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VIEWPOINT

The atlastin paralogs: The complexity in the tails

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Atlastins are mechanochemical GTPases that catalyze homotypic fusion of ER tubules. Recent work has demonstrated that tethering and fusion by the three mammalian atlastin paralogs are differentially regulated by their variable N- and C-terminal extensions. These new findings have profound implications for atlastin-mediated homeostasis of the tubular ER network.

The ER is a morphologically diverse, dynamic, membrane-bound compartment that is essential for many fundamental cellular processes, including calcium storage and synthesis of protein and lipids. The ER consists of the nuclear envelope and the peripheral ER, which is made of an interconnected network of tubules and sheets. The tubular structure of the ER allows the network to spread throughout the cytosol and to make extensive contacts with other cellular compartments. The large surface area of the ER sheets provides a suitable location for protein translocation, trafficking, and folding.

Over the years, several proteins have been identified that are required for the formation and maintenance of ER tubules. The reticulons stabilize membrane curvature, and the atlastins, members of the dynamin superfamily of membrane remodeling proteins, are required for homotypic fusion of ER tubules. Finally, the conserved protein lunapark is involved in maintaining three-way junctions in the tubular ER network (Wang et al., 2016). Little is known about how these proteins collaborate to shape the ER.

Atlastins are conserved from yeast to humans. Their importance in maintaining ER morphology and function is underscored by the finding that human atlastins 1 and 3 (ATL1, ATL3) are frequently mutated in hereditary spastic paraplegia (HSP; ATL1) and hereditary sensory neuropathy (HSN; ATL1 and 3), both of which are length-dependent axonopathies. Of the three mammalian atlastin paralogs, ATL1 is predominantly neuronal, while ATL2 and ATL3 are more broadly expressed. All three atlastins localize to the ER, with some reports also indicating an additional cis-Golgi cisternae localization for ATL1 (Rismanchi et al., 2008). All atlastins share a common domain structure (Fig. 1, A and B), with a cytoplasmic dynamin-like GTPase (G) domain connected to a three-helix bundle (3HB) by a flexible linker, a membrane-inserted hairpin, immediately followed by an amphipathic helix that is essential for membrane fusion (Liu et al., 2012; Faust et al., 2015). More distant family members include yeast Sey1 and plant RHD3, which extend the 3HB into four interconnected tandem 3HBs (Yan et al., 2015). Early work with the *Drosophila melanogaster* atlastin ortholog, DATL, showed that its loss results in ER fragmentation (Orso et al., 2009). Conversely, overexpression of DATL results in expansion of sheet-like ER, perhaps due to excessive membrane fusion. Hence, balancing the activity of DATL is required for maintenance of ER morphology.

Crystallographic work on the soluble core of ATL1 (Bian et al., 2011; Byrnes et al., 2013; Byrnes and Sondermann, 2011) has offered tantalizing insights into the mechanisms of atlastin-mediated fusion. The structures presented in these studies captured the end points of a large conformational shift in the 3HB relative to the G domain (Fig. 1 C), which were immediately interpreted as pre- and post-ER membrane fusion states. Biochemical characterization refined the roles of GTP binding and hydrolysis in the atlastin conformational cycle and resulted in a general model for atlastin-mediated homotypic membrane fusion: In the pre-fusion state (Fig. 1 C, pre-fusion), the 3HB is docked against its G domain. In this conformation, G domain dimerization and homotypic membrane tethering is precluded. Nucleotide exchange on the G domain releases the 3HB that enables atlastin G domains in opposing membranes to dimerize, thereby tethering them. The 3HB conformational shift results in the 3HBs from opposing, tethered atlastins rotating to align in a domain-swapped parallel manner (Fig. 1 C, post-fusion), a shift reminiscent of a myosin-like power stroke that may force membranes into sufficiently close proximity to enable membrane merging. Hydrolysis of GTP then disassembles the G domain dimers, perhaps via an intermediate with 3HBs in even closer proximity (Byrnes et al., 2013), restarting the cycle. Several GTPase cycles lead to repeated rounds of fusion (Crosby and Lee, 2022).

