER tubules (2). Furthermore, purified atlastin was shown to catalyze membrane fusion in a GTPase-dependent fashion (1) and interact with proteins from the reticulon and deleted in polyposis 1 (DP1) families (2), which are also involved in maintaining ER shape (3). The work by Byrnes and Sondermann (4) in PNAS now sheds light on the structural basis of how atlastins perform this function.

Three atlastin isoforms exist in humans, the neuronal atlastin-1/Spg3a and the ubiquitous atlastin-2 and -3. They are built of a 45-kDa cytosolic part composed of a large guanine nucleotide binding (G) domain and a helical middle domain (MD) followed by two predicted transmembrane (TM) segments and a short C-terminal cytosolic portion of about 60 aa in length. Sequence comparison indicates that atlastins belong to the dynamin superfamily of GTPases, which are involved in cellular membrane remodeling events including membrane tubulation, fission, and fusion (5). Members of this superfamily oligomerize around target membranes and show oligomerization-stimulated GTPase activity. The closest homologs of atlastins, the guanylate binding proteins (GBPs), have been implicated in the defense against intracellular pathogens but also in the control of cell proliferation and adhesion (6).

Byrnes and Sondermann (4) present two independent X-ray structures of the first cytosolic part of human atlastin-1, encompassing the G domain in the guanosine diphosphate (GDP)-bound state and the MD. Not surprisingly, the G domain is closely related to that of human GBP1, whose structure and catalytic mechanism have been extensively characterized (7, 8). The MD forms an elongated three helical bundle with a similar architecture as other dynamin superfamily members, such as dynamin (9), myxovirus resistance protein 1 (MxA) (10), GBP1 (7), bacterial dynamin-like protein (BDLP) (11), and the Eps15 homology domain-containing proteins (EHD) ATPases (12). Strikingly, the orientation of the MDs relative to the G domains differs dramatically in the two crystal forms of atlastin-1 (Fig. 1 *Left*). This includes an almost 90° rotation in the linker region between the G domain and the MD around a conserved proline residue that has previously been recognized as a hinge region mediating similar large-scale rearrangements in BDLP and possibly, other dynamins (13). The solved atlastin-1 construct ends just before the start of the predicted transmembrane segments (Fig. 1). It should be noted, however, that the second cytosolic part of atlastins bears limited sequence similarity to the GTPase effector domain (GED) of GBP1. As in the other dynamin family members, it might, therefore, adopt a GED-like structure consisting of a long α-helix that folds back to the G domain.

Atlastin-1 dimerizes in solution only in the GTP- but not in the GDP-bound form. Interestingly, both crystal forms of atlastin-1 dimerize through the same highly conserved interface in the G

