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review

Further assembly required: construction and dynamics of the endoplasmic reticulum network

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The endoplasmic reticulum (ER) is a continuous membrane system comprising the nuclear envelope, ribosome-studded peripheral sheets and an interconnected network of smooth tubules extending throughout the cell. Although protein biosynthesis, transport and quality control in the ER have been studied extensively, mechanisms underlying the notably diverse architecture of the ER have only emerged recently; this review highlights these new findings and how they relate to ER functional specializations. Several protein families, including reticulons and DP1/REEPs/Yop1, harbour hydrophobic hairpin domains that shape high-curvature ER tubules and mediate intramembrane protein interactions. Members of the atlastin/RHD3/Sey1 family of dynamin-related GTPases mediate the formation of three-way junctions that characterize the tubular ER network, and additional classes of hydrophobic hairpin-containing ER proteins interact with and remodel the microtubule cytoskeleton. Flat ER sheets have a different complement of proteins implicated in shaping, cisternal stacking and microtubule interactions. Finally, several shaping proteins are mutated in hereditary spastic paraplegias, emphasizing the particular importance of proper ER morphology and distribution for highly polarized cells.

Keywords: endoplasmic reticulum; hereditary spastic paraplegia; morphology; reticulon; shaping
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Introduction

The modern architectural principle that form follows function is equally applicable to organelles in eukaryotes. These cellular compartments have diverse but highly characteristic shapes that are typically conserved across species, which suggests that their structural features are tied closely to their roles in the cell. The endoplasmic reticulum (ER) is particularly notable for its obvious heterogeneity of construction and utility. It has crucial roles in the synthesis, modification, quality control and transport of integral membrane and soluble proteins destined for secretion; the sequestration and regulated release of Ca^{2+}; sterol synthesis; lipid synthesis and distribution; and detoxification (Baumann & Walz, 2001; Levine & Rabouille, 2005). Reflecting these many functions, the ER comprises a continuous membrane system that includes the inner and outer nuclear membranes, peripheral sheet-like structures and a network of interconnected tubules extending promiscuously into the cell periphery (Fig 1; Vedrenne & Hauri, 2006; Shibata *et al*, 2006, 2009; English *et al*, 2009; Sparkes *et al*, 2009). This architecture is evolutionarily retained broadly among eukaryotes, and this endomembrane system might have evolved by plasma membrane invaginations of the type seen in bacterial mesosomes.

Perhaps unsurprisingly, there exist prominent functional specializations among ER domains that correlate closely with these distinct morphologies. For example, peripheral ER sheets are often studded with polyribosomes—so-called rough ER (RER)—and are associated with the biosynthesis and quality control of integral membrane and secreted proteins. The first membrane transport step in the biosynthetic secretory pathway, the export of proteins and lipids from the ER, is mediated by COPII-coated vesicles, and this occurs through a subdomain in RER that is distinct biochemically and morphologically from the surrounding RER—the transitional ER (tER). Proteins and lipids are exported from these ribosome-free, smooth tER sites through COPII vesicles, for transport to the Golgi apparatus (Fig 1). In contrast to ER sheets, tubules comprise mostly smooth ER (SER) and are associated with lipid synthesis and delivery, establishing contact with other organelles, detoxification and lipid droplet formation.

Befitting the different roles of these domains, the ER can be markedly different across cell types. For instance, professional secretory cells such as pancreatic acinar cells exhibit extensive stacks of ribosome-studded ER sheets, which are responsible for the production and release of secreted proteins (Shibata *et al*, 2006). By contrast, liver hepatocytes are characterized by an extensive SER network that is mostly devoid of ribosomes but is enriched in enzymes for the metabolism and detoxification of biosynthetic products and exogenous substances. Uniquely, muscle cells are packed with a specialized form of SER known as the sarcoplasmic reticulum (SR), which is important for the sequestration of Ca^{2+} and its regulated release during muscle contraction. As a final example, the nuclear envelope (NE) is riddled with several thousand large, multimeric nuclear pore complexes (NPCs) that regulate protein transport between the cytoplasm and the nucleoplasm and help to stabilize the internal structure of the nucleus (Baumann & Walz, 2001; Shibata *et al*, 2006).

