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A Class of Dynamin-like GTPases Involved in the Generation of the Tubular ER Network

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SUMMARY

The endoplasmic reticulum (ER) consists of tubules that are shaped by the reticulons and DP1/Yop1p, but how the tubules form an interconnected network is unknown. Here, we show that mammalian atlastins, which are dynamin-like, integral membrane GTPases, interact with the tubule-shaping proteins. The atlastins localize to the tubular ER and are required for proper network formation in vivo and in vitro. Depletion of the atlastins or overexpression of dominant-negative forms inhibits tubule interconnections. The Sey1p GTPase in *S. cerevisiae* is likely a functional ortholog of the atlastins; it shares the same signature motifs and membrane topology and interacts genetically and physically with the tubule-shaping proteins. Cells simultaneously lacking Sey1p and a tubule-shaping protein have ER morphology defects. These results indicate that formation of the tubular ER network depends on conserved dynamin-like GTPases. Since atlastin-1 mutations cause a common form of hereditary spastic paraplegia, we suggest ER-shaping defects as a neuropathogenic mechanism.

INTRODUCTION

The atlastins comprise a family of highly related, integral membrane GTPases (Zhao et al., 2001; Rismanchi et al., 2008; Zhu et al., 2003). They belong to the dynamin family of GTPases that associate with different intracellular membranes (for review, see Praefcke and McMahon, 2004). The prototypical member of this family, dynamin-1, is involved in vesicle budding from the plasma membrane during clathrin-mediated endocytosis; dynamin-related proteins are also required for the fusion and fission of mitochondria (Hoppins et al., 2007; Praefcke and McMahon, 2004). The functions of the atlastins are largely unknown. Mutations in *atlastin-1* (SPG3A) are the second most frequent cause of pure hereditary spastic paraplegia (HSP), and the most common cause of early-onset HSP (for review, see Salinas et al., 2008). The cardinal feature of these disorders is progressive spasticity and weakness of the lower limbs due to a length-dependent, retrograde axonopathy of the corticospinal motor neurons. Pure forms of HSP are characterized by spastic paraplegia alone, while additional neurological features are present in complicated forms. The two other genes most frequently associated with pure HSP encode for spastin, a microtubule-severing ATPase, and REEP1 (Beetz et al., 2008), a member of the DP1/Yop1p protein family involved in endoplasmic reticulum (ER) tubule formation (Voeltz et al., 2006). Because atlastin-1 (ATL1) and REEP1 are both associated with pure HSPs, this raises the possibility that the disease is caused by ER morphology defects.

The ER is a continuous membrane system that is comprised of the inner and outer nuclear membranes as well as peripheral ER sheets and a network of interconnected tubules (for review, see Shibata et al., 2006). The ER network is very dynamic, with tubules undergoing continuous fusion and fission to generate and eliminate three-way junctions (Lee and Chen, 1988; for review, see Du et al., 2004). Some insight has been gained into how the tubular ER is generated and maintained. ER tubules are frequently pulled out from a membrane reservoir by molecular motors as they move along microtubules in mammalian cells or along actin filaments in plant and yeast cells, or else tubules can be pulled out by their association with the tips of microtubules or actin filaments as they grow by polymerization (Prinz et al., 2000; Waterman-Storer and Salmon, 1998). However, the tubules are ultimately stabilized by cytoskeleton-independent mechanisms, since the alignment of membrane tubules with the cytoskeleton is not perfect (Terasaki et al., 1986), and actin depolymerization in yeast does not result in the disruption of the tubular network (Prinz et al., 2000). In addition, ER tubules can be generated in vitro through the fusion of small vesicles without involvement of the cytoskeleton (Dreier and Rapoport, 2000).

