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Hereditary spastic paraplegias: membrane traffic and the motor pathway

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Abstract

Voluntary movement is a fundamental way in which animals respond to, and interact with, their environment. In mammals, the main CNS pathway controlling voluntary movement is the corticospinal tract, which encompasses connections between the cerebral motor cortex and the spinal cord. Hereditary spastic paraplegias (HSPs) are a group of genetic disorders that lead to a length-dependent, distal axonopathy of fibres of the corticospinal tract, causing lower limb spasticity and weakness. Recent work aimed at elucidating the molecular cell biology underlying the HSPs has revealed the importance of basic cellular processes — especially membrane trafficking and organelle morphogenesis and distribution — in axonal maintenance and degeneration.

In humans, the pathways that comprise the voluntary motor system are arranged in two tiers (BOX 1). First, axons of the upper motor neurons, which originate in the cerebral motor cortex, pass through the medullary pyramids, where most axons decussate to form the lateral corticospinal tract in the spinal cord. These axons establish synapses directly or indirectly with the lower motor neurons in the spinal cord anterior horn. In the second tier, axons of the lower motor neurons synapse at neuromuscular junctions to mediate skeletal muscle contraction.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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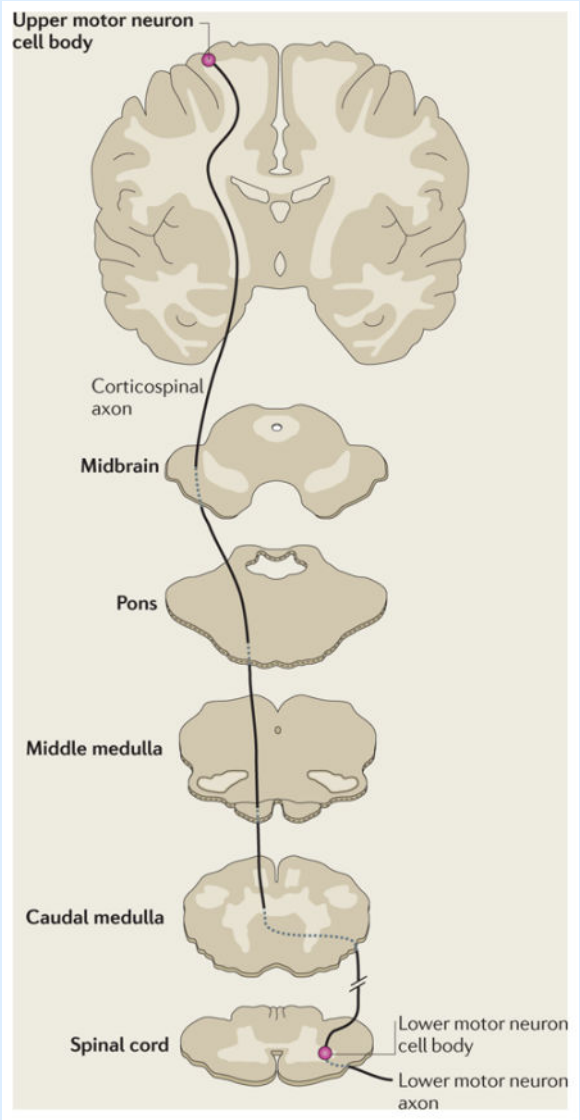
Box 1

The corticospinal tract and hereditary spastic paraplegias

The main CNS motor pathway controlling voluntary movement is the corticospinal tract (see the figure). Its axons originate from upper motor neuron cell bodies in the cerebral motor cortex and pass to the medulla, where they form the medullary pyramids (hence the tract is sometimes termed the pyramidal tract). Most of the axons decussate at the caudal medulla, forming the lateral corticospinal tract. The fibres that do not decussate form the ventral corticospinal tract, and most of these axons decussate lower in the spinal cord. Axons of the lateral and ventral corticospinal tracts make direct or indirect synaptic connections (indirect connections are via interneurons) with lower motor neurons in the spinal grey matter. Lower motor neurons innervate skeletal muscles. Lesions of the corticospinal tracts cause spasticity.

The hereditary spastic paraplegias (HSPs) are in most cases caused by a length-dependent, distal degeneration of corticospinal tract axons. HSPs are estimated to have a prevalence of up to 18 per 100,000 of a population (5 per 100,000 is probably more accurate for North America and Northern Europe) and thus afflict several hundred thousand people worldwide[95,96]. HSPs can be divided into ‘pure’ forms, in which only spastic paraplegia occurs, and ‘complex’ or ‘complicated’ forms, characterized by additional clinical features[2,5,97]. Seminal studies recognized autosomal dominant, autosomal recessive and X-linked recessive inheritance patterns in both pure and complex HSPs. In Northern Europe and North America, autosomal dominant pure HSP is the most prevalent subtype, although in Mediterranean and North African countries autosomal recessive HSPs seem to be more common[2,5,98].

More than 40 HSP loci have been mapped (named spastic gait or spastic paraplegia (*SPG*), from *SPG1* to *SPG48*) and 20 HSP genes identified. Although the distinction between pure and complex forms of HSP remains clinically useful, this distinction is not always maintained at the molecular level. For example, although mutations in the gene encoding spastin (*SPG4*) usually cause pure HSP, they can also (although rarely) cause a complex phenotype[99].



Distances traversed by the upper and lower motor neurons are extremely long, with axons reaching up to 1 m in length. This length is both an asset and a liability; it permits rapid relay of signals that mediate voluntary movement, but requires complex machineries for the proper intracellular sorting and distribution of proteins and organelles over long distances. The axoplasm can comprise over 99% of the total cell volume and is equipped with an elaborate cytoskeletal scaffold, mainly comprised of microtubules along which motor proteins mediate the selective transport of components. The interaction of intracellular cargos with a large number of diverse kinesin motor proteins — themselves associated with various adaptor proteins and other modulators of transport — permits tightly regulated, selective transport of organelles to growth cones during axonal development and to presynaptic terminals in mature neurons[1].

Hereditary spastic paraplegias (HSPs) are a large and diverse group of genetic disorders characterized by progressive lower limb spasticity and weakness[2] (BOX 1). These conditions highlight the clinical importance of understanding the mechanisms underlying axon maintenance and function, because in most HSP subtypes — including those on which this Review focuses — the spasticity is caused by a progressive distal axonopathy, mainly involving the longest corticospinal tract axons[2,3]. Interestingly, there is typically little neuronal cell death in HSPs. Studying HSPs therefore provides an important means to understand the specific molecular mechanisms of axonal maintenance and degeneration[2]. The insights gained may also be of relevance to more common neurological conditions in which axonopathy is a contributing feature, such as peripheral neuropathies, multiple sclerosis and motor neuron disease[3,4].