These morphological and functional variations of the ER do not capture fully the broad range of its specializations, as ER domains are also highly dynamic. The ER undergoes prominent shape transitions

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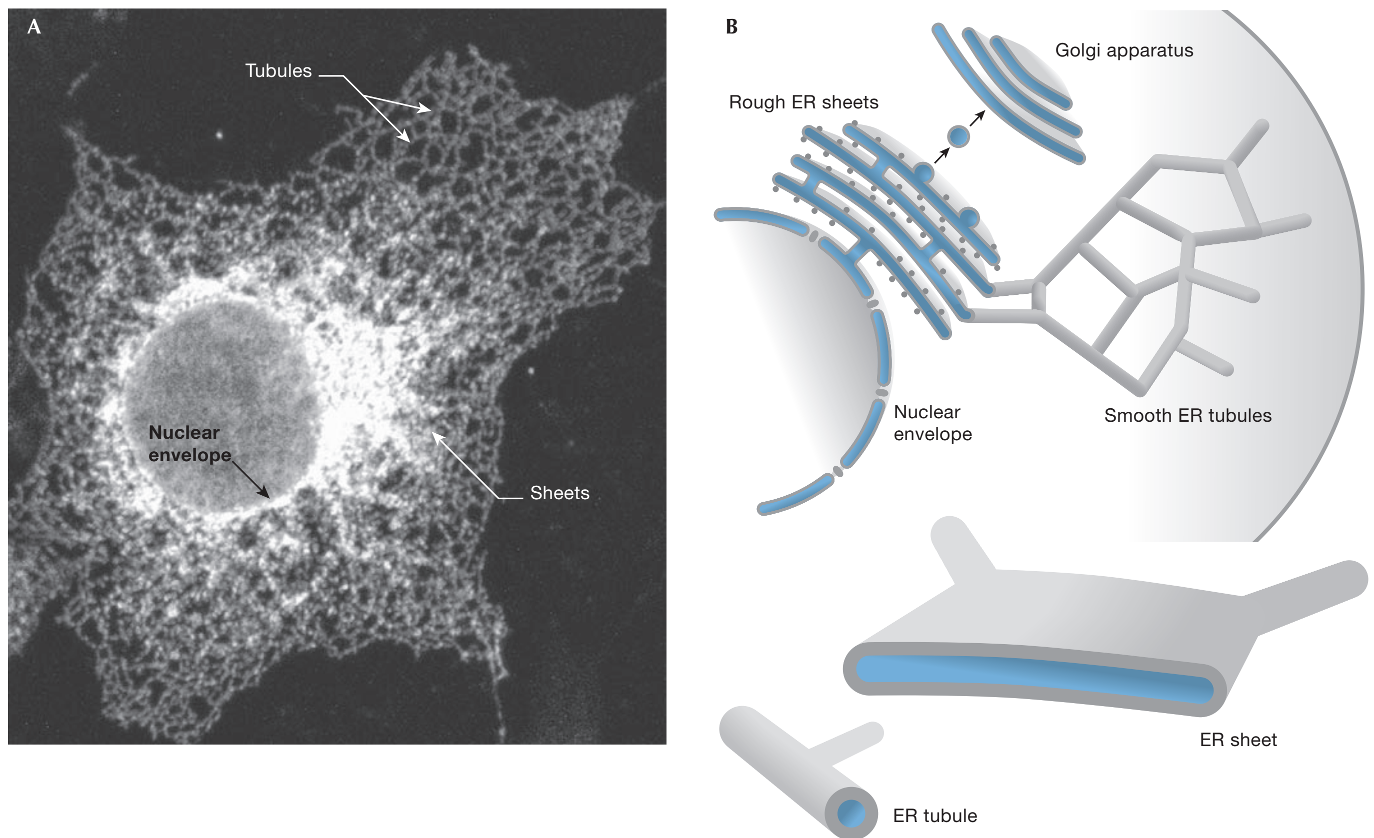

Fig 1 | Morphology of the endoplasmic reticulum. (**A**) A COS7 cell was immunostained with the ER markers Sec61β and DP1/REEP5 to reveal the different domains, as labelled. (**B**) Schematic diagram showing the domain organization of the ER, with tubular and sheet morphologies emphasized below. DP1, deleted in polyposis locus 1; ER, endoplasmic reticulum; REEP5, receptor expression-enhancing protein 5.

during events such as cell division and differentiation, and ongoing fusion reactions of ER tubules to form new three-way junctions have been observed in interphase cells (Lee & Chen, 1988; Prinz *et al*, 2000; Du *et al*, 2004; Puhka *et al*, 2007; Lu *et al*, 2009). In addition, the ER is interdependent with the cytoskeleton and other cellular organelles such as mitochondria, which also change constantly. In neurons, ER shape changes in dendritic spines both influence and are regulated by synaptic signalling pathways (Spacek & Harris, 1997; Park *et al*, 2008). In the dynamic variability of ER morphology, it is implicit that mechanisms must exist to shape the various ER domains. Identifying the machineries that mediate and regulate ER shape is crucial for addressing these questions, and we summarize here the latest insights into these dynamic processes.