One challenge arising from the existing body of atlastin work is to understand why mammals have three atlastin paralogs (ATL1-3), whereas insects have just one (Bryce et al., 2023). Mammalian atlastins are highly conserved in their core regions (G, 3HB, transmembrane hairpin, and amphipathic helix) and differ only in their N-terminal extensions (the aptly named

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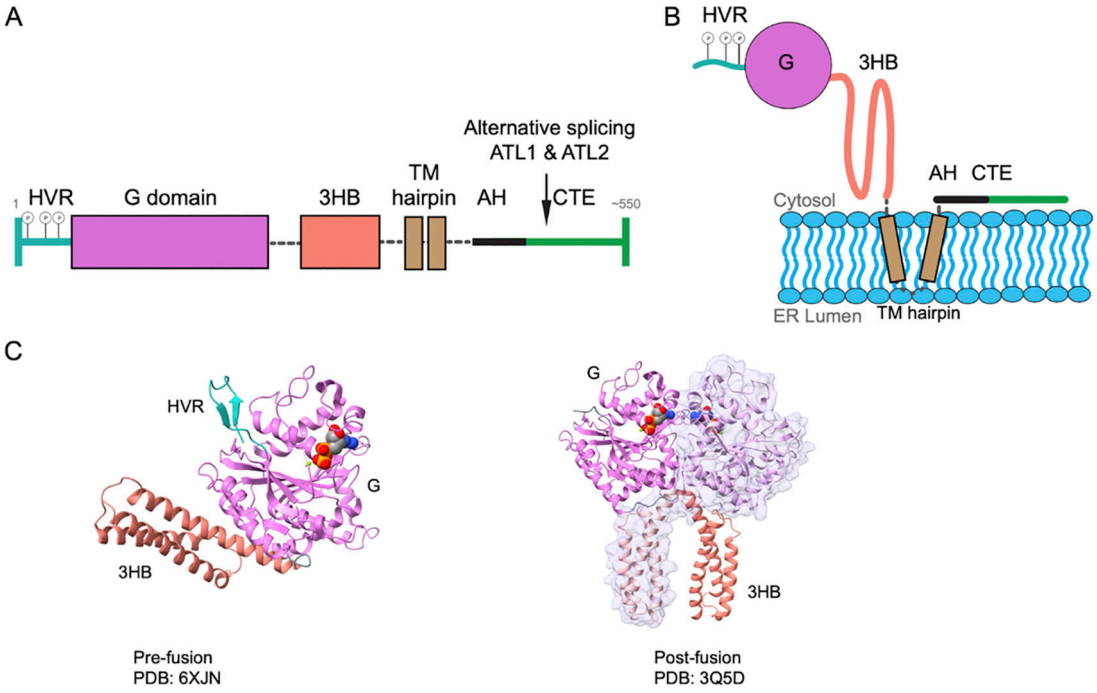

Figure 1. **Atlastins. (A)** All atlastins share a common domain structure. Mammalian paralogs ATL1, ATL2, and ATL3 have a highly conserved core consisting of the G domain, 3HB, TM hairpin, and AH. The paralogs differ in the HVR and CTE, the latter being alternatively spliced in ATL1 and 2. The HVR phosphorylation sites are shown. **(B)** Structural model of ATL. **(C)** Pre- and post-fusion structures of soluble ATL1. Formation of the post-fusion structure requires a 3HB conformational shift. The post-fusion structure is shown with its dimerization partner.

hypervariable region, HVR) and in the sequence downstream of their amphipathic helices (the C-terminal extension, CTE). The CTE is also alternatively spliced in ATL1 and 2. What are the functions of these flanking tails? How are tethering and fusion regulated and how is atlastin activity coordinated? Such questions were tough to address to date as purified mammalian ATLs were not active in early in vitro fusion assays (Wu et al., 2015). Recent studies from several groups (Kelly et al., 2021; Crosby et al., 2022; Jang et al., 2023; Bryce et al., 2023) have shed much-needed light on these questions and have collectively made considerable advances to understanding the mechanistic basis for atlastin regulation and its roles in ER dynamics and homeostasis.