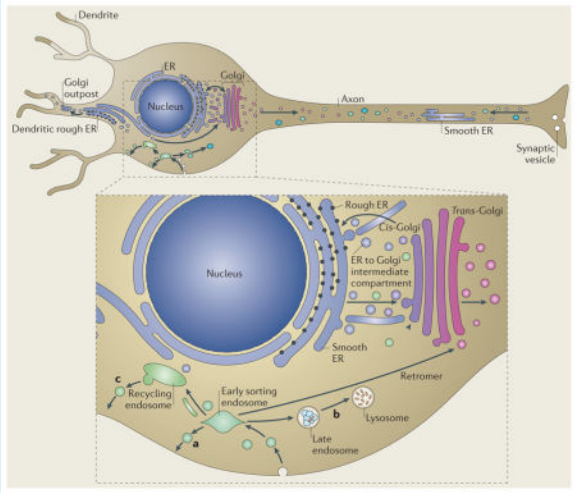
The identification of genes that are implicated in HSPs has been fundamental to understanding the cellular biology and pathogenesis of this group of disorders. We now appreciate that most proteins encoded by ‘HSP genes’ fall into four main functional groups (TABLE 1). The largest group of HSP proteins are either known or thought to be involved in the intracellular trafficking, localization or shaping of membrane compartments (BOX 2), highlighting these processes as crucial to the maintenance of axonal health.

Box 2

Membrane traffic pathways in non-polarised cells and neurons

The secretory and endocytic pathways are the main membrane traffic pathways in non-polarized cells (see the figure, blown-out part). In the secretory pathway (shown by a blue–purple gradient), cargo proteins are synthesised in the endoplasmic reticulum (ER). Cargoes are then trafficked to the Golgi through a tubulovesicular network, the ER to Golgi intermediate compartment. Post Golgi secretory vesicles and tubules are transported to several sites, including the plasma membrane and endosomes. In the endocytic pathway (shown in green), endocytosed material is delivered to early sorting endosomes. Cargo can then take three main routes: direct recycling back to the plasma membrane (a), delivery to the late endosomal and lysosomal degradative pathway (b), or a slow pathway back to the plasma membrane via perinuclear recycling endosomes (c). There are numerous cross-connections between the secretory and endocytic pathways — for example, the retromer complex sorts cargoes from early endosomes back to the *trans*-Golgi network. Long range movement of tubulovesicular cargoes occurs on cytoskeletal elements and uses motor proteins. For clarity, the recycling endosomal compartment and the Golgi are separated, but these organelles are pericentrosomal in most cells. In neurons the situation is more complex because the cells are polarized, with the somatodendritic membrane differing in composition to the axonal membrane. In addition to the core secretory pathway present in the cell soma, some dendrites also possess Golgi outposts. Cargo from ER within the dendrite may be transported to this, or may be transported to the somatic Golgi apparatus. The axon contains smooth ER continuous with the somatodendritic ER but is generally not regarded as containing rough ER or Golgi outposts. It also has endosomal components, including recycling endosomes, late endosomes and lysosomes, which are moved bidirectionally. At least some of these

endosomes differ at the molecular level from somatodendritic endosomes — for example, the somatic early endosomal marker early endosome antigen 1 (EEA1) is not present in axons.



Recently, there have been substantial advances in our understanding of the function of this large group of HSP-associated proteins involved with membrane trafficking, and in this article we will review these advances, including the roles of HSP proteins in membrane modelling events, regulation of receptor-mediated signalling and membrane transport. Readers wishing to learn more about the clinical features, genetic epidemiology, pathology and molecular genetics of the HSPs are referred to other recent reviews[2,5–10].

Membrane modelling and shaping

At least four HSP-associated proteins, including spastin (encoded by *SPG4*), atlastin-1 (encoded by *SPG3A*), receptor expression-enhancing protein 1 (REEP1; encoded by *SPG31*) and strumpellin (encoded by *SPG8*), are involved in membrane shaping and modelling events. This is an important subgroup, as mutations in the genes encoding these proteins cause up to 60% of HSP cases in North America and Northern Europe. This section will describe the role of these proteins and how their dysfunction might lead to HSPs.

Spastin: coupling membrane modelling to microtubule severing

The gene encoding spastin was the first gene to be identified as a cause for autosomal dominant pure HSP[11] (BOX 1). The gene encodes four main cellular isoforms of the spastin protein, which are generated by a combination of different promoter use, alternative initiation of translation from two different AUG start codons and differential exon splicing[12,13] (FIG. 1). These four isoforms comprise a 616 amino-acid full-length protein (M1 spastin), a shorter isoform that lacks the first 86 amino acids of the full-length protein (M87 spastin) and splice variants of both of these, which lack a 32 amino-acid stretch encoded by exon 4 (REF. 12).

There is overwhelming evidence from overexpression and knockdown studies in mammalian cultured cell lines, *in vitro* studies, structural studies and animal models that spastin can sever microtubules[10,14]. It is one of a small group of proteins that influence the cytoskeleton by causing internal breaks in microtubules, thereby regulating cellular functions that are dependent on these structures[15]. The carboxy-terminal half of spastin — from residue 270 onwards — contains the domains that are required for microtubule severing. This section of the protein contains a microtubule binding domain, situated between residues 270 and 328, and an AAA (ATPases that are associated with diverse cellular activities) ATPase domain, spanning residues 342–599 (REFS 11,16). AAA ATPase domains are found in a large family of proteins and typically use energy from ATP hydrolysis to catalyse conformational changes in target proteins[17]. Structural studies of recombinant spastin domains of *Drosophila melanogaster* and *Caenorhabditis elegans* have provided insights into its mode of action in microtubule severing (FIG. 1). Like most AAA domains, the AAA domain of spastin contains an α- and β-nucleotide-binding domain and a smaller four-helix bundle domain (HBD), which together comprise the enzymatic core of the protein. It contains an additional two helices, not found in AAA domains of other proteins, that interact with the nucleotide-binding domain. In the unbound state the nucleotide pocket is open and ATP binding is relatively inefficient because an extended loop that is likely to be involved in nucleotide contact and protomer–protomer interactions is positioned away from the nucleotide-binding pocket. In this non-ATP-bound state, spastin exists mainly as a monomer or a weak dimer. By contrast, ATP-bound spastin forms a hexameric ring with a prominent central pore. It has been suggested that polymerized spastin docks onto microtubules and destabilizes them by tugging the C terminus of tubulin through this central pore[18].

The amino-terminal region of spastin contains sequences that mediate interaction with various adaptor proteins that recruit spastin to sites of action (FIG. 1). Most of these interactors are integral membrane or membrane-associated proteins, suggesting that spastin's microtubule severing activity is targeted to specific membranes. In addition to showing partial colocalization with microtubules, endogenous spastin also localizes to the endoplasmic reticulum (ER) and can be recruited to endosomes[19]. Indeed, spastin shows an isoform-specific intracellular localization pattern, with the membrane-bound, larger M1 isoform predominantly found at structures of the early secretory pathway and to a much lesser extent on endosomes, whereas the shorter M87 isoform is present in a cytosolic pool that can be recruited to endosomes, but not to the early secretory pathway[19].