Bending and fusing tubules; flattening sheets

Much of the morphological variation exhibited by ER domains is the result of differences in the curvature of the membranes—a feature that enables domains to maintain distinct shapes despite being physically continuous. In this context, it seems that mechanisms behind the shapes of the various ER domains are conserved among eukaryotic cells, with striking cross-species similarity. The luminal diameters of ER tubules, for example, are similar across a range of organisms: about 30 nm in yeast up to around 60–100 nm in mammalian cells—for reference, the thickness of the phospholipid bilayer is about 4 nm. Similarly, ER sheets in most species are similar in luminal thickness to the tubules but can extend several micrometres and curve only at the edges. Finally, although the NE is spherical in cells, its large diameter of around 5–8 μm means that the inner and outer nuclear membranes take on a sheet-like appearance with little curvature (Shibata *et al*, 2009).

Lipid bilayers tend to remain relatively flat because generating curvature requires energy. As such, mechanisms must exist to both generate and stabilize curvature. Although lipids alone could suffice if the composition were highly asymmetrical, there is little evidence that this occurs *in vivo* and proteins are widely held to be responsible for shaping the ER (Zimmerberg & Kozlov, 2006; Shibata *et al*, 2009). Indeed, proteins participate in a number of mechanisms that generate high-curvature tubules. These include membrane deformation by force-generating proteins (for example, molecular motors), bending tubules by using scaffolding formed from highly curved protein networks that interact with the phospholipid bilayer, and hydrophobic insertion of proteins into the outer leaflet of the bilayer, curving the ER in on itself (Shibata *et al*, 2009). Each of these mechanisms might be involved in shaping ER tubules, and they are not mutually exclusive. In fact, ER tubules can be formed by membranes sliding along microtubules or attached to polymerizing microtubules. In this context, TACs, in association with polymerizing microtubules, pull the membrane in the plus-end direction and motor proteins mediate transport along established microtubules (Waterman-Storer & Salmon, 1998).

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Within these and other scenarios, it is important to determine how ER tubules are ultimately stabilized. Pioneering work by Rapoport and colleagues identified several classes of protein that seem necessary and sufficient for the generation and maintenance of ER tubules in eukaryotic cells and lipid tubules *in vitro* (Voeltz *et al*, 2006; Hu *et al*, 2008; Shibata *et al*, 2008). These ER-shaping proteins comprise two main protein families, the DP1/REEPs/Yop1 proteins and the reticulons, which share little overall homology but do exhibit an important common structural feature—paired, elongated hydrophobic segments that appear to form partially membrane-spanning hairpin domains (Fig 2; Voeltz *et al*, 2006). In these extended hydrophobic domains are charged amino acids or proline residues, and the composition and length of these segments might be important both for targeting to different ER domains and for shaping them (Letourneur & Cosson, 1998; Voeltz *et al*, 2006; Ronchi *et al*, 2008). It is predicted that the bulk of the hydrophobic portions of these ER-shaping proteins resides in the outer leaflet of the phospholipid bilayer, and thus they might induce curvature by hydrophobic wedging. As these proteins form large, immobile oligomers (Shibata *et al*, 2008), scaffolding might also have a role in curvature induction and stabilization. Finally, although the hydrophobic hairpins have important roles in mediating the interactions required for the formation of these large multimeric structures (Voeltz *et al*, 2006; Shibata *et al*, 2008), some specificity might be provided by interactions among their cytoplasmic domains as many of these proteins can be crosslinked extensively using hydrophilic, membrane-impermeable crosslinkers.

