Hereditary spastic paraplegias comprise a group of clinically heterogeneous syndromes characterized by lower extremity spasticity and weakness, with distal axonal degeneration in the long ascending and descending tracts of the spinal cord. The early onset hereditary spastic paraplegia SPG3A is caused by mutations in the atlastin/human guanylate-binding protein-3 gene (renamed here atlastin-1), which codes for a 64-kDa member of the dynamin/Mx/guanylate-binding protein superfamily of large GTPases. The atlastin-1 protein is localized predominantly in brain, where it is enriched in pyramidal neurons in the cerebral cortex and hippocampus. In cultured cortical neurons, atlastin-1 colocalized most prominently with markers of the Golgi apparatus, and immunogold electron microscopy revealed a predominant localization of atlastin-1 to the cis-Golgi. Yeast two-hybrid analyses and co-immunoprecipitation studies demonstrated that atlastin-1 can self-associate, and gel-exclusion chromatography and chemical cross-linking studies indicated that atlastin-1 exists as an oligomer in vivo, most likely a tetramer. Membrane fractionation and protease protection assays revealed that atlastin-1 is an integral membrane protein with two predicted transmembrane domains; both the N-terminal GTP-binding and C-terminal domains are exposed to the cytoplasm. Together, these findings indicate that the SPG3A protein atlastin-1 is a multimeric integral membrane GTPase that may be involved in Golgi membrane dynamics or vesicle trafficking.

Hereditary spastic paraplegias (HSPs) are a group of neurological disorders characterized principally by progressive spasticity and weakness of the lower limbs (1–5). They typically exhibit axonal degeneration in the distal portions of long ascending dorsal column fibers and descending corticospinal tracts of the spinal cord, which constitute the longest motor and sensory axons in the central nervous system (6). HSPs have historically been classified as “pure” or “uncomplicated” if spastic paraplegia occurs in isolation and “complicated” if other neurological abnormalities are present (7). More recently, the identification of new genetic loci for HSPs has permitted a molecular classification of HSPs (2–5). Of the 20 known loci (SPG1–20), 11 are autosomal dominant, six are autosomal recessive, and three are X-linked. Although most HSP patients experience progressive worsening of their symptoms, for some, the disorder does not appear to be progressive (3, 4). Thus, although some HSPs are clearly neurodegenerative disorders (e.g. SPG4), others such as SPG1 (due to mutations in the L1 cell adhesion molecule) and SPG3A (due to mutations in the atlastin GTPase) may be neurodevelopmental disorders (3, 4).

Eight disease genes for HSPs have now been identified; and based on the proteins involved, several mechanisms for pathogenesis have been advanced. These include aberrant cell signaling or migration, abnormalities of mitochondrial chaperones, abnormalities of myelination, and defects in intracellular trafficking and transport (2–5). Proteins mutated in HSPs that have been implicated in cellular protein or vesicle trafficking include KIF5A (SPG10), spastin (SPG4), spartin (SPG20; Troyer syndrome), and atlastin (SPG3A) (reviewed in Refs. 2–5). KIF5A is a neuronal kinesin heavy chain motor protein involved in the transport of macromolecules and membranous organelles along the axon (8). Spastin, a member of the AAA (for ATPases associated with a variety of cellular activities) protein family, associates with microtubules, and spastin overexpression causes microtubule disassembly (9). The spartin protein is similar to the VPS4, SNX15 (sorting nexin-15), and SKD1 proteins, which are involved in endosome morphology and protein trafficking of endosomal compartments (10). These latter proteins share with both spastin and spartin a region called the MIT (contained within microtubule-interacting and trafficking molecules) or ESP (present in End13/VPS4, SNX15, and PalB) domain (2, 11). Based on its similarity to members of the dynamin/Mx/guanylate-binding protein (GBP) superfamily of large GTPases (12), the SPG3A protein atlastin-1 has been implicated in intracellular trafficking, yet little is known regarding its cellular localization or function (13).