The N-terminal region of spastin contains two well-characterized interaction domains that can explain its isoform-specific localization. First, a hydrophobic region that mediates interactions with at least three classes of early secretory pathway proteins — namely Atlastins, REEPs and Reticulons (see below) — lies within the beginning of spastin's N-terminal region and is absent in the short M87 isoform[20–22]. Second, a microtubule interacting and trafficking (MIT) domain is present in all known spastin isoforms. It consists of a three-helix bundle formed by residues 116–194 of the full-length M1 protein (FIG. 1)[23]. MIT domains have been described in a number of proteins and typically mediate interactions with a group of proteins termed charged multivesicular body proteins (CHMPs)[24]. These CHMP proteins form a complex termed endosomal sorting complex required for transport III (ESCRT-III), and roles for this complex have been described in various membrane

modelling processes, including viral budding, formation and sorting of cargoes (such as the epidermal growth factor (EGF) receptor) into the internal vesicles of late endosomal multivesicular bodies, and the final abscission stage of cytokinesis[25,26]. It has been suggested that ESCRT-III is a membrane scission machinery[27]. The MIT domain is necessary for the recruitment of spastin to endosomes, and spastin interacts strongly, through its MIT domain, with two ESCRT-III proteins, charged multivesicular body protein 1B (CHMP1B) and IST1[19,28,29]. The crystal structure of the spastin MIT–CHMP1B complex reveals a non-canonical interaction site between the first and third helices of the spastin MIT domain and the C-terminal helical domain of the CHMP1B protein (FIG. 1). Interestingly, this C-terminal helix, which functions as an autoinhibitory domain that is exposed upon ESCRT-III oligomerization, provides an oligomerization-dependent switch for interaction with MIT-domain proteins, permitting selective recruitment[30].

Spastin is required for completion of abscission at the end stage of cytokinesis, when the tubular midbody — which connects newly divided cells and is densely packed with an anti-parallel array of microtubules as well as a central collection of proteins known as the Flemming body — is resolved[31,32]. In cells lacking spastin, a microtubule disruption event that normally accompanies abscission does not occur, suggesting that it is caused by spastin-mediated microtubule severing[19,30]. The ESCRT proteins are also required for abscission, and there is evidence that these proteins participate in the resolution of the midbody membrane[33,34]. Endogenous spastin is localized at the periphery of the Flemming body within the midbody, and as its recruitment there depends on the MIT domain, it is thought to be mediated by one or more ESCRT-III proteins[19,30]. Thus, cytokinesis provides an example of how microtubule regulation can be linked to membrane modelling events through spastin. This link may be of relevance to the role of spastin in the nervous system, as loss of spastin reduces the rate of axonal branching in cultured primary neurons, and axonal branching also involves coordinated microtubule regulation and membrane modelling[35,36].

Although spastin and the ESCRT-III proteins both localize to endosomes, a role for spastin in the known endosomal functions of the ESCRT complex has not been identified. For example, lack of spastin does not have a major effect on EGF receptor degradation[19]. Spastin also interacts with another endosomal protein, protrudin (also known as ZFYVE27), in HeLa cells[37]. Although this interaction has not yet been verified with the endogenous proteins in neurons, it is nonetheless interesting as lack of protrudin in cultured neurons inhibits neurite extension, whereas overexpression of protrudin promotes neurite extension[38]. Mechanistically, the effect of protrudin in neurite extension is mediated by Ras-related protein RAB11. Protrudin preferentially binds to the GDP-bound, inactive form of RAB11, which promotes directional membrane traffic[38]. The relevance of spastin to the action of protrudin on directional membrane traffic remains to be defined, but as neurite extension involves coordinated cytoskeletal remodelling and membrane trafficking, it is tempting to speculate that this might be another example of these processes being coupled through spastin.

Atlastin: a GTPase of the ER

Mutations in *SPG3A* are the second most common cause of HSP, and the most common cause of early-onset disease. Atlastin-1, the protein encoded by *SPG3A*, is a member of a superfamily of dynamin-related GTPases, and recent studies have highlighted the crucial role of the Atlastin family in the formation of the ER network. Atlastin-1 is one of three mammalian Atlastin proteins that are thought to represent functional paralogues, and it is the only Atlastin that is highly expressed in the CNS; *Drosophila* spp. and *C. elegans*, for example, have only one Atlastin protein. Atlastin-related GTPases are present in all eukaryotic cells and are characterized by an N-terminal GTP-binding domain and two very closely spaced hydrophobic segments near the C terminus that probably form a hairpin transmembrane domain (see below)[21,39]. These multimeric, integral membrane GTPases localize predominantly to the tubular ER, but also to the ER to Golgi intermediate compartment (ERGIC) and *cis*-Golgi membranes. Atlastin GTPases are required for the formation of the three-way junctions in ER tubules — which give the characteristic, polygonal appearance of the ER in the cell periphery (FIG. 2) — by directly mediating homotypic fusion of ER tubules[21,39,40]. Consistent with their proposed role in the formation of the ER network, Atlastins localize to discrete sites along ER tubules, including at three-way junctions[21,39].

Depletion of atlastin-1 in rat cortical neurons in primary culture inhibits axon elongation[41], and the importance of ER morphology in the formation and maintenance of long processes is supported by studies of the Atlastin orthologue in *Arabidopsis thaliana*, RHD3. Mutant *rhd3* plants have short, wavy root hairs and abnormal-appearing tubular ER bundles. Furthermore, the morphology of the ER changes noticeably during the elongation phase of root hair growth[42]. Thus, long cellular protrusions, such as plant root hairs and neuronal axons, are structures that are highly dependent on the dynamic morphology of the tubular ER.

Spastin, Atlastins and REEP1 are ER morphogens

The ER is a continuous membrane system that comprises the nuclear envelope, ribosome-studded peripheral sheets and a polygonal network of smooth tubules that extend throughout the cell. The mechanisms underlying the heterogeneous architecture of the ER have been clarified recently[43]. Several classes of proteins are important for the generation of tubular ER membranes, most notably the ER-shaping proteins of the REEP and Reticulon families. Although these families have little overall sequence homology to one another, they exhibit a common structural feature — elongated, hydrophobic segments that are predicted to form paired hairpin domains that partially span the membrane. With the majority of the protein domain localized to the outer leaflet of the phospholipid bilayer, ER-shaping proteins may generate curvature through hydrophobic wedging[43,44].