Several lines of evidence indicate that these ER-shaping proteins are sufficient to generate the diverse ER morphologies observed. Overexpression of the ER-shaping protein reticulon 4a (Rtn4a) leads to prominent tubules and the disappearance of ER sheets (Voeltz *et al*, 2006; Shibata *et al*, 2009). Similarly, in plant cells the overexpression of reticulons markedly reduced the luminal diameter of ER tubules, probably by further increasing curvature (Tolley *et al*, 2008). Conversely, depletion of tubule-shaping proteins of the DP1/REEP/Yop1 and reticulon families in mammalian or yeast cells converts tubules to sheets (Voeltz *et al*, 2006; Anderson & Hetzer, 2008). A direct demonstration of the tubule-shaping properties of these proteins was provided by Hu *et al* (2008), who showed that recombinant yeast Yop1 and Rtn1 deformed a lipid bilayer into 15–17 nm proteolipid tubules *in vitro*.

Generating highly curved ER tubules is only part of the equation, however, as the tubular ER forms an interconnected network that ultimately requires the fusion of tubules. Several proteins have been proposed to mediate this fusion, including the p97/p47/VCIP135 complex, syntaxin 18 and BNIP1/Sec20 (Vedrenne & Hauri, 2006). More recent work has highlighted the important role of the atlastin family of dynamin-related GTPases in the formation of the ER network. These proteins are large, multimeric, integral membrane GTPases that localize predominantly to the tubular ER, but also to ERGIC and *cis*-Golgi apparatus (Zhu *et al*, 2003, 2006; Rismanchi *et al*, 2008). In mammals, there are three closely related atlastins, atlastin 1/2/3, each of which harbours an amino-terminal GTP-binding domain and two closely spaced hydrophobic segments near the carboxyl terminus (Fig 2; Rismanchi *et al*, 2008). The atlastin family of GTPases is ubiquitous and includes the functional orthologues Sey1 in *Saccharomyces cerevisiae* and RHD3 in *Arabidopsis thaliana* (Hu et al, 2009). These large GTPases interact with ER-shaping proteins of both the DP1/REEP/Yop1 and the reticulon families and are required for the formation of three-way junctions in the ER (Rismanchi *et al*, 2008; Barlowe, 2009; Farhan & Hauri, 2009; Hu *et al*, 2009). Why does the cell need three-way ER junctions? Surprisingly, the reasons are largely unknown. One possibility is that this arrangement might maximize the coverage of ER tubules in the cytoplasm, positioning them for intimate contact with the plasma membrane, cytoskeleton, organelles and signalling complexes as needed—see Sidebar A for more outstanding questions.

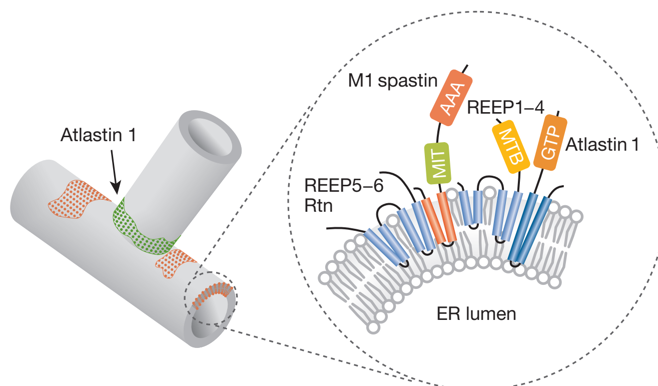

Fig 2 | Schematic diagram of the tubular endoplasmic reticulum. Left: ER-shaping reticulon and DP1/REEPs/Yop1 proteins form large oligomers that shape ER tubules (Shibata *et al*, 2009). Atlastin/RHD3/Sey1 proteins are shown at three-way junctions. Right: Proposed membrane topologies for proteins involved in shaping the tubular ER network. DP1, deleted in polyposis locus 1; ER, endoplasmic reticulum; REEP, receptor expression-enhancing protein; RHD3, root hair defective 3; Rtn, reticulon; Sey1, synthetic enhancer of Yop1. Adapted with permission from Park *et al* (2010).