Among the HSPs, SPG3A is particularly notable for the very early onset of pure spastic paraplegia. Five missense mutations and one single base insertion with premature termination of the predicted 558-amino acid coding region of atlastin-1 have been reported (13–16). It has been speculated that these mutations may alter the structure, interactions, or GTPase activity of atlastin-1 (13–16). Of the members of the dynamin/Mx/GBP superfamily, atlastin-1 is most similar to GBPs. Like
GBPs, atlastin-1 possesses an RD loop instead of the classical GDP binding consensus triad (13). However, atlastin-1 lacks a C-terminal isoprenylation motif and the C-terminal α12/13 helix motif, two characteristic structural features of many of the GBPs (12). Here, we demonstrate that atlastin-1 is an oligomeric GTPase that, unlike GBPs, is composed of subunits that aggregate to form a protein complex with both N- and C-terminal exposed to the cytoplasmic compartment. Atlastin-1 localizes prominently to the Golgi apparatus and is enriched in cerebral cortical pyramidal cells, a subpopulation of which exhibit a “long amyopathy” in patients with SPG3A.

EXPERIMENTAL PROCEDURES

Sequence Analysis—Atlastin-1 homologs were identified by BLAST (17). Chromosomal assignments were made using the NCBI Map Viewer. Transmembrane helical domains were predicted using the SOSUI system (18). Protein sequence similarities were calculated using BESTFIT analysis (Wisconsin Package Version 10.3, Accelrys, San Diego, CA). Protein alignments were performed with ClustalW (19). Protein sequence similarities were calculated using BLAST. The full coding sequence of the atlastin-1 GTPase (GenBankTM/EBI accession number NM_015915) was amplified by PCR using primers on 10 or 14 acrylamide gels, stained with Alexa Fluor 488- and Alexa Fluor 568-conjugated secondary antibodies (1:500 dilution). The post-nuclear supernatant was sonicated at 1330 × g for 3 min, generating a P3 pellet and an S3 supernatant. The S3 supernatant was centrifuged at 133,000 × g for 1 h, generating a P3 pellet and an S3 supernatant. Protein concentrations were determined by the BCA assay (Pierce) with bovine serum albumin as the standard.

Gel Electrophoresis and Immunoblotting—Proteins were resolved by SDS-PAGE (4% acrylamide for the gel and 0.5% for the stacking gel) followed by electrotransferred to nitrocellulose (Hybond ECL, Amersham Biosciences). After blocking with 5% nonfat milk, 0.1% Tween 20, and Tris-buffered saline (pH 7.4) overnight, antibodies (0.1–1.0 μg/ml) were added for 1 h at 25 °C. After several washes with 0.1% Tween 20 and Tris-buffered saline with horseradish peroxidase-conjugated secondary antibodies (1:3000 dilution; Amersham Biosciences) were added for 30 min. Finally, after several washes with the blocking buffer, the antibody was visualized with 4-chloro-1-naphthol (Sigma) and enhanced chemiluminescence reagent (PerkinElmer Life Sciences). The post-nuclear supernatant was sonicated and then recentrifuged at 200,000 × g for 30 min, yielding a pellet (pellet 2) and a supernatant (S2). The S2 supernatant was then centrifuged at 200,000 × g for 1 h, generating a P3 pellet and an S3 supernatant. Protein concentrations were determined by the BCA assay (Pierce) with bovine serum albumin as the standard.

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to generate a final pellet and supernatant. In other experiments, soluble and membrane fractions were prepared from COS-7 cells expressing Myc-targeted atlastin-1 or various Myc-tagged atlastin-1 deletion constructs. Equal proportions of the soluble and membrane fractions were then immunoblotted with anti-Myc antibodies. Membranes from COS-7 cells expressing untagged atlastin-1 and Pr protein fractions from rat brain were subjected to phase partitioning with Triton X-114 as described by Bordier (24), and equal proportions of the aqueous and detergent phases were immunoblotted with anti-atlastin-1 antibodies (No. 5409).

**Protease Digestion and Deglycosylation Assays**—COS-7 cells expressing untagged atlastin-1 were washed twice with 10 mM Tris-HCl (pH 7.5) and then collected in 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 1.5 mM MgCl2, 0.5% Triton X-100, and 10% glycerol, and the homogenate was centrifuged at 130,000 × g for 60 min. This latter pellet was resuspended in the same buffer with or without proteinase K (EC 3.4.21.64; Sigma) at either 50 μM (30 min, 25 °C) or 200 μM (15 min, 37 °C). Reactions were terminated with 2 mM phenylmethylsulfonyl fluoride, followed immediately by lysis in SDS-PAGE sample buffer. Samples were then immunoblotted with antibodies against the C terminus (No. 4735) or N terminus (No. 5409) of atlastin-1 or with anti-calreulin antibodies. Protein deglycosylation with peptide N-glycosidase F (EC 3.5.1.52; New England Biolabs Inc., Beverly, MA) was performed as described previously (25).