An enabling finding from the Lee lab (Crosby et al., 2022) was that ATL1 expressed in, and purified from, mammalian cells was active in an in vitro fusion assay. The most prevalent ATL2 splice form (ATL2-1) was, however, inactive in this assay, unless its CTE was removed, whereupon it acquired robust activity. Furthermore, removal of the CTE from ATL1 increased its efficiency in the fusion assay to that observed with the truncated ATL2. Hence, ATL2-1 and ATL1 both have inhibitory CTEs. The less abundant ATL2 splice forms (ATL2-2 and 2-3) have CTEs that lack inhibitory activity. ATL3 also had activity in the fusion assay and removal of its slightly shorter CTE had no effect, suggesting that ATL3 may be constitutive. Mammalian cells, therefore, have a range of options for controlling ER fusion. Incidentally, early in vitro studies on atlastin fusion relied upon *D*ATL. Bryce and colleagues showed that removal of the *D*ATL CTE had no effect on activity (Bryce et al., 2023), suggesting that it is also constitutive. *D*ATL was therefore a serendipitous choice for early analysis! Indeed, further sequence analysis indicates that insect atlastins are, in general, constitutive (Bryce et al., 2023).

How might the CTEs of ATL2-1 and ATL1 mediate inhibition? As the inhibitory ATL2-1 and ATL1 CTEs vary at the sequence level (Bryce et al., 2023), mechanistic details will differ. Jang and colleagues (Jang et al., 2023) demonstrated that addition of CTE inhibitory peptide from ATL2-1 blocked the in-vitro fusion of ATL2-1 lacking its CTE. Furthermore, the ATL2-1 inhibitory CTE directly interacted with the ATL2 G domain and inhibited its GTPase activity, though the details of the binding site on the G domain remain unknown. The Lee lab used cryo-electron tomography to compare the structures formed by liposomes containing either full-length ATL2-1 or a truncation lacking the CTE. With full length ATL2-1, after addition of GTP, extensive "tight" electron-dense tethers were observed (Crosby et al., 2022). These apparently rigid tethers deformed the host liposomes into closely associated polygonal structures. In the absence of the CTE, both tight and less extensive, more "diffuse" tethers were observed, and the host liposomes were not deformed. Together, these observations suggest that CTE may participate in both inhibition and tethering. How does the ATL2-1 CTE inhibit and tether? Does the CTE engage the G domain in cis or that of a neighboring protomer? For both possibilities, flexibility at one of the linkers connecting the G domain to the 3HB or the cytosolic regions to the transmembrane domains will be necessary. High-resolution structural information will be required to fully understand the mechanisms of both inhibition and tethering. A related question is how inhibition might be lifted. Jang and colleagues (Jang et al., 2023) observed that the presence of ATL3 may

alleviate ATL2-1 inhibition in vitro. Perhaps the G domain of ATL3 acts as a receptor for the ATL2-1 CTE in this scenario. Other mechanisms may contribute in vivo. Also, it remains unclear what the functions are—if any—of non-inhibitory CTEs. More fundamentally, why do mammalian cells need to regulate some of their atlastins in this manner while insects do not?

How might a group of atlastin molecules at a site of fusion be coordinated? The HVR was partially ordered in the recent crystal structure of the soluble parts of GDP-bound ATL1 (Kelly et al., 2021). Residues 18–31 formed a β-hairpin that contacted the G domain of the neighboring protomer in the lattice, binding to a small patch. The G domains in the lattice were dimerized, as would be the case in tethered membranes. The HVR of ATL1 may therefore coordinate the catalytic and conformational cycles of a cluster of ATL1 within a membrane. Using a light scattering-based tethering assay, the authors showed that loss of the ATL1 HVR does indeed affect tethering. By contrast, no fusion deficit was noted by the Lee lab on removal of the ATL1 or two HVRs (Crosby et al., 2022). Perhaps biochemical differences may account for this: the Sondermann lab used C-terminally tagged bacterially expressed protein that was itself tethered to the liposome surface via an affinity tag, while the Lee lab used N-terminally tagged full-length protein expressed in mammalian cells.