Glossary

ALS	amyotrophic lateral sclerosis
BNIP1	Bcl2/adenovirus E1B 19 kDa interacting protein 1
CHO	Chinese hamster ovary
CLIMP63	cytoskeleton linking membrane protein of 63 kDa
COPII	coat protein complex II
DP1	deleted in polyposis locus 1
EF-hand	helix–loop–helix structural domain in Ca^{2+}-binding proteins
ERGIC	ER–Golgi intermediate complex
FAM134	family with sequence similarity 134
Grp75	glucose-regulated 75 kDa protein
HSAN	hereditary sensory and autonomic neuropathy
IP_3	inositol trisphosphate
Mdm10/12/34	mutants defective in mitochondrial distribution 10/12/34
Mfn2	mitofusin 2
Mmm1	maintenance of mitochondrial morphology 1
PACS	phosphofurin acidic cluster sorting protein
REEP	receptor expression-enhancing protein
RHD	root hair defective
Sey1	synthetic enhancer of yop1
STIM1	stromal interaction molecule 1
TAC	tip attachment complex
VAP	vesicle-associated membrane-protein associated protein
VCIP135	p97/p47 complex-interacting protein of 135 kDa
VDAC	voltage-dependent anion-selective channel
Yop1	Yip one partner 1

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Sidebar A | In need of answers

(i) How are atlastin/RHD3/Sey1 GTPases functionally regulated?
(ii) How is the specificity of interactions determined among different hydrophobic hairpin-containing proteins in the tubular ER?
(iii) Why do cells need a tubular ER? Do three-way junctions represent important functional domains?
(iv) What are the molecular mechanisms underlying atlastin GTPase-dependent formation of three-way junctions?
(v) Do FAM134 proteins constitute a new family of organelle-shaping proteins?
(vi) What are the actual structures of the ER-shaping proteins and atlastin/Sey1 within the membrane?

Consistent with their role in ER network formation, the atlastins and Sey1 localize to distinct puncta along ER tubules, including at three-way junctions (Fig 3; Hu *et al*, 2009; Park *et al*, 2010). A recent study by Orso *et al* (2009) demonstrated the role of atlastin GTPases in homotypic ER membrane fusion in *Drosophila melanogaster*. In fact, atlastin was able to form *trans*-oligomeric complexes and promote membrane fusion of proteoliposomes in a GTPase-dependent manner *in vitro*. Thus, atlastin mediates a GTP-dependent tethering step through *trans*-oligomer formation, followed by fusion of the bilayers (Orso *et al*, 2009). Further studies are required to uncover the structural basis of this fusion, and whether other proteins such as SNAREs participate in this process *in vivo*.

Atlastins interact directly with the AAA ATPase spastin, which is involved in microtubule severing (Evans *et al*, 2006; Sanderson *et al*, 2006), thus physically linking membrane and cytoskeletal remodelling proteins. This interaction occurs between the paired hydrophobic domain of atlastins and a hydrophobic domain of 23 amino-acid residues present only in its larger M1 spastin isoform (Park *et al*, 2010). Protease protection assays indicate that this segment might also exist as an intramembrane hairpin, and it harbours one internal charged residue (Park *et al*, 2010). In addition to interacting with tubule-shaping reticulons, atlastins and M1 spastin interact with DP1/REEPs in the tubular ER (Connell *et al*, 2009; Park *et al*, 2010). This latter family comprises six members in humans—REEP1–6 (REEP5 is also known as DP1)—and until recently, all were considered to be functionally similar. However there are clear distinctions between REEP1–4 and REEP5–6, both phylogenetically and structurally: the yeast ER-shaping protein Yop1 is more similar to REEP5–6 (Park *et al*, 2010), whereas REEP1–4 proteins have hydrophobic hairpins but also interact with microtubules through an extended C-terminal cytoplasmic domain enriched in basic amino acids (Park *et al*, 2010). Although interactions among REEPs, atlastins and M1 spastin through hydrophobic segments provide a compelling mechanism for coupling ER membrane remodelling with cytoskeletal dynamics, it is unclear whether these proteins are components of structures such as the TACs.

ER sheets have a similar degree of curvature at their edges as do ER tubules, possibly owing to the presence of ER-shaping reticulons (Shibata *et al*, 2009), but mechanisms used to form and stabilize the sheet-like morphology remain less well understood. The constant luminal thickness of extensive ER sheets might be stabilized through several means including polyribosome complexes or intraluminal bridges formed through proteins such as CLIMP63, which forms large oligomeric complexes in ER sheets (Klopfenstein *et al*, 2001; Shibata *et al*, 2006, 2009). Stacks of sheets might also be stabilized through protein interactions at the cytoplasmic face (Snapp *et al*, 2003). Clarification of the proteins mediating ER sheet formation and stabilization is an important area of future investigation. The NE has the overall appearance of a sheet, except in the areas abutting the NPCs, which are highly curved. ER-shaping proteins of the reticulon and DP1/REEP/Yop1 families are required for nuclear pore formation (Dawson *et al*, 2009), probably because of their membrane-curving functions. Flatter areas of the NE are stabilized by interactions of nuclear membrane proteins with chromatin and the nuclear lamina (Farhan & Hauri, 2009). Thus, the formation of stabilizing complexes that assemble and help to maintain the degree of curvature of an ER domain is a common mechanistic theme.