**Yeast Two-hybrid Assays**—Yeast two-hybrid tests were performed using the L40 yeast strain harboring the reporter genes HIS3 and β-galactosidase under the control of upstream LexA-binding sites as described previously (20). Atlastin-1 deletion constructs were produced by PCR amplification using Pfu Turbo and cloned in-frame into pGAD10 prey and pBHA bait vectors (Clontech). All constructs were confirmed by DNA sequencing. Strength of interaction was assayed by β-galactosidase and HIS3 induction as described previously (20).

**Immunoprecipitation and Chemical Cross-linking**—COS-7 cells cotransfected with HA- and Myc-atlastin-1 or transfected with Myc-atlastin-1 alone were washed twice with PBS and then harvested in 0.5% Triton X-100 and PBS and clarified by centrifugation at 130,000 × g for 30 min. The 50 μg of protein was incubated for 1–2 h with 5 μg of rabbit polyclonal anti-HA antibodies (Amersham Biosciences). Beads were washed three times with 0.5% Triton X-100 and PBS. Bound proteins were resolved by SDS-PAGE and immunoblotted with mouse monoclonal anti-Myc antibodies. Chemical cross-linking with diithiothreitol (sucinimidyl propionate) (Pierce) was used to cross-link proteins. Protein A-Sepharose CL-4B (Amersham Biosciences) affinity resin (Stratagene). Expression of CBP-atlastin-1 in *Escherichia coli* BL21(DE3) was induced by 100 μM isopropyl-β-D-thiogalactopyranoside for 4.5 h at 25 °C. After pelleting, cells were resuspended in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM MgCl2, 2 mM CaCl2, 10% glycerol, 10 μM β-mercaptoethanol, 1% Triton X-100, and 0.5 mM phenylmethylsulfonyl fluoride and ruptured by two passages through a 25-gauge French pressure cell at 10,000 p.s.i. The extract was clarified by centrifugation at 50,000 × g for 30 min and then applied to calmodulin affinity resin (Stratagene). After washing with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM MgCl2, 2 mM CaCl2, 10 μM β-mercaptoethanol, and 0.1% Triton X-100, bound fusion proteins were eluted with 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM MgCl2, 2 mM CaCl2, 10 μM β-mercaptoethanol, 2 mM EGTA, and 0.1% Triton X-100. Affinity-purified CBP-atlastin-1 fusion protein was dialyzed against assay buffer (20 mM HEPES [pH 7.2], 2 mM MgCl2, and 1 mM dithiothreitol). The reaction mixture for the GTPase assay included dialyzed CBP-atlastin-1 with 0.05% bovine serum albumin and 0.825 μM [γ-32P]GTP (3000 Ci/mmol; ICN Biomedicals, Irvine, CA) in assay buffer. Samples of the reaction mixture at various time points (0–60 min) were spotted onto polyethyleneimine cellulose on polyester TLC plates (Sigma). Guanine nucleotides were separated by ascending chromatography in 1 M LiCl and 1.2 M formic acid. The [γ-32P]GDP and [γ-32P]GTP spots were identified, and intensities were quantified using PhosphorImager and ImageQuant software (Amersham Biosciences). GTPase activity was expressed as a ratio of GDP to total guanine nucleotides (GTP + GDP) at each time point.