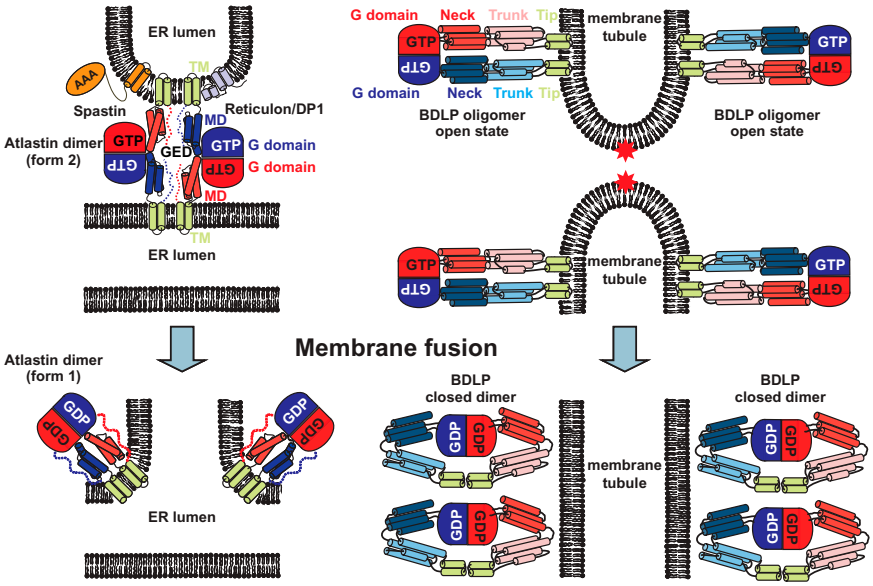

Fig. 1. Model for membrane fusion catalyzed by atlastins (*Left*) and BDLP (*Right*). Two atlastin molecules located at opposing membranes assemble through their G domains in a GTP-dependent fashion, with a dimer arrangement as seen in crystal form 2. The second cytosolic domain of atlastin is indicated as a dashed line. Reticulons, DP1 family proteins, and spastin facilitate the creation of membrane curvature. After GTP hydrolysis-dependent membrane fusion and establishment of a three-way junction, the two atlastin molecules might adopt a conformation as seen in crystal form 1. In BDLP, the MD contributes to the neck, trunk, and tip domains. BDLP molecules in the GTP-bound form oligomerize *in cis*, thus inducing membrane tubulation and extreme membrane curvature (indicated by the red stars), especially at or close to the tip of the membrane tubule (13). After GTP hydrolysis, the BDLP coat collapses allowing membrane fusion. Interestingly, the relative arrangement of G domain and MD in atlastin-1 crystal form 1 resembles the open oligomerized conformation of BDLP, whereas crystal form 2 of atlastin-1 has a similar relative domain arrangement as the closed state of BDLP.

Author contributions: O.D. and G.J.K.P. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 2216.

[1]To whom correspondence may be addressed. E-mail: oliver.daumke@mdc-berlin.de or gpraefck@uni-koeln.de.

domain, suggesting that high protein concentrations during crystallization might have favored low-affinity dimerization in the GDP-bound form. The same dimerization interface is not only used in many dynamin-related GTPases such as GBP1 (8), dynamin (9), and BDLP (11), but also in the septin and paraseptin families (summarized in ref. 14). This G domain dimer may, thus, represent a feature of a primordial membrane-associated GTPase that further evolved into the septin and dynamin branches (14). In GBP1 and dynamin, dimerization leads to rearrangements of catalytic residues inducing GTP hydrolysis. For GBP1, this includes a flip of an arginine residue in the phosphate-binding loop. This *cis* arginine finger stabilizes the transition state of the reaction and is conserved in most GBPs and atlastins (8). In the structure of GDP-bound atlastin-1, however, it is part of the dimer interface. A hallmark for a dimerization-induced GTPase mechanism is the cooperativity of the reaction. Although this has not been examined for atlastin-1, the structural parallels suggest that atlastins also use such a catalytic mechanism. In accordance with this, interference with dimerization by single point mutations led to reduced GTPase activity (4).

Atlastin-1/*Spg3* constitutes one of several genes affected in hereditary spastic paraplegia (HSP), a progressive spasticity of the lower extremities resulting from neuronal dysfunction (15). The 28 identified missense mutations in HSP patients cluster predominantly in the dimer interface, the nucleotide binding pocket, and the interface between G domain and the MD. Whereas some mutations interfere with the activation of the GTPase reaction, others compromise protein stability; still others are predicted to interfere with binding of interaction partners such as reticulons/DP1 proteins (2) or the triple ATPases associated with a variety of cellular activities (AAA) domain containing spastin, whose genes are also found mutated in HSP patients (16).

Byrnes and Sondermann present two independent X-ray structures of the first cytosolic part of human atlastin-1.

The dimerization mode of the G domains, combined with the two different crystal forms, offers exciting insights into the mechanism of atlastin-catalyzed membrane fusion. In the extended atlastin-1 dimer of crystal form 2, the two tips of the MD connecting to the transmembrane helices are located at opposing sides (Fig. 1 *Upper Left*). This dimer agrees with X-ray scattering data of atlastin-1 in solution in the presence of a non-hydrolyzable GTP analogue (4), with the proposed model of GBP1 and biochemical data showing that atlastin can cross-link two opposing membranes in vitro and mediate membrane fusion in a GTP binding and hydrolysis-dependent fashion (1). Interestingly, crystal form 1 of atlastin-1 features a cross-over of the linker and helical domain with an additional interface in the helical domain. This topology represents an attractive model for an end state of atlastin after GTP hydrolysis-mediated fusion (Fig. 1 *Left*) and resembles the postfusion SNARE complex, where two transmembrane spanning segments, initially at opposing membranes, are located next to each other (17).