Implicit in the maintenance of the different ER domains is the proper segregation of shaping proteins. In this regard, the hydrophobic hairpins seem to function as ER tubule-sensing as well as shaping motifs. These proteins are excluded from peripheral sheets and the NE—even when overexpressed—through a combination of stabilizing interactions and their curve-inducing properties. Hydrophobic segments of the atlastins and M1 spastin that localize to ER tubules and the hydrophobic hairpin domains in reticulons and DP1/REEPs/Yop1 are required and sufficient for this distribution (Voeltz *et al*, 2006; Hu *et al*, 2009). Thus, a mechanism by which to alter the proportions of ER sheets and tubules in cells is through modulating the levels of various ER-shaping proteins, which supports a fundamental role for these proteins in generating and maintaining ER shape (Shibata *et al*, 2009).

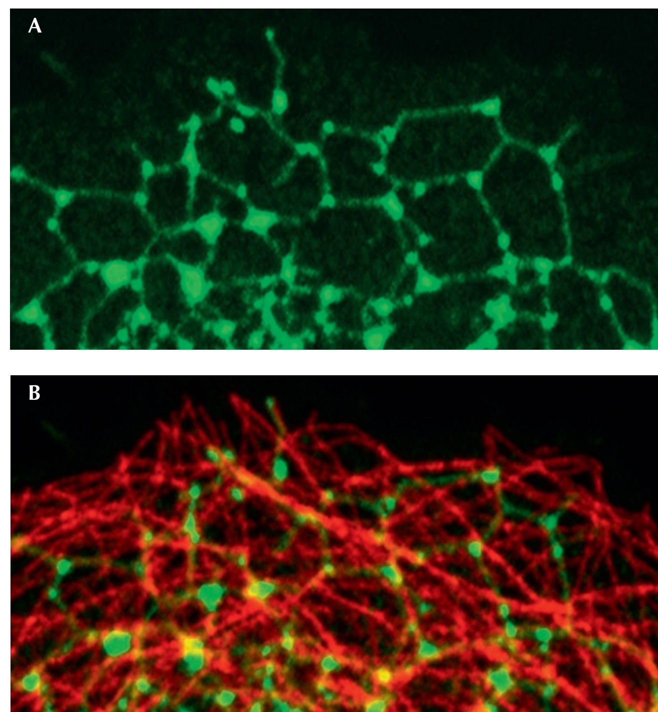

Fig 3 | Interdependence of endoplasmic reticulum tubules and microtubules. (**A**) Atlastin 1 GTPase overexpressed in COS7 cells shows punctate enrichment along ER tubules (green) in the cell periphery, including at three-way junctions. (**B**) Microtubules are identified by co-immunostaining for β-tubulin (red). ER, endoplasmic reticulum. Adapted with permission from Park *et al* (2010).

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Cytoskeletal and organellar interactions

Another way in which ER morphological domains and their spatial distribution in the cell might be stabilized is through interactions with the cytoskeleton, plasma membrane or other organelles such as mitochondria. In animal cells, there is a close association of the ER with the microtubule cytoskeleton, and the ER is formed along microtubules by several mechanisms (Terasaki *et al*, 1986; Waterman-Storer & Salmon, 1998). In plant cells and yeast, the ER is associated with actin fibres (Prinz *et al*, 2000; Du *et al*, 2004). However, the cytoskeleton is not absolutely required for tubule formation, and an interconnected tubular network can be generated from a *Xenopus laevis* microsomal membrane fraction in the absence of microtubules (Fig 4; Dreier & Rapoport, 2000). Even so, cytoskeletal interactions are important for the characteristic appearance of ER in cells, as disruption of the microtubule cytoskeleton with nocodazole causes the collapse of the ER by retraction from the cell periphery and conversion of peripheral ER tubules to extended sheet-like structures (Lu *et al*, 2009). In animal cells, CLIMP63 might mediate the attachment of the sheets to the microtubules (Klopfenstein *et al*, 1998). In a complementary manner, spastin and the REEP1–4 class of proteins probably mediate the interaction of ER tubules with the microtubule cytoskeleton (Park *et al*, 2010).

