Lipid interaction of the C terminus and association of the transmembrane segments facilitate atlastin-mediated homotypic endoplasmatic reticulum fusion

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AUTHOR SUMMARY

Homotypic fusion, which involves the merging of identical membranes, is required for the remodeling of organelles, including the endoplasmic reticulum (ER) and mitochondria. These organelles contain membrane tubules that are connected into a network by homotypic fusion. The homotypic fusion of ER membranes is catalyzed by the atlastins (ATLs) (1, 2), membrane-bound GTPases of the dynamin family. The physiological importance of the ATLs is indicated by the fact that mutations in one of the isoforms are known to cause a dominantly inherited form of hereditary spastic paraplegia (HSP), a neuromuscular disorder. The ATLs contain an N-terminal cytosolic domain comprising a GTPase module and a three-helix bundle, two closely spaced transmembrane (TM) segments, and a C-terminal tail (CT) (Fig. P1). Here, we demonstrate that membrane fusion by ATL is achieved by the cooperation of a conformational change in the cytosolic domain with protein–lipid and protein–protein interactions within the membrane mediated by its CT and TM segments, respectively.

Two crystal structures of the cytosolic domain of ATL (3, 4), which likely represent pre- and postfusion conformations, suggest that ATL molecules undergo a GTP hydrolysis-induced conformational change that pulls the membranes together so that they can fuse (Fig. P1). The differences in interaction surface area in the pre- and postfusion structures indicate that the energy gain from the conformational change is not large, raising the possibility that the TMs and CT, which are not included in the crystal structures, could be important for ATL-mediated fusion.

Our present results show that the CT is required for efficient membrane fusion. The key feature of the CT is a conserved amphipathic helix that immediately follows the TMs. Deletion of the CT or point mutations in the helix greatly reduce the GTP-dependent fusion of ATL-containing vesicles. A synthetic peptide corresponding to the helix (CTH), but not to unrelated amphipathic helices, can act in trans to restore the fusion activity of tailless ATL. This reaction is strictly GTP dependent, as with wild-type ATL, and involves fusion of both leaflets of the bilayer and a concomitant size increase of the ATL-containing vesicles. Using biophysical assays, we demonstrated that the CTH promotes vesicle fusion by interacting directly with and perturbing the lipid bilayer. However, disturbance of the bilayer by the C-terminal helix does not cause significant lysis during fusion, as shown by an assay that measures the mixing of vesicle contents during fusion: No leakage of content was detected in the reaction with wild-type ATL, and only a low level was observed with tailless ATL in the presence of the CTH. The TM segments also play an important role in ATL-mediated membrane fusion. They do not serve as mere membrane anchors for the cytosolic domain, because they cannot be replaced by unrelated TMs. Further, point mutations in the TMs can affect ATL’s ability to catalyze fusion.

Using coimmunoprecipitation experiments, we showed that the

Fig. P1. A model for ATL-mediated homotypic ER fusion. The membrane-bound GT-Pase ATL is postulated to mediate the fusion of ER membranes in a process involving the following steps. (A) ATL molecules in the same membrane associate in a nucleotide-independent manner through their transmembrane segments. (B) These complexes bind GTP and interact with similarly assembled ATL molecules in another membrane. (C) GTP hydrolysis and phosphate (Pi) release triggers a large conformational change that pulls the two membranes toward each other. The amphipathic helix in the CT of ATL, shown as magenta and yellow circles, facilitates fusion between the approaching membranes by perturbing the lipid bilayers. (D) and (E) Once fusion has occurred, GDP release allows nucleotide-dependent ATL dimers to dissociate and start a new round of fusion. 3HB, three-helix bundle; G, GTPase domain.


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TMs mediate nucleotide-independent oligomerization of ATL molecules.

Although wild-type human ATL1 can replace its functional ortholog Sey1p in *Saccharomyces cerevisiae* to maintain ER morphology, fusion-defective point mutants in the CT or the TMs cannot, indicating that these domains are important for fusion in vivo. The physiological relevance of the CT is supported further by the fact that C-terminal truncation mutants of human ATL1 cause HSP.

Our results suggest a refined model for ATL-mediated membrane fusion in which the CT and TMs of ATL cooperate with the N-terminal cytosolic domain. First, several ATL molecules in a membrane associate with each other through their TM segments (Fig. P1A). Second, these complexes interact with similarly assembled ATL molecules in another membrane (Fig. P1B); the interaction of ATL molecules across the two membranes requires GTP binding. It also is conceivable that the first and second steps are coordinated rather than occurring in a strictly consecutive manner. Third, GTP hydrolysis and the release of inorganic phosphate trigger a conformational change that pulls the membranes toward each other for fusion (Fig. P1C and D). The nucleotide-independent oligomerization of ATL molecules might increase the efficiency of fusion by allowing several ATL molecules in each membrane to undergo the conformational changes synchronously. Local perturbation of the membrane bilayer by the CT (Fig. P1C; magenta and yellow circles) also could contribute to the process by lowering the energy barrier for the approach and eventual merging of the membranes. Finally, once fusion is completed and the postfusion conformation is reached, GDP is released (Fig. P1D and E), allowing the nucleotide-dependent ATL dimers to dissociate and to start a new round of fusion.

Aspects of the mechanism proposed for ATL are likely to be applicable to other fusion reactions, including ER fusion mediated by the functional orthologs of ATL in yeast and plants, Sey1p, and RHD3, and the fusion of mitochondrial outer membranes by the mitofusins/Fzo1p. As in the case of ATLs, membrane fusion mediated by SNARE proteins during intracellular vesicular transport or by viral proteins often involves lipid-interacting amphipathic helices as well as a specific function for the TMs that goes beyond a role as mere membrane anchors.