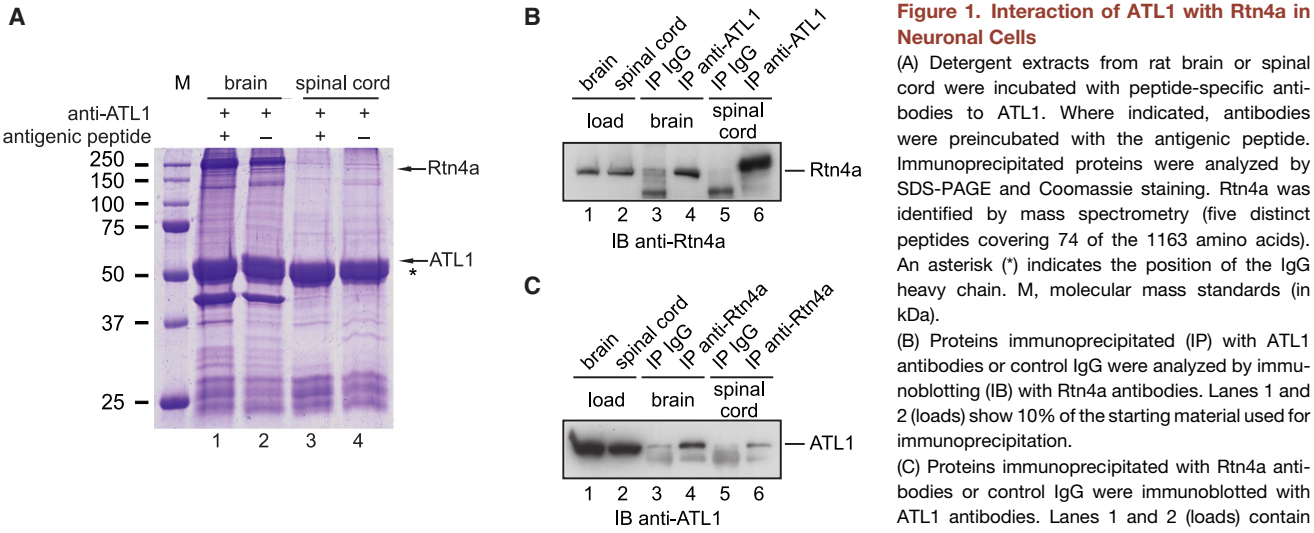

Figure 1. Interaction of ATL1 with Rtn4a in Neuronal Cells

(A) Detergent extracts from rat brain or spinal cord were incubated with peptide-specific antibodies to ATL1. Where indicated, antibodies were preincubated with the antigenic peptide. Immunoprecipitated proteins were analyzed by SDS-PAGE and Coomassie staining. Rtn4a was identified by mass spectrometry (five distinct peptides covering 74 of the 1163 amino acids). An asterisk (*) indicates the position of the IgG heavy chain. M, molecular mass standards (in kDa).

(B) Proteins immunoprecipitated (IP) with ATL1 antibodies or control IgG were analyzed by immunoblotting (IB) with Rtn4a antibodies. Lanes 1 and 2 (loads) show 10% of the starting material used for immunoprecipitation.

(C) Proteins immunoprecipitated with Rtn4a antibodies or control IgG were immunoblotted with ATL1 antibodies. Lanes 1 and 2 (loads) contain 10% of the starting material used for the immunoprecipitations.

Two families of integral membrane proteins were recently identified that appear to be responsible for shaping the tubular ER (Voeltz et al., 2006). The first is the reticulons, comprising four reticulon genes in mammals (*RTN1–4*) and two in yeast (*RTN1* and *RTN2*). The other family consists of the DP1/Yop1p proteins, which includes six mammalian *DP1/REEP* genes and the yeast ortholog *YOP1*. Members of both families are ubiquitously expressed in all eukaryotes and localize predominantly to the tubular ER (Voeltz et al., 2006). Their overexpression renders the mammalian ER network resistant to the rearrangement that follows microtubule depolymerization (Shibata et al., 2008), and overexpression of certain reticulon isoforms leads to long and unbranched tubules (Voeltz et al., 2006). Conversely, the lack of reticulons and Yop1p in yeast results in the loss of tubular ER (Voeltz et al., 2006). In addition, purified yeast Rtn1p and Yop1p deform reconstituted proteoliposomes into tubules (Hu et al., 2008). Together, these results indicate that the reticulons and DP1/Yop1p are both necessary and sufficient for ER tubule formation.

The reticulons and DP1/Yop1p do not share primary sequence homology, but both have a conserved domain of ~200 amino acid residues, comprising two long hydrophobic segments that sit in the membrane as hairpins (Voeltz et al., 2006). This domain is also responsible for oligomerization of these proteins (Shibata et al., 2008). The reticulons and DP1/Yop1p probably deform the lipid bilayer into tubules through "wedging" and "scaffolding" mechanisms (Hu et al., 2008; Shibata et al., 2008). The double-hairpin transmembrane segments may form a wedge that occupies more space in the outer leaflet of the bilayer than in the inner leaflet, thus generating high curvature that is characteristic of cross-sections of tubules. Oligomerization of the reticulons and DP1/Yop1p may generate arc-like structures that could serve as scaffolds along the tubules. Although the reticulons and DP1/Yop1p appear to be the minimal components required for ER tubule formation, in vivo there are likely additional factors that determine the shape of the ER network. These components might be involved in forming branched interconnections or in modulating ER morphology during the cell cycle or in response to external signals.

Here, we demonstrate that the atlastin GTPases interact with the ER tubule-shaping proteins, the reticulons and DP1 and provide evidence supporting a role for atlastins in the formation of an interconnected tubular network. We propose the GTPase Sey1p as a functional ortholog of the atlastins in *S. cerevisiae*, since it has similar signature motifs and membrane topology, interacts genetically and physically with the tubule-shaping proteins, and is involved in ER network formation. These results indicate that the morphology of the tubular ER network depends on a ubiquitous class of dynamin-like, membrane-bound GTPases and suggest that pure HSP may be caused by defects in ER morphology.

RESULTS

Atlastins Interact with the Reticulons and DP1

An early indication that atlastins play a role in ER network formation came from experiments seeking to identify interaction partners of the atlastins. We used detergent extracts from rat brain, where the ATL1 isoform is abundantly expressed (Zhu et al., 2003). The extracts were incubated with anti-peptide antibodies against ATL1, and immunoprecipitated proteins were analyzed by SDS-PAGE and Coomassie staining (Figure 1A, lane 2). A small number of coprecipitated proteins were absent from samples in which the antibodies were preincubated with the antigenic peptide (Figure 1A, lane 1). Mass spectrometry identified reticulon 4a (Rtn4a) as an ATL1-interacting protein. This protein was also found in similar pull-down experiments with extracts from rat spinal cord (Figure 1A, lane 4).

To confirm the interaction of ATL1 and Rtn4a, we incubated brain or spinal cord extracts with ATL1 antibodies, and we resolved precipitated proteins by SDS-PAGE. Immunoblot analysis demonstrated that Rtn4a was coprecipitated (Figure 1B, lanes 4 and 6; nonimmune IgG controls are shown in lanes 3 and 5), in contrast to two abundant control membrane proteins