**RESULTS**

**Atlastin Family of GTP-binding Proteins**—We searched nucleotide and protein data bases for human proteins similar to atlastin (renamed atlastin-1) and found two highly related proteins (Fig. 1). One, which was previously identified as ARL-6-interacting protein-2 (GenBank/EMBI accession number NM_022374), we have renamed atlastin-2. The other we have named atlastin-3 (accession number AK097588). Whereas the atlastin-1 gene is found on chromosome 1q42.21 (locus ID 51062), the atlastin-2 gene localizes to chromosome 2p22.3 (locus ID 64225), and the atlastin-3 gene to chromosome 11q13.1 (locus ID 25923). These proteins show extensive homology, with a large central core of higher similarity that includes the GTP-binding region in the N-terminal portion; the most divergence occurs at the C and N termini (Fig. 1). There is 69% amino acid identity and 79% similarity between atlastin-1 and atlastin-2 over the central 533 residues and 66% identity and 75% similarity between atlastin-1 and atlastin-3 over 476 residues. Atlastin-2 and atlastin-3 share 67% identity and 78% similarity over 482 residues. Also, two predicted transmembrane helices (SOSU118) are conserved in all three proteins. Using the same SOSUI prediction program, human GPB1 (accession number NM_002053) and the related GTPase MAG-2 (accession number M81128) lack any predicted transmembrane domains, consistent with previous reports (12). Consensus sequences for N-linked glycosylation and Myb DNA binding previously reported for atlastin-1 (13) are not conserved in atlastin-2 and atlastin-3 (Fig. 1).

**Identification and Subcellular Localization of the Atlastin-1 Protein**—We generated specific anti-peptide antibodies against the divergent N (No. 5409) and C (No. 4705) termini of atlastin-1. Atlastin-1 was detected at ~64 kDa on immunoblots of homogenates from atlastin-1-overexpressing COS-7 cells, consistent with its predicted size, but not in those from untransfected cells (Fig. 2A). Similar results were obtained with anti-peptide antibodies raised against both the N (Fig. 2A) and C (data not shown) termini. Atlastin-1 was also detected in tissue homogenates from rat and human brain, with no cross-reacting protein bands (Fig. 2A). The immunoreactive signal was abolished when the antibodies were preadsorbed with the immunogenic peptide (1 μM) (data not shown). Atlastin-1 was most enriched in brain, but was also present in several other human tissues at much lower levels (Fig. 2B). By comparison, the mitochondrial intermembrane space protein DDP1/TIMMM8a had a more homogeneous tissue distribution (Fig. 2B). We examined the subcellular localization of atlastin-1 using rat brain fractions prepared by differential centrifugation (Fig. 2C). Both atlastin-1 and the endoplasmic reticulum protein calreulin were enriched in the microsomal P3 fraction. In contrast, the mitochondrial protein OPA1/Mgm1 was most abundant in the P2 pellet, with much lower levels in the P3 fraction.

We examined the localization of endogenous atlastin-1 in rat brain sections using antibodies generated against the N terminus of atlastin-1 (No. 5409) (Fig. 3). Highly immunopositive cells were most abundant in lamina V of the cerebral cortex, including the motor cortex. Although the neuronal soma was most strongly labeled, the dendritic tree was also stained (Fig.
Immunostaining was eliminated when the anti-atlastin-1 antibodies were first preadsorbed with the immunogenic peptide (Fig. 3D). Labeling was also prominent within the hippocampus, mainly in pyramidal neurons in CA1 and CA3 (Fig. 3, E and F). On the other hand, there was no detectable staining in the cerebellum. Although little immunoreactivity was present in the striatum, with moderate immunostaining limited to a subpopulation of large cholinergic neurons, more robust staining was evident in neurons within the amygdala and several thalamic nuclei. A number of cells in several mesopontine nuclei, including the dorsal substantia nigra pars compacta and the retrorubal area, stained moderately.

To establish the localization of atlastin-1 at the subcellular level, cultured cerebral cortical neurons were examined using double-label immunofluorescence. Atlastin-1 staining was found most prominently in the cell soma, with weaker staining in neuronal processes, comprising both axons and dendrites (Fig. 4). Co-localization was very limited with markers for the endoplasmic reticulum (anti-KDEL antibodies), and no overlap of atlastin-1 labeling was seen with mitochondria stained with MitoTracker CMXRos (data not shown). However, p115, a marker for the Golgi apparatus, tightly co-localized with atlastin-1 (Fig. 4, A–C). Co-localization with atlastin-1 was also seen with the Golgi marker GM130, but to a lesser extent (data not shown). The atlastin-1 immunoreactive signal was blocked by preadsorption of the antibodies with the immunogenic peptide (data not shown). At longer exposures, punctate staining was seen in dendrites and axons (Fig. 4, D–F). However, this staining was far less intense than that seen associated with the Golgi apparatus in the neuronal cell body.