Stabilization of the ER network might occur in part through interactions with other cellular organelles. Such interactions might also be important for the interorganelle exchange of lipids, and a large number of proteins have been implicated in this regard. Mitochondrial–ER attachment sites are among the most extensively studied; these organelles are among the most abundant and their interorganellar transfer of Ca^{2+} and phospholipids is an area of intense interest. Proteins including Mfn2, the IP_3 receptor, VDAC, the chaperones Grp75 and sigma 1 receptor, and the sorting protein PACS2 have been implicated in ER–mitochondrial interactions over the years (Giorgi *et al*, 2009). More recently, a synthetic biology screen in *S. cerevisiae* revealed that a complex containing the four proteins Mmm1 and Mdm10/12/34 acts as a molecular tether between the ER and mitochondria (Kornmann *et al*, 2009). Similarly, interactions of the ER with the plasma membrane are of interest for their role in responding to the depletion of ER Ca^{2+} stores. In particular, STIM1 in the ER acts as a Ca^{2+} sensor that oligomerizes in response to Ca^{2+} depletion, translocates to the plasma membrane through a polybasic C-terminal domain, and activates store-activated Ca^{2+} influx to replenish stores (Liou *et al*, 2007). It is important to establish how such connections with the ER are mediated and regulated for other organelles as such interactions are a powerful means for the ER to communicate with the rest of the cell (Park *et al*, 2008).

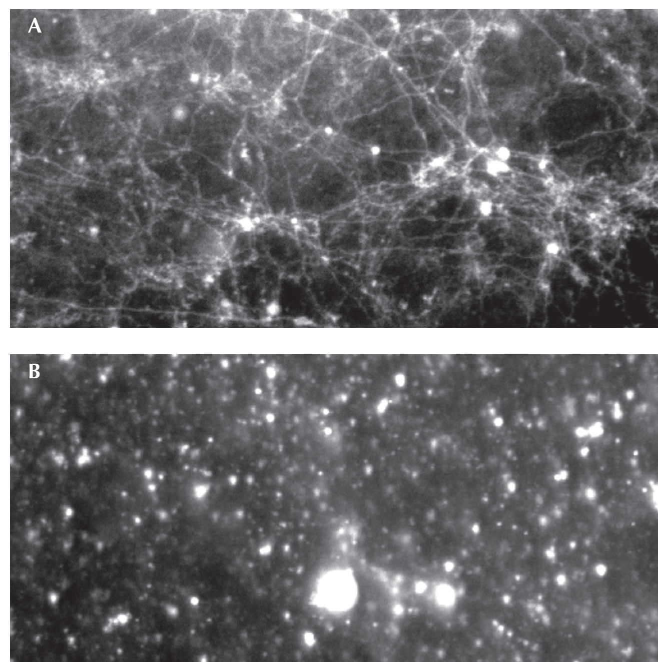

Fig 4 | *In vitro* generation of a tubular endoplasmic reticulum network. (**A**) An ER network can be generated from a *Xenopus* microsomal fraction on addition of ATP and GTP in the absence of microtubules. (**B**) Network formation is inhibited by neutralizing atlastin antibodies. Similar inhibition is seen with neutralizing antibodies against reticulons and DP1/REEPs, but not against ER proteins such as IP_3 receptor (Voeltz *et al*, 2006; Park *et al*, 2010). DP1, deleted in polyposis locus 1; ER, endoplasmic reticulum; IP_3, inositol trisphosphate; REEP, receptor expression-enhancing protein. Images are courtesy of Dr Peng-Peng Zhu.

ER network defects and neurological disease

Highly polarized cells present special challenges for the ER. A remarkable example of the importance of ER morphology and distribution in highly polarized cells is provided by a class of human diseases known as the hereditary spastic paraplegias, which are characterized by a length-dependent axonopathy of the corticospinal motor neurons, which can reach up to 1 m in length. Although these disorders comprise more than 40 distinct genetic loci (*SPG1–45*), the most common forms, representing about 60% of all affected individuals, are due to autosomal dominant mutations in one of three proteins already discussed for their roles in ER network formation: spastin (SPG4), atlastin 1 (SPG3A) and REEP1 (SPG31; Salinas *et al*, 2008). These proteins interact directly with one another and are localized to the ER in the corticospinal neurons that are important for disease pathogenesis, where they mediate ER shaping and interactions of the tubular ER network with the microtubule cytoskeleton (Park *et al*, 2010).