Both the Sondermann and the Lee labs noted that serines within the ATL1 HVR were phosphorylated. Three serines in the HVR, two captured in the structure, were sites of phosphorylation and manipulation of the sites affected ER morphology and ATL1 distribution when expressed in a triple atlastin knockout line (Kelly et al., 2021). The serines were particularly responsive to p21-activated kinase, Aurora A, PKA C-α, CKII, and CaMKII, immediately suggesting signaling pathways that may regulate ATL1 activity and ER morphology. The HVR also contains the binding site for the neuronally enriched M1 isoform of spastin (Jang et al., 2023), which is required to recruit ATL1 to three-way junctions on the ER. M1-spastin also increased the rate of fusion of ATL1 when reconstituted into liposomes with ATL1. Several outstanding questions remain. Does the HVR adopt the same structural motif on M1-spastin interaction? How is the HVR released to interact with neighboring ATL1 protomers in a tethered spot? Does the HVR functionally interact with its CTE? As no ATL2 structures have been reported, it is also unknown whether the ATL2 HVR functions in tethering.

While much is now known about the conformational and regulatory cycles of atlastins, clearly the biggest remaining challenge is to understand exactly how the known spectrum of HSP and HSN mutations compromise ATL function and cause disease. Almost half of all autosomal dominant HSP cases have mutations in the transmembrane hairpin loops of ER shaping proteins (including ATL1, reticulon 2, REEP1, and spastin; Voeltz et al., 2006; Park et al., 2010). While this suggests a crucial role for shaping and maintenance of ER morphology in axonal biology, the contribution of the transmembrane regions of the atlastins and other ER shaping proteins to their regulation and fundamental roles are perhaps their least understood aspects. One avenue for exploration is to clarify the role of cholesterol in atlastin-mediated fusion. The Jun lab observed that a lipid mixture incorporating cholesterol could support in vitro fusion by *Escherichia coli*-expressed atlastins (Jang et al., 2023), and its omission impaired ATL2-2-mediated fusion. The transmembrane hairpins of the atlastins harbor cholesterol-binding motifs (Moon and Jun, 2020) and, likewise, the transmembrane hairpin of Sey1 has an ergosterol binding domain that aids in the Sey1 concentration at the site of fusion (Lee et al., 2019). However, cholesterol is not always required to support fusion as the Lee lab omitted it in their model membrane. Hence, the requirement for cholesterol in atlastin-mediated fusion may depend on the system used for purification or on posttranslational modifications that may be available with mammalian expression.

For ATL1, HSP-causing mutations are distributed evenly throughout all its domains and most of these mutations give rise to dominant negative phenotypes (O'Donnell et al., 2018), though effects on fusion in vitro may differ. For example, R239C and H258R decrease and increase fusion rates respectively using the in vitro fusion assay (Crosby et al., 2022). Precisely how these mutations affect ATL function and how ER membrane fusion is linked to HSP onset still remain to be explored. Such ambiguity does not affect all mutations, however. For example, two known ATL3 HSN-causing mutations lie at the base of the G domain and in the linker connecting it to its 3HB (Fischer et al., 2014; Kornak et al., 2014), respectively, which likely compromise the 3HB conformational change.

The pioneering structural studies on the soluble parts of ATL1 (Bian et al., 2011; Byrnes and Sondermann, 2011) established several mechanistic motifs, particularly nucleotide-mediated G domain dimerization and the 3HB conformational shift, that were profoundly influential: the mechanistic principles of membrane remodeling were subsequently seen to recur in membrane remodeling reactions catalyzed by all members of the dynamin superfamily. With new insights into tethering and regulation, it will be of enormous interest to see how these findings will influence membrane remodeling fields in general.

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