To confirm the Golgi predominance of the endogenous atlastin-1 protein, we examined the localization of atlastin-1 in cultured cortical neurons by immunogold electron microscopy.

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3. B. Lavoie and C. Blackstone, unpublished data.
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(Fig. 5). An abundance of immunogold labeling decorated the Golgi apparatus, predominantly the cis-Golgi cisternae (Fig. 5, A and B). This labeling was not present in control sections in which the primary antibody was omitted (Fig. 5, C and D). No specific staining was seen in mitochondria or in the nucleus.

Atlastin-1 Is an Integral Membrane Protein—Based on the results of subcellular fractionation and immunolabeling, we examined whether atlastin-1 is an integral membrane or tightly membrane-associated Golgi protein, comparing it with known proteins under different membrane extraction conditions. Atlastin-1 overexpressed in COS-7 cells partitioned exclusively to the membrane fraction, which had been lysed by sonication to release soluble luminal proteins and subjected to ultracentrifugation (Fig. 6A). Notably, a significant percentage of the soluble luminal protein calnexin was found in the supernatant, confirming the efficient lysis of microsomes. The integral membrane protein calnexin was exclusively found in the membrane fraction (Fig. 6A). This membrane fraction was subjected to various extraction conditions (i.e. high salt, low pH, high pH, and detergents) to determine whether atlastin-1 is an integral membrane protein. Atlastin-1 partitioned to the supernatant in a manner similar to the integral membrane protein calnexin; neither atlastin-1 nor calnexin was released from the membrane fraction by low or high pH buffers, but both atlastin-1 and calnexin were efficiently solubilized by detergents (Fig. 6A). In contrast, the membrane-associated fraction of calnexin was released from membranes not only by detergents, but also by both high and low pH buffers (Fig. 6A). Phase partitioning with Triton X-114 demonstrated a distribution of atlastin-1 predominantly to the detergent phase, whereas the soluble membrane-associated protein calnexin was found in the aqueous phase (Fig. 6B).

To determine whether the two hydrophobic domains in atlastin-1 represent transmembrane domains, we used several Myc-tagged N- and C-terminal atlastin-1 deletions to assess localization to supernatant or membrane fractions (Fig. 6C). Wild-type atlastin-1 was present in the membrane fraction, and small deletions at the N and C termini did not change the membrane association significantly (Fig. 6C). However, a C-terminal deletion that removed the two predicted transmembrane domains, atlastin-1-(1–447), caused a redistribution of the protein to the supernatant fraction (Fig. 6C), indicating that these domains are required for membrane attachment. A 111-residue C-terminal atlastin-1 fragment containing the predicted transmembrane domains (residues 448–558) was sufficient for membrane association (Fig. 6C).

To evaluate the transmembrane topology of the atlastin-1 protein, we used intact microsomal vesicles from COS-7 cells overexpressing atlastin-1 in a protease protection assay (Fig. 7A). Immunoreactivity to both N and C termini was lost upon treatment of microsomes with proteinase K, although higher concentrations were required for full removal of the N-terminal domain (Fig. 7A). These results indicate that both N and C termini are exposed to the cytoplasmic face of the membrane. In control experiments, the luminal protein calnexin was completely protected from proteolysis in the same samples (Fig. 7A), even at higher concentrations of protease (data not shown). Consistent with this finding is that mutation of the three consensus sites for N-linked glycosylation in atlastin-1 (N177Q, N236Q, and N436Q) did not alter the migration of atlastin-1 upon SDS-PAGE, nor did treatment with peptide N-glycosidase F (Fig. 7B). In control experiments, the known
glycoprotein torsin A (26, 27) overexpressed in COS-7 cells was efficiently deglycosylated by peptide N-glycosidase F (Fig. 7B). Thus, we have no evidence that these consensus sites in atlastin-1 are N-glycosylated in vivo, consistent with our proposed transmembrane topology model (Fig. 7C).