Other recent studies have highlighted a possible broader role for shaping proteins in neurological disease. Kurth *et al* (2009) identified loss-of-function mutations in FAM134B—a *cis*-Golgi apparatus-enriched member of the FAM134 family of proteins that also includes FAM134A and FAM134C—in patients with HSAN2, a severe hereditary sensory and autonomic neuropathy. The FAM134 proteins each have a pair of long hydrophobic segments reminiscent of those in the reticulons and DP1/REEPs/Yop1. Furthermore, FAM134B depletion in cells causes prominent changes in Golgi morphology in neurons and neuron-like cells—although ER morphology was not examined (Kurth *et al*, 2009). More work is needed to establish whether these proteins have direct shaping functions and, if so, for which organelles. Even so, these disorders might highlight the significance of morphological defects in the ER and the early secretory pathway in the pathogenesis of length-dependent axonopathies.

Additional studies suggest a role for ER-shaping mechanisms in the pathogenesis of other related neurological disorders. In the superoxide dismutase 1 (SOD1) G93A transgenic mouse model for ALS, which involves both corticospinal and lower motor neurons,

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the ER-shaping protein RTN4A selectively redistributed the ER chaperone protein disulphide isomerase intracellularly, and this RTN4A overexpression protects against neurodegeneration. Conversely, knockout of RTN4A/B on this SOD1 G93A background worsened disease in mice (Yang *et al*, 2009). Further supporting a role for ER morphogenesis in disease pathogenesis, the VAPB mutant P56S, which underlies another familiar form of ALS, is associated with the production of a novel form of organized SER (Fasana *et al*, 2010). Together, these studies identify tantalizing links that might support a converging neurological disease mechanism comprising alterations of ER shape.

Dynamic changes in ER shape

Much of this review has emphasized how proteins establish and maintain the morphology of ER domains. However, the ER is highly dynamic and undergoes continuous fusion reactions and interactions with the cytoskeleton and other organelles as well as transitions between tubules and sheets. It is well known that microtubules are involved in the distribution and formation of ER tubules, and that the cytoskeleton is involved in transport of ER-derived vesicles. One candidate regulatory protein is the cytoplasmic protein p22, an EF-hand Ca^{2+}-binding protein that binds to microtubules in an *N*-myristoylation-dependent manner and exhibits increased interactions with microsomes in a Ca^{2+}-dependent manner (Andrade *et al*, 2004). More marked changes in morphology occur during cellular events such as fertilization and cell division. For instance, within minutes after fertilization, the ER in starfish eggs becomes extensively fragmented (Terasaki *et al*, 1996). This is accompanied by release of Ca^{2+} from internal stores, and in fact Ca^{2+}-induced reversible ER fragmentation has been reported in cell lines and neurons (Subramanian & Meyer, 1997; Kucharz *et al*, 2009), prefiguring a role for signalling pathways in the modification of ER morphology.

A prominent change in ER morphology occurs during cell division. Although Puhka *et al* (2007) reported that the ER of CHO cells loses its sheet-like structure and becomes almost completely tubular during mitosis, a more recent study by Lu *et al* (2009) demonstrated that, from prometaphase to telophase in various mammalian cell types, most of the ER is organized as extended cisternae. This tubule-to-sheet transition is reminiscent of the changes in ER morphology upon nocodazole-induced microtubule depolymerization in interphase cells, providing additional support for a fundamental role of the microtubule cytoskeleton in regulating ER morphology and distribution (Lu *et al*, 2009).

Conclusion

With a host of proteins now identified in crucial roles in the generation and shaping of the ER network, the stage is set not only for the identification of additional proteins, but also for investigations into the mechanisms regulating ER morphology and distribution in the cell. It is tempting to speculate that signalling pathways linked to modifications of several of these proteins will have important roles in modulating the structure of the ER in cells, dynamically positioning functional ER domains. Finally, as ER morphology defects seem to be the prominent mechanism underlying the length-dependent axonopathies known as hereditary spastic paraplegias, the identification of additional genes for these disorders, as well as other heritable long axonopathies, might continue to provide new tools to deconstruct the ER network.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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