This suggested mechanism of membrane fusion in atlastin is strikingly different from that proposed for BDLP (and possibly, the related mitofusins) (13). By assembling on the surface of the same membrane, BDLP polymers create high membrane curvature, a prerequisite for membrane fusion (Fig. 1 *Upper Right*). After GTPase-dependent release of the BDLP coat, membrane fusion was suggested to occur particularly at or close to the tips of the membrane tubule where curvature is maximal and the membrane is most fusogenic (Fig. 1 *Lower Right*). Although these models of membrane fusion appear mutually exclusive, there are also a number of similarities between atlastins and BDLP. They can both bind to phosphatidylserine containing liposomes, inducing membrane tubulation and high membrane curvature (13, 18). Furthermore, GDP-bound full-length BDLP also dimerizes through the G domains, with the MDs stretching away in opposing orientations, similar to crystal form 2 of atlastin-1 (Fig. 1) (11). As in atlastins, the BDLP homologue from *B. subtilis* can tether opposing membranes and mediate membrane fusion in vitro (19). Finally, crystal form 1 of atlastin-1 resembles, in the relative orientation of G domain and MD, the GTP-bound form of BDLP oligomerized around a lipid tubule (Fig. 1) (13). Additional experiments are needed to address whether atlastins and BDLP indeed use different strategies or whether there is a universal mechanism shared by all dynamin-related proteins with membrane fusion activity.

1. Orso G, et al. (2009) Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. *Nature* 460:978–983.
2. Hu J, et al. (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* 138:549–561.
3. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA (2006) A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* 124:573–586.
4. Byrnes LJ, Sondermann H (2011) Structural basis for the nucleotide-dependent dimerization of the large G protein atlastin-1/SPG3A. *Proc Natl Acad Sci USA* 108:2216–2221.
5. Praefcke GJ, McMahon HT (2004) The dynamin superfamily: Universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* 5:133–147.
6. Vestal DJ, Jeyaratnam JA (2010) The guanylate-binding proteins: Emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *J Interferon Cytokine Res* 20:991–1000.
7. Prakash B, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C (2000) Structure of human guanylate-binding protein 1 representing a unique class of GTP-binding proteins. *Nature* 403:567–571.
8. Ghosh A, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C (2006) How guanylate-binding proteins achieve assembly-stimulated processive cleavage of GTP to GMP. *Nature* 440:101–104.
9. Chappie JS, Acharya S, Leonard M, Schmid SL, Dyda F (2010) G domain dimerization controls dynamin's assembly-stimulated GTPase activity. *Nature* 465:435–440.
10. Gao S, et al. (2010) Structural basis of oligomerization in the stalk region of dynamin-like MxA. *Nature* 465:502–506.
11. Low HH, Löwe J (2006) A bacterial dynamin-like protein. *Nature* 444:766–769.
12. Daumke O, et al. (2007) Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. *Nature* 449:923–927.
13. Low HH, Sachse C, Amos LA, Löwe J (2009) Structure of a bacterial dynamin-like protein lipid tube provides a mechanism for assembly and membrane curving. *Cell* 139:1342–1352.
14. Schwefel D, et al. (2010) Structural basis of oligomerization in septin-like GTPase of immunity-associated protein 2 (GIMAP2). *Proc Natl Acad Sci USA* 107:20299–20304.
15. Zhao X, et al. (2001) Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. *Nat Genet* 29:326–331.
16. Park SH, Zhu PP, Parker RL, Blackstone C (2010) Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *J Clin Invest* 120:1097–1110.
17. Stein A, Weber G, Wahl MC, Jahn R (2009) Helical extension of the neuronal SNARE complex into the membrane. *Nature* 460:525–528.
18. Muriel MP, et al. (2009) Atlastin-1, the dynamin-like GTPase responsible for spastic paraplegia SPG3A, remodels lipid membranes and may form tubules and vesicles in the endoplasmic reticulum. *J Neurochem* 110:1607–1616.
19. Bürmann F, et al. (2011) A bacterial dynamin-like protein mediating nucleotide independent membrane fusion. *Mol Microbiol*, doi: 10.1111/j.1365-2958.2011.07523.x.