In highly polarized cells such as neurons, distribution of ER domains is coordinated with cytoskeletal dynamics, mostly involving microtubules. Atlastins interact directly with spastin[20,45], specifically through a predicted hydrophobic hairpin domain that is present only in the larger M1 isoform of spastin and possibly also through flanking hydrophilic sequences[22]. Expression of ATPase-defective M1 spastin causes a dramatic tubulation of the

ER and redistribution of ER markers, including atlastin-1, onto abnormally thickened microtubule bundles[20]. In addition to interactions with tubule-shaping Reticulons, Atlastins and M1 spastin also seem to interact directly with REEPs in the tubular ER[19,22,46] (FIG. 2). REEPs comprise six members in humans — REEP1 through to REEP6, with phylogenetic and structural distinctions between REEP1 through to REEP4 versus REEP5 and REEP6 (REF. 22). REEP1 through to REEP4 proteins harbour hydrophobic hairpins but also interact with microtubules through an extended C-terminal cytoplasmic domain that is enriched in basic amino acids[22]. These proteins may help to mediate the formation or stabilization of the tubular ER network, as deletion of the microtubule-interacting domain of REEP1 decreases the number of three-way junctions in the ER[22]. These interactions among REEPs, Atlastins, and spastin via hydrophobic hairpins provide a compelling mechanism for coupling ER membrane remodelling to cytoskeletal dynamics.

Strumpellin and endosomal tubulation

Mutations in *KIAA1096* (also known as *SPG8*), the gene that encodes strumpellin, were identified as a cause of HSP in 2007 (REF. 47). Strumpellin has recently been identified as part of a large protein complex that associates with endosomes through an interaction with vacuolar protein sorting-associated protein 35 (VPS35). VSP35 is a component of retromer, an endosomal complex that is responsible for sorting certain cargoes from endosomes to the *trans*-Golgi network[48–50] (BOX 2). Depletion of members of the strumpellin complex increases tubulation at early endosomes, resulting in impaired trafficking through early endosomal compartments, exemplified by impaired recycling of the transferrin receptor[48–50]. At least three members of the complex — Wiskott–Aldrich syndrome protein and SCAR homologue (WASH), family with sequence similarity 21 (FAM21) and actin capping protein — regulate actin dynamics. The complex generates an actin network on subdomains of early endosomes, for example by activating the actin-related protein 2 (ARP2)–ARP3 complex to initiate new actin filaments branching off existing filaments[48,49,51] (FIG. 3). The increased tubulation associated with depletion of the strumpellin complex is thought to be due to a lack of the actin-driven force that is required for fission of tubular transport intermediates from the endosomal body[48,49]. The precise role of strumpellin in the complex remains unclear. However, the strumpellin–WASH complex does provide another example of an HSP protein being associated with coordinated membrane modelling and cytoskeletal organization. More recently, strumpellin has been shown to interact with valosin-containing protein (VCP; also known as p97), which is encoded by the gene that is mutated in frontotemporal dementia with Paget's disease of bone and inclusion body myopathy[52], although the relationship of this protein to the strumpellin–WASH complex has not yet been explored.

Shaping defects and axonopathy

Spastin is a microtubule-severing protein that is recruited to membranes that are undergoing remodelling processes, atlastin-1 is a large integral-membrane GTPase of the ER that mediates homotypic fusion of ER tubules, REEP1 is an ER morphogen that links ER membranes to microtubules and strumpellin participates in a complex that is thought to link actin regulation to the fission of tubules from early endosomes. The common function of these HSP proteins in membrane shaping suggests that membrane modelling is

mechanistically important in the pathophysiology of the disease. Defects in axonal growth or transport in animal models of HSP based on mutations in spastin and atlastin-1 also support a functional role for these proteins in axons[41,53–55]. How could membrane modelling events be linked to axonopathy? One possibility is that defects in membrane modelling events within the axon — events that are perhaps required for axonal functions such as synaptic plasticity or efficient axonal transport — are the primary cause of the disease. Although the link between these axonal functions and membrane shaping is currently unclear, it might be speculated that, for example, tubulation events at early endosomes could be part of a process required to distribute membranes from one site to another during synaptic plasticity. Correct morphogenesis of ER or other membrane compartments could be required to deliver membrane components with a particular composition to the distal axon, or could be important for bioenergetically efficient axonal transport, failure of which might lead to protein or lipid starvation of the distal axon. Alternatively, membrane modelling defects associated with mutated HSP proteins could affect the traffic of receptors that control specific signalling pathways that are important for axonal function. In fact, several proteins that, in mutated form, have been associated with HSPs regulate signalling pathways that are important for axonal function, as discussed in the following section.

Regulating receptor-mediated signalling

Bone morphogenetic protein signalling

Bone morphogenetic proteins (BMPs) are ligands of the transforming growth factor β (TGFβ) superfamily. BMP signalling has crucial roles in many developmental processes, including organogenesis, dorsoventral patterning, cellular differentiation and tissue remodelling. In *Drosophila melanogaster* and in mammals, the BMP signalling pathway is an important determinant of axonal growth and synaptic function[56–60]. Interestingly, impairment of BMP signalling in *Drosophila melanogaster* leads to axon transport defects[61–63]. In rodents, BMP signalling is upregulated after lesion of the corticospinal tract, and inhibition of this upregulation promotes axonal regrowth[64].

HSP-associated mutations are found in at least four proteins — atlastin-1, non imprinted in Prader-Willi/Angelman syndrome 1 (NIPA1), spastin and spartin — that are inhibitors of BMP signalling[65,66]. The best characterized example is NIPA1, a polytopic integral membrane protein. Fly larvae lacking the *Drosophila melanogaster* homologue of NIPA1, spichthyin, show an increased number of synaptic boutons at neuromuscular junctions[63]. This axonal phenotype is associated with increased neuronal concentrations of phosphorylated mothers against decapentaplegic (MAD), a downstream messenger of BMP signalling, and it is suppressed by genetic manipulations that block BMP signalling[63]. NIPA1 and spichthyin are predominantly endosomal proteins that are also found at the plasma membrane, where they are subject to clathrin-mediated endocytosis[65,67]. They are thought to inhibit BMP signalling by binding to the type II BMP receptor and promoting its endocytic internalization and, at least in mammals, degradation in lysosomes[63,65] (FIG. 4).

There has been some controversy over the pathological mechanism of NIPA1 in the causation of HSP. Studies examining the effect of NIPA1 disease-associated missense mutations showed that they cause the retention of NIPA1 in the ER and affect traffic of the

protein through the secretory pathway[67,68]. This might cause disease through a gain-of-function induction of ER stress and the unfolded protein response (UPR) or by altering the trafficking of BMP receptors, with consequent effects on BMP signalling[65,68].

The importance of dysregulated BMP signalling as a cause of axonal abnormalities in an *in vivo* vertebrate model of HSP has been confirmed by a recent study investigating atlastin-1 (REF. 66). Zebrafish morphants depleted of atlastin-1 had abnormal spinal motor axon morphology, particularly increased branching, as well as severely decreased larval mobility. The BMP signalling pathway was upregulated in these larvae, and inhibition of BMP signalling rescued the anatomical and behavioural phenotype of the *atl1* knockdown zebrafish. Atlastin-1 partially colocalized with type I BMP receptors in neurite endosomes, suggesting that it may play a part in BMP receptor traffic[66]. Furthermore, depletion of spastin or spartin, which can both localize to endosomes, upregulates BMP signalling in mammalian cell lines through an as-yet-unknown mechanism[65]. Considered together, these results suggest that abnormal BMP signalling, probably caused by abnormal BMP receptor trafficking in many cases, could be a unifying mechanism in causing axonopathy in some classes of HSP. It will be important to determine whether the axonal phenotypes observed following depletion of several HSP proteins in primary neuronal cultures are caused by dysregulated BMP signalling. In particular, investigation of relevant HSP mouse models will now be crucial in determining whether abnormal BMP signalling has a pathological role in HSP and, if so, whether inhibition of BMP signalling by existing small-molecule inhibitors can rescue the disease phenotype[69].