Atlastin-1 Is an Oligomeric Protein—Members of the dynamin/Mx/GBP family form homo-oligomeric protein complexes in vivo, and we sought to determine whether atlastin-1 forms oligomers as well. Using yeast two-hybrid tests, we detected a robust interaction of full-length atlastin-1 with itself (Fig. 8A). We were unable to narrow down the domains required for self-interaction by deletion analyses; interactions were decreased or eliminated with both N- and C-terminal deletions (Fig. 8A). Also, we did not find an interdomain association among several different N- and C-terminal domain constructs (Fig. 8A), although interdomain and intramolecular interactions have been demonstrated for other members of the dynamin/Mx/GBP superfamily (12, 28–31). In co-immunoprecipitation experiments, Myc-atlastin-1 interacted with HA-atlastin-1 (Fig. 8B), demonstrating self-association of atlastin-1 overexpressed in COS-7 cells. Based on the results of chemical cross-linking experiments, atlastin-1 appears most likely to be a homotrimer, with products at ~110, ~160, and ~230 kDa in addition to the monomer band at 64 kDa (Fig. 8C). Using gel-exclusion FPLC, we found that detergent-solubilized atlastin-1 fractionated in the lysed membrane pellet (Membr), but not in the soluble or membrane-associated proteins calnexin, DOC, Triton X-114 phase as indicated by the absence of a band at 60 kDa when Triton X-114 phase was immunoblotted with anti-atlastin-1 antibodies. This pellet was subsequently resuspended in the indicated salts or detergents and then fractionated by high speed centrifugation into the supernatant (S) and membrane pellet (P). Atlastin-1 was released from membranes in a manner similar to the integral membrane protein calnexin, but not to the soluble and membrane-associated protein calregulin, DOC, Triton X-114 phase partitioning. Membranes from atlastin-1-transfected COS-7 cells and rat brain P3 fractions were partitioned with Triton X-114 (TX-114), and aliquots of the starting material (Input) as well as aqueous and detergent (Triton X-114) phases were immunoblotted with anti-atlastin-1 antibodies. This pellet was subsequently resuspended in the indicated salts or detergents and then fractionated by high speed centrifugation into the supernatant (S) and membrane pellet (P). Atlastin-1 was released from membranes in a manner similar to the integral membrane protein calnexin, but not to the soluble and membrane-associated protein calregulin, DOC, Triton X-114 phase partitioning. Membranes from atlastin-1-transfected COS-7 cells and rat brain P3 fractions were partitioned with Triton X-114 (TX-114), and aliquots of the starting material (Input) as well as aqueous and detergent (Triton X-114) phases were immunoblotted with anti-atlastin-1 antibodies. 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![Image](http://www.jbc.org/Downloaded from July 24, 2018)
Atlastin-1 eluted at a native size of ~280 kDa, consistent with the proposed tetrameric structure (Fig. 8D). However, because the cross-linked atlastin-1 product at ~230 kDa was weakly visible, and it was difficult to estimate the contribution of detergent to the size estimate for the atlastin-1 oligomer by gel-exclusion chromatography, we cannot rule out a trimeric quaternary structure for the atlastin-1 protein in vivo. Interestingly, atlastin-1-(1–516), which contains the putative transmembrane domains, also migrated at a similar native size upon gel-exclusion chromatography. However, atlastin-1-(1–447), which lacks the transmembrane domains, eluted as multiple peaks (Fig. 8D), demonstrating that the two predicted transmembrane domains are required for proper conformation and/or oligomerization of atlastin-1.

Atlastin-1 Is a GTPase—Using purified CBP-atlastin-1 fusion proteins, we examined whether atlastin-1 possesses GTPase activity. Increasing amounts of atlastin-1 protein successively increased the percentage of GTP hydrolysis (Fig. 9). Thus, atlastin-1 not only binds GTP, as shown in previous studies (32), but also functions as a GTPase (Fig. 9).