Other signalling pathways

In addition to BMP signalling, there is evidence that HSP-associated proteins regulate other signalling pathways. For example, the endosomal protein spartin is required for efficient EGF receptor degradation, and so probably regulates EGF signalling[70]. Spartin might also have a role in the regulation of signalling pathways by ubiquitin modification, as it interacts with a family of ubiquitin E3 ligases, including atrophin-1 interacting protein 4 (AIP4) and AIP5, which are known to regulate numerous pathways[70–73]. It is therefore possible that abnormality of spartin function might affect many axonal signalling pathways. Interestingly, spartin also regulates lipid droplet biogenesis by promoting AIP4-mediated ubiquitination of lipid droplet proteins[71,73]. In this regard it parallels the function of seipin, encoded by *SPG17*, which is an ER protein that functions in the formation of lipid droplets[74]. Although overexpression studies have suggested that the pathogenicity of seipin mutations is mediated by ER stress[75,76], they could conceivably also affect lipid droplet biogenesis. Very little is known about the parts (if any) that lipid droplets play in axons. However, lipid droplets have been implicated in membrane biogenesis and cellular signalling, and could conceivably be involved in shaping organelles or regulating signalling pathways important for axonal function.

Mutations in the amyotrophic lateral sclerosis 2 (ALS2) protein alsin have been associated with spastic paraplegia and some patients with these mutations have a disease course that is more similar to the HSPs than to ALS. Mice that lack the gene encoding alsin show motor impairments and a distal axonopathy of the corticospinal tract that is characteristic of

HSPs[77,78]. Alsin is a guanine nucleotide exchange factor for the small GTPases RAB5 and Ras-related C3 botulinum toxin substrate 1 (RAC1)[79]. Overexpression of alsin in neurons stimulated RAB5-dependent endosomal fusion, resulting in enlarged endosomes[79], whereas RAB5-dependent endosomal fusion was impaired in neurons from alsin knockout mice[80]. Thus, abnormal endosomal function may be important in ALS2 pathogenesis, possibly through effects on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor trafficking, insulin-like growth factor 1 (IGF1) receptor trafficking or brain-derived neurotrophic factor (BDNF) receptor trafficking, which are disrupted in mice lacking alsin and which are components of a number of intracellular signalling cascades that control neuronal survival, plasticity and axonal morphology[80,81].

Motor-based axonal transport

The identification of mutations in the gene kinesin family member 5A (*KIF5A*, also known as *SPG10*), which encodes the kinesin heavy chain isoform 5A (KIF5A), in families with pure and complex HSP indicates the importance of motor-based transport in the disease[82,83]. KIF5s are microtubule plus-end-directed, ATP-dependent motors that move cargo in the anterograde direction in axons[84,85]. Axonal transport fails in mutants of the *Drosophila* orthologue of *KIF5A*, resulting in axonal ‘log jams’ of immotile anterograde and retrograde cargo organelles[86]. In mammals, KIF5A has been characterized as a motor that is necessary for axonal transport of neurofilament subunits, although it has not been excluded from contributing to the transport of other anterograde cargoes, such as membrane vesicles[87]. KIF5 proteins also regulate transport of cargoes in dendrites and have roles in a number of membrane traffic pathways in the cell body, including in ER-to-Golgi and Golgi-to-ER traffic, Golgi to plasma membrane transport, and transport of recycling endosomes and of lysosomes[84,88]. It is not yet certain which of these diverse functions is impaired in patients with HSP who carry mutations of kinesin.

Nearly all KIF5A mutations are missense mutations and typically impair transport as they affect the kinesin motor domain[82,83,89]. The efficiency of cargo transport to the distal axon is thought to be affected either because the mutated KIF5A are slower motors or because they have reduced microtubule binding affinity and compete with other, wild-type motors for cargo binding sites[90].

Axonal transport is also affected in mice that lack spastin, which have axonal swellings in which accumulated organelles are found[54,55]. Studies of axons from these mice have shown defects in both anterograde and retrograde transport, and these defects are associated with altered microtubule architecture within the axon. However, it is not clear whether these axonal transport defects are a direct result of dysregulation of axonal microtubules owing to lack of spastin, or an indirect effect of loss of spastin on membrane traffic pathways that control axonal signalling pathways[54,55].

Membrane traffic and other axonopathies

The importance of organelle morphology and distribution for the maintenance of axons is also emphasized by genes that have been associated with other inherited, non-HSP

axonopathies. These can include disorders involving peripheral nerves such as Charcot–Marie–Tooth (CMT) neuropathies and polyneuropathies with associated autonomic neuropathies (known as hereditary sensory and autonomic neuropathies (HSANs)). In a recent study[91], mutations in the gene encoding protein FAM134B, a *cis*-Golgi apparatus-enriched member of the FAM134 family of proteins, were identified in patients with HSAN type II. FAM134B is enriched in dorsal root ganglion neurons, consistent with the clinical presentation of the disease[91]. The FAM134 proteins each have a pair of long hydrophobic segments, reminiscent of those in the Reticulons and REEP proteins. Furthermore, depletion of FAM134B causes prominent changes in Golgi morphology in neurons and in a tumour cell line derived from autonomic ganglion neurons, although effects on ER morphology have not been described[91]. More work will be needed to establish whether these proteins have a direct shaping function and how their dysfunction causes axonopathies. Even so, these disorders may highlight the implications of morphological defects in the ER and early secretory pathway in the pathogenesis of length-dependent axonopathies.

Studies into the pathogenesis of CMT neuropathies may be instructive, because these are also length-dependent axonopathies — although they primarily affect peripheral, rather than central, neurons. CMT1 is comprised of demyelination disorders and CMT2 of those that cause axonopathies. Axonal forms of CMT can be caused by mutations in a number of genes that encode proteins with functions in trafficking and organelle morphogenesis. In particular, CMT2A is caused by mutations in the gene encoding mitofusin 2 (*MFN2*), which regulates mitochondrial morphology by mediating mitochondrial fusion. Also, the CMT2B protein RAB7 is a small GTPase that regulates vesicle trafficking. Interestingly, RAB7 interacts with the SPG21 protein maspardin, another HSP-associated protein that localizes to endosomes[92].

Lastly, ER-shaping mechanisms may have roles in related neurologic disorders such as familial ALS, in which both corticospinal and lower motor neurons are affected. In the superoxide dismutase 1 (SOD1) G93A transgenic mouse model for ALS, overexpression of the ER-shaping protein reticulon-4A selectively redistributed the ER chaperone protein disulphide isomerase and protected against neurodegeneration. Conversely, loss of reticulon-4A increased the severity of disease in *SOD1* G93A mice[93]. Further supporting a role for ER morphogenesis in neurologic disorders, a mutant variant of vesicle-associated membrane protein-associated protein B (VAPB) that underlies another familial form of ALS (ALS8) is associated with the production of a novel form of organized, smooth ER[94]. Taken together, these studies support dysfunctional ER morphogenesis as a potential mechanism for multiple neurological diseases.

Conclusion

Over the past decade, many HSP-associated gene products have been identified and a small number of common themes for HSP pathogenesis are emerging. A large group of proteins associated with the majority of HSPs is involved in membrane trafficking processes; several of the proteins within this group, including those involved in the most common forms of HSP — spastin and atlastin-1 — shape membranes of the ER or endosomes. In addition, many proteins within this membrane traffic group are regulators of BMP signalling, a

pathway that is probably regulated by endosomal or secretory pathway trafficking of BMP receptors.

However, many questions remain to be answered. Do other HSP-associated proteins that localize to the ER or endosomes also contribute to the shape of membranes? How might defects in organelle shaping cause axonopathy? There could be a direct and crucial requirement for membrane modelling events in the axon, or alternatively, abnormal membrane modelling could cause axonopathy by altering axonal BMP signalling. Is dysregulation of BMP signalling sufficient to cause axonopathy in mammals and, if so, by what mechanism? What mechanisms underlie phenotypic differences in HSPs? Perhaps the answer to this last question could be related to the specificity with which membrane modelling events are affected in different HSPs. Alternatively, uncomplicated HSPs could arise if BMP signalling is dysregulated in isolation, whereas complex HSPs might arise if additional signalling pathways are also involved. A variety of animal and cellular models are currently being developed to address these questions in the near future. This will raise the prospect of rationally designed therapies based on a thorough knowledge of the molecular and cellular pathology of distal axonopathies.

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Glossary

Upper motor neurons
Neurons whose fibres comprise descending pathways in the CNS and that are involved in voluntary control of skeletal muscle contraction. Corticospinal neurons are a type of upper motor neuron.

Decussate
To cross the midline to reach the contralateral side of the nervous system.

Paraplegia
Muscle weakness involving both legs.

Spasticity
Increased muscle tone and deep tendon reflexes resulting from damage to the corticospinal tract.

Protomer
A structural unit of an oligomeric protein.

Early secretory pathway

A pathway through the endoplasmic reticulum (ER), ER-to-Golgi intermediate compartment and the *cis*-Golgi apparatus.

Viral budding
The process by which an enveloped virus particle is released from the plasma membrane of a host cell.

Abscission
The final stage of cytokinesis, when the midbody connecting two daughter cells is broken and sealed.

Cytokinesis
The stage in cell division when the cytoplasm of a single cell is divided to form two daughter cells.

Midbody
The tubular plasma membrane-bound structure that connects two daughter cells in the late stage of cytokinesis.

Anti-parallel
Running side-by-side, but in opposite directions. A bundle of microtubules is anti-parallel if the microtubules of which it is comprised have plus ends facing both directions.

Paralogues
Similar DNA and protein sequences (often distinct genes) within a species.

Hydrophobic wedging
A mechanism for inducing membrane curvature by partitioning the bulk of a hydrophobic domain within the outer leaflet of the bilayer.

Tubular transport intermediates
Membrane-bound, small, cigar-shaped organelles that are trafficked from one intracellular membrane compartment to another. They are distinguished by their shape from vesicular transport intermediates, which are spherical.

Polytopic integral membrane protein
A protein that spans the membrane more than once because it has more than one transmembrane domain.

Clathrin-mediated endocytosis
The major endocytic pathway, in which cells internalize extracellular or plasma membrane molecules into clathrin-coated vesicles. Once uncoated, the vesicles are capable of fusing with internal organelles, such as endosomes.

Unfolded protein response
A cellular stress response that is triggered by excess of unfolded or misfolded proteins in the endoplasmic reticulum.

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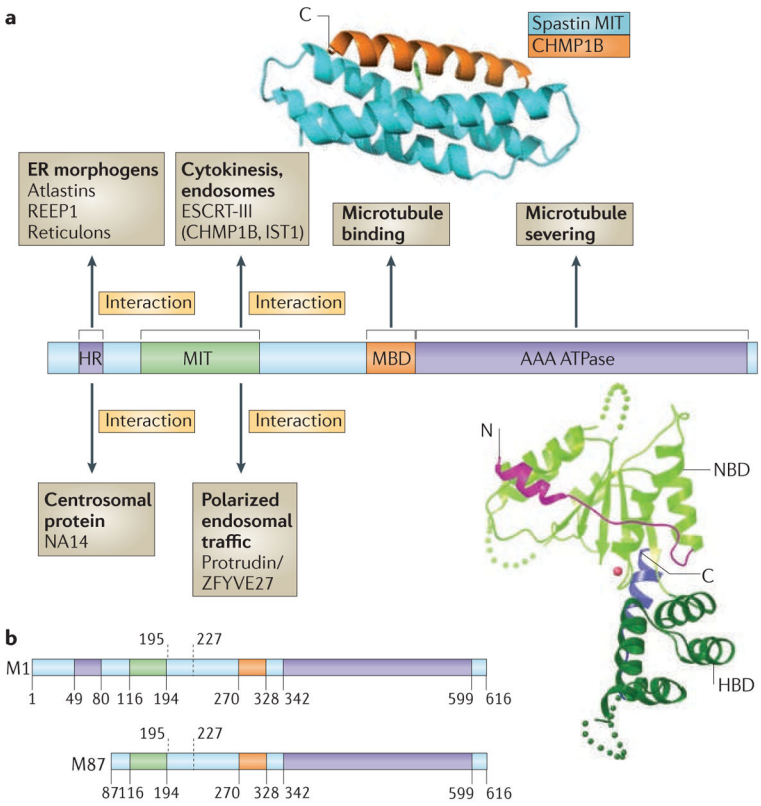

Figure 1. Spastin domain structure and interacting proteins

Domains in the spastin protein. **a** | The hydrophobic region (HR) possibly forms an intramembrane hairpin loop. The microtubule interacting and trafficking domain (MIT) forms a three-helix bundle that interacts with a helix in the endosomal sorting complex required for transport III (ESCRT-III) proteins charged multivesicular body protein 1B (CHMP1B) and IST1. The microtubule binding domain (MBD) is necessary for spastin to bind to microtubules and is required for microtubule severing. The AAA (ATPases associated with diverse cellular activities) ATPase domain contains the enzymatic activity of the protein that is essential for microtubule breakage. The regions to which interaction sites with known binding partners have been narrowed are indicated. The structure of the interaction between the spastin MIT domain and CHMP1B is shown, as is the structure of the *Drosophila* AAA ATPase domain. The amino (N)- and carboxy (C)-terminal helices are shown in magenta and blue, respectively. NBD, nucleotide binding domain; HBD, helix bundle domain; REEP1, receptor expression-enhancing protein 1. The spastin structure and interaction with CHMP1B are reproduced, with permission, from REF. 30 © (2008) Macmillan Publishers Ltd. All rights reserved. The structure of the *Drosophila* AAA ATPase domain is reproduced, with permission, from REF. 18 © (2008) Macmillan Publishers Ltd. All right reserved. **b** | The domain structure of the M1 spastin isoform and the M87 spastin isoform, with amino-acid numbers indicated. The position of the alternatively spliced exon 4 is shown by the dashed lines.