**DISCUSSION**

Mutations in the SPG3A gene cause a very early onset pure HSP (2–5). In this study, we have found that the SPG3A gene product atlastin-1 is an oligomeric GTPase composed of subunits that are integral membrane proteins, with both C and N termini exposed to the cytoplasm. We have also identified two other members of the atlastin family of GTPases that share significant sequence similarity with atlastin-1 and likely the same structural features: atlastin-2 and atlastin-3 (Fig. 1). These proteins are of similar size and have the same highly conserved GTP-binding motifs as well as two predicted transmembrane domains. Interestingly, the atlastin-3 gene maps to an area within the SPG17 (Silver syndrome; MIM 270685) locus (33) and thus may be a candidate gene for SPG17. The atlastin-2 gene is in the vicinity of the SPG4 gene spastin on chromosome 2.

The transmembrane structure we propose for atlastin-1 (Fig. 7C) is not typical of GBPs, the family of large GTPases to which the atlastins are most homologous (11, 12, 30). However, although the atlastins and GBPs all share an RD loop instead of (N/T)KXD in the third motif within the classical guanylate-binding triad, there are several structural differences between the atlastins and GBP families (12, 13, 32). First, unlike atlastins, GBPs have an additional C-terminal α-helical domain that folds back to interact with more proximal areas (12). Second, many GBPs have a C-terminal CAAX (where A represents an aliphatic amino acid) motif for isoprenylation, which all of the atlastins lack (Fig. 1). Third, our study has demonstrated that atlastin-1 and likely atlastin-2 and atlastin-3 are integral membrane proteins with two putative transmembrane domains; by contrast, GBPs are not integral membrane proteins. In this regard, the atlastins are more reminiscent of the Fzo/mitofusins, large GTPases that span the outer mitochondrial membrane twice, with both N and C termini facing the cytoplasm (34, 35). Because the Fzo/mitofusins are critical for proper mitochondrial fusion, atlastin-1 may also have roles in
Atlastin-1 is a GTPase. Upper panels, purified CBP-atlastin-1 fusion protein (1.1–4.4 μM, as indicated) or bovine serum albumin (control) was incubated with [γ-32P]GTP for 0–60 min. [32P]GDP and [32P]GTP were resolved by separation on TLC plates. Lower panel, [32P]GDP and [32P]GTP were quantified, and the percentage of GTP hydrolysis was calculated as described under “Experimental Procedures.”

The atlastin-1 protein is most abundant in brain, although it is also present at much lower levels in other tissues, including lung, smooth muscle, adrenal gland, kidney, and testis. Within brain, atlastin-1 is prominently enriched in the lamina V pyramidal neurons in the cerebral cortex, a subpopulation of which exhibit a distal axonopathy in patients with SPG3A. These upper motor neurons project to lower motor neurons in the lumbar spinal cord, and their dysfunction results in a spastic paraparesis, the cardinal feature of HSPs. Because these neurons have among the longest axons in the central nervous system, their dysfunction in SPG3A patients may reflect a critical role for proper atlastin-1 function in this subpopulation of “long axon” upper motor neurons. Based on the subcellular localization of atlastin-1 to the Golgi apparatus and its structural similarity to members of the dynamin/Mx/GBP superfamily of GTPases, atlastin-1 may be important for proper Golgi membrane dynamics or vesicle trafficking.

how might defective Golgi membrane structure or vesicle trafficking cause a distal axonopathy in upper motor neurons with long axons? Defective transport along axons has been directly implicated in a number of hereditary long axonopathies, including the HSP SPG10 (mutations in the neuronal kinesin KIF5A) (8) and the hereditary neuropathy Charcot-Marie-Tooth type 2A (mutations in the KIF1Bβ motor protein) (40), as well as in an autosomal dominant form of lower motor neuron disease (mutations in p150 subunit of dynactin) (41). Although these motor proteins directly affect transport, proper formation of intracellular cargoes is important as well. Given the early onset of SPG3A and suggestions that it may be due to abnormalities of neuronal development (3, 4), it is intriguing that treatment of hippocampal neurons with brefeldin A, a fungal metabolite that disrupts the Golgi apparatus, inhibits axonal growth (42). Thus, an intact Golgi apparatus is required for axonal growth (42), which may be particularly relevant for long axon formation during development of the central nervous system. Conceivably, mutations of atlastin-1 in patients with SPG3A function may result in impaired Golgi structure or function, leading to impaired axonal growth. Future studies of the effects of SPG3A patient mutations on Golgi structure and function as well as axonal transport and growth may clarify the cellular pathogenesis of the HSP SPG3A.

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