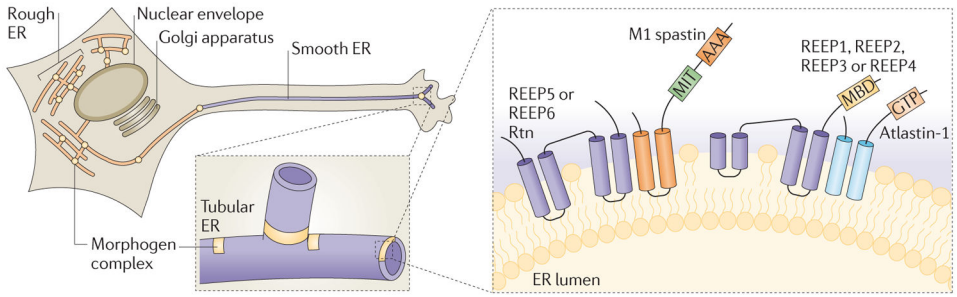

Figure 2. The spastin–Atlastin–REEP–Reticulon complex at the ER

Receptor expression-enhancing proteins (REEPs; Yop1p in yeast) and Reticulon proteins form large oligomers, referred to here as morphogen complexes, to shape the tubular endoplasmic reticulum (ER) network. Atlastin proteins (Sey1p in yeast) interact with REEPs and Reticulons and are enriched in puncta along the tubules (shown by yellow circles), including at three-way junctions. A blown-out image of the axon shows a tubular ER three-way junction. A nested blown-out image of a presumptive ER morphogen complex depicts the proposed membrane topologies for proteins involved in generating curvature of ER tubules, as well as mediating microtubule interactions and fusion of ER tubules. AAA, ATPases associated with diverse cellular activities (AAA) ATPase domain; GTP, Atlastin GTPase domain; MIT, microtubule-interacting and trafficking protein domain; MBD, microtubule-binding domain; REEP, receptor expression-enhancing protein; Rtn, reticulon.

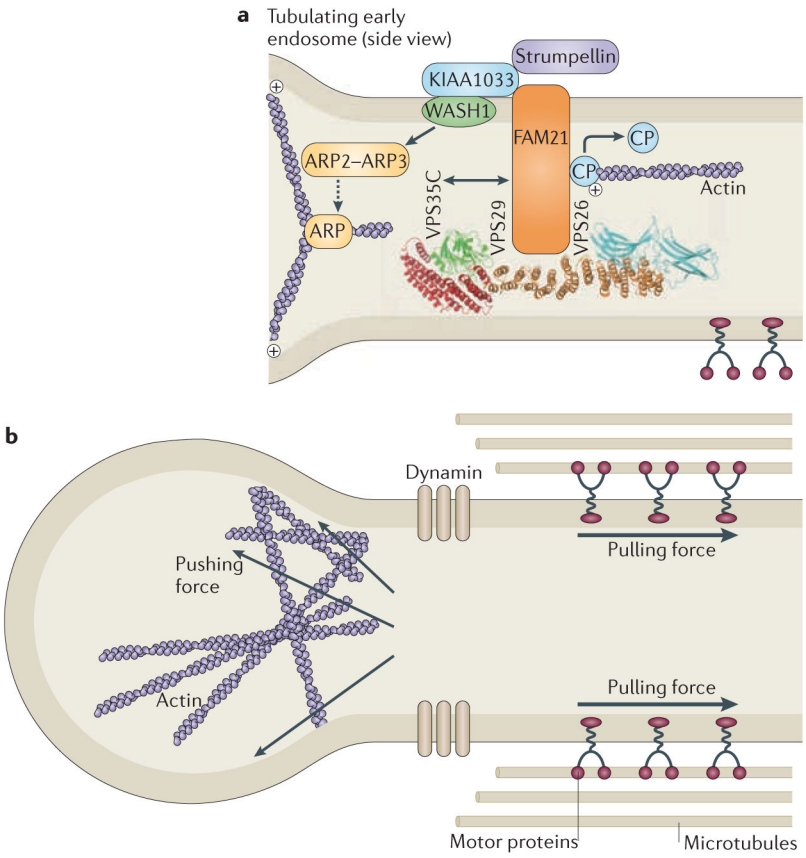

Figure 3. The strumpellin–WAsH complex

a | The strumpellin–WASH (Wiskott–Aldrich syndrome protein and SCAR homologue) complex is recruited to endosomes by interaction between family with sequence similarity 21 (FAM21) and the vacuolar protein sorting-associated protein 35 (VPS35) component of the VPS26–VPS29–VPS35 cargo-selective retromer. Strumpellin probably interacts with the WASH complex via KIAA1033, but this remains to be proven. WASH interacts with, and activates, the actin-related protein 2 (ARP2)–ARP3 complex, which nucleates the formation of branched actin filaments. FAM21 has been proposed to interact with capping protein (CP) and promote its removal from the actin plus end, thus enhancing actin polymerization. **b** | In the absence of components of the WASH complex, early endosomal tubulation is enhanced, leading to the suggestion that the actin network generated by the complex is required to generate a pushing force on the tubule, which, combined with a pulling force generated by microtubule-based motors, promotes fission of the tubule by dynamin. The structure of the VPS26–VPS29–VPS35 complex is reproduced, with permission, from REF. 100 © (2007) Macmillan Publishers Ltd. All rights reserved.

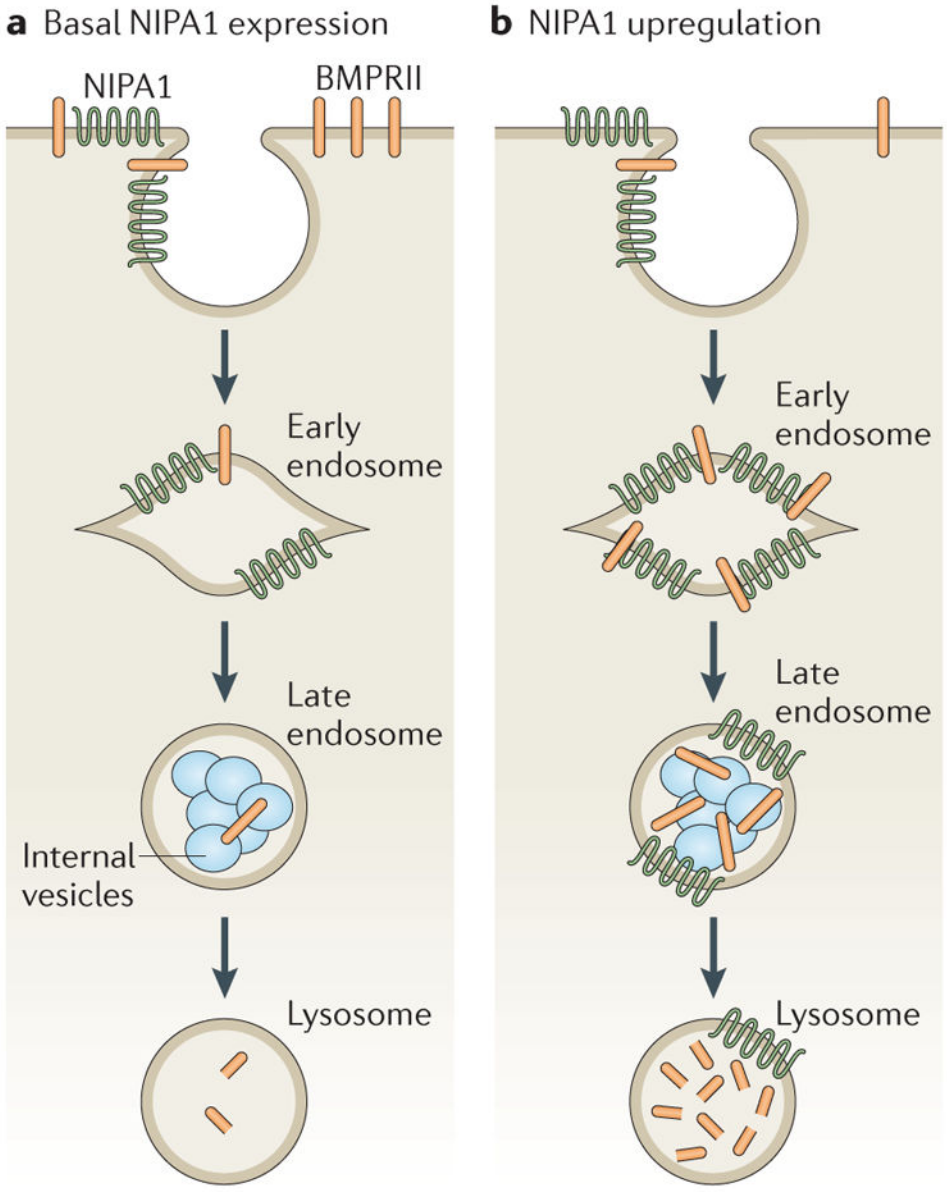

Figure 4. Model of NIPA1 action on bone morphogenetic protein receptor type-2 (BMPRII) traffic

a | In cells that express normal levels of non imprinted in Prader-Willi/Angelman syndrome 1 (NIPA1), most bone morphogenetic protein receptor type-2 (BMPRII) is situated on the plasma membrane, with small amounts in early and recycling endosomal compartments. NIPA1 is found in endosomal compartments and at the plasma membrane. Lysosomal inhibition and cellular depletion of NIPA1 both increase cellular BMPRII levels, suggesting that NIPA1 promotes degradation of BMPRII in the lysosome. **b** | Consistent with this idea, overexpression of NIPA1 causes dramatic internalization of BMPRII into endosomal and lysosomal compartments, accompanied by increased lysosomal degradation of BMPRII. Certain features in these diagrams are conjectural — for example, it is not known whether the initial interaction between NIPA1 and BMPRII occurs at the plasma membrane or

endosomes, whether NIPA1 is degraded in lysosomes or whether it can recycle back to the plasma membrane.

Table 1

Known HSP genes, divided into functional groups

Gene symbol	Protein name	Main phenotype	Cell biological function
Membrane traffic-related			
SPG3A[101]	Atlastin-1	AD Pure	• ER morphogenesis • BMP signalling
SPG4 (also known as *SPAST*)[11]	Spastin	AD Pure	• ER morphogenesis • Endosomal traffic • BMP signalling • Cytokinesis • Cytoskeletal regulation
SPG6 (also known as *NIPA1*)[102]	NIPA1	AD Pure	• Endosomal traffic • BMP signalling
SPG8 (also known as *KIAA0196*) [47]	Strumpellin	AD Pure	• Endosomal morphogenesis • Cytoskeletal regulation
SPG10 (also known as *KIF5A*) [82]	KIF5A	AD Complex	• Microtubule-based motor protein
SPG11 (REF. 103)	Spatacsin	AR Complex	• Membrane traffic?
SPG15 (also known as *ZFYVE26*)[104,105]	Spastizin (also known as ZFYVE26 or FYVE-CENT)	AR Complex	• Membrane traffic? • Cytokinesis
SPG17 (also known as *BSCL2*)[106]	Seipin	AD Complex	• ER membrane protein • Lipid droplet biogenesis
SPG20 (REF. 107)	Spartin	AR Complex	• Endosomal traffic • BMP signalling • Lipid droplet biogenesis • Mitochondrial?
SPG21 (REF. 108)	Maspardin	AR Complex	• Endosomal traffic
SPG31 (also known as *REEP1*)[109]	REEP1	AD Pure	• ER morphogenesis
Mitochondrial			
SPG13 (also known as *HSPD1*)[110]	HSP60	AD Pure	• Mitochondrial chaperone
SPG7 (REF. 111)	Paraplegin	AR Complex	• Mitochondrial protease
Myelination			
SPG2 (also known as *PLP1*)[112]	PLP	XLR Complex	• Myelin protein
SPG35 (also known as *FA2H*)[113,114]	Fatty acid 2-hydroxylase	AR Complex	• Hydroxylation of myelin lipids
Miscellaneous			
SPG1 (also known as *L1CAM*)[115]	L1CAM	XLR Complex	• Cell adhesion and signalling
SPG5 (also known as *CYP7B1*)[116]	CYP7B1	AR Pure	• Cholesterol metabolism
SPG39 (REF. 117)	Neuropathy target esterase	AR Complex	• Phospholipid homeostasis • Target of organophosphates
SPG42 (also known as *SLC33A1*)[118]	SLC33A1	AD Pure	• Acetyl-CoA transporter
SPG48 (also known as *KIAA0415*)[119]	KIAA0415	AR Complex	• DNA repair

AD, autosomal dominant; AR, autosomal recessive; BMP, bone morphogenetic protein; CYP7B1, 25-hydroxycholesterol 7-alpha-hydroxylase; ER, endoplasmic reticulum; FA2H, fatty acid 2-hydroxylase; HSP60, heat shock protein 60; KIF5A, kinesin heavy chain isoform 5A; L1CAM, neural cell adhesion molecule L1; NIPA1, non imprinted in Prader-Willi/Angelman syndrome 1; PLP, myelin proteolipid protein; REEP1, receptor expression-enhancing protein 1; SPG, spastic paraplegia; SLC33A1, solute carrier family 33 (acetyl-CoA transporter), member 1; XLR, X-linked recessive.