Untangling the web: Mechanisms underlying ER network formation

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Abstract

The ER is a continuous membrane system consisting of the nuclear envelope, flat sheets often studded with ribosomes, and a polygonal network of highly-curved tubules extending throughout the cell. Although protein and lipid biosynthesis, protein modification, vesicular transport, Ca^{2+} dynamics, and protein quality control have been investigated in great detail, mechanisms that generate the distinctive architecture of the ER have been uncovered only recently. Several protein families including the reticulons and REEPs/DP1/Yop1p harbor hydrophobic hairpin domains that shape high-curvature ER tubules and mediate intramembrane protein interactions. Members of the atlastin/RHD3/Sey1p family of dynamin-related GTPases interact with the ER-shaping proteins and mediate the formation of three-way junctions responsible for the polygonal structure of the tubular ER network, with Lunapark proteins acting antagonistically. Additional classes of tubular ER proteins including some REEPs and the M1 spastin ATPase interact with the microtubule cytoskeleton. Flat ER sheets possess a different complement of proteins such as p180, CLIMP-63 and kinectin implicated in shaping, cisternal stacking and cytoskeletal interactions. The ER is also in constant motion, and numerous signaling pathways as well as interactions among cytoskeletal elements, the plasma membrane, and organelles cooperate to position and shape the ER dynamically. Finally, many proteins involved in shaping the ER network are mutated in the most common forms of hereditary spastic paraplegia, indicating a particular importance for proper ER morphology and distribution in large, highly-polarized cells such as neurons.

Keywords

Atlastin; Endoplasmic reticulum; Hereditary spastic paraplegia; Morphology; REEP; Reticulon; Shaping

1. Introduction

It is the pervading law of all things organic and inorganic,

Of all things physical and metaphysical,

Of all things human and all things super-human,

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Of all true manifestations of the head,

Of the heart, of the soul,

That the life is recognizable in its expression,

That form ever follows function.This is the law.

■ Louis Sullivan, architect [1]

Cellular organelles have diverse but highly characteristic shapes that are typically conserved across species, suggesting that their structural features are intimately tied to their specific roles within the cell. The ER is particularly striking for its heterogeneity of form and function. It plays critical roles in the synthesis, modification, quality control, and trafficking of integral membrane proteins and soluble proteins destined for secretion, the mobilization and regulated release of Ca^{2+}, sterol/lipid synthesis and distribution, signaling, carbohydrate metabolism, and detoxification of harmful substances [2]. Reflecting these diverse functions, the ER comprises a continuous membrane system that includes the inner and outer nuclear membranes, sheet-like cisternae, and a network of interconnected tubules extending promiscuously into the cell periphery (Figs. 1 and 2A) [3-8]. The ER is the largest continuous organelle, with its membranes comprising about half of the total membrane and its lumen enclosing about 10% of the volume of a typical eukaryotic cell [9].

Prominent functional specializations appear to correlate with the distinct morphologies of the ER. For instance, ER sheets studded with polyribosomes (so-called "rough" ER) are associated with the biosynthesis, modification, and quality control of secreted and integral membrane proteins. The initial membrane trafficking step in the biosynthetic secretory pathway, the export of proteins and lipids from the endoplasmic reticulum (ER), is mediated by COPII-coated vesicles arising from specialized ER exit sites for trafficking to the Golgi apparatus. By contrast with ER sheets, tubules are mostly smooth ER and associated with lipid synthesis and delivery, metabolism of carbohydrates, establishing contacts with other organelles, detoxification, and lipid droplet formation [3].

Befitting the distinct functional specializations of these domains, the ER can appear drastically different across cell types. For instance, professional secretory cells such as pancreatic acinar cells and plasma cells harbor abundant stacks of ribosome-studded ER sheets which are involved in the production and secretion of proteins, up to thousands of molecules per second. On the other hand, hepatocytes exploit an extensive smooth ER network mostly devoid of ribosomes but enriched in enzymes for the metabolism of carbohydrates and detoxification of biosynthetic products as well as exogenous substances such as drugs and poisons. Muscle cells possess a specialized form of smooth ER, the sarcoplasmic reticulum, that is important for the mobilization and regulated release of Ca^{2+} into the cytoplasm during muscle contractions. Retinal pigment epithelial cells exhibit stacked ER sheets mostly devoid of ribosomes, the organized smooth ER. As a last example, the nuclear envelope is pierced by several thousand large, multimeric nuclear pore complexes that regulate trafficking between the cytoplasm and the nucleoplasm and help to stabilize the internal structure of the nucleus [8, 10].

These morphologic variations still do not encompass fully the broad range of ER specializations, as the ER is highly dynamic. It undergoes prominent shape transitions during events such as cell division and differentiation, and fusion reactions of ER tubules to form new three-junctions have been observed in interphase cells across many cell types and species [11-15]. The ER interacts with the cytoskeleton and the plasma membrane as well as other dynamic cellular organelles such as endosomes and mitochondria [4, 16-18]. In highly-polarized and specialized neurons, ER shape changes in dendritic spines both influence and are regulated by synaptic signaling pathways [19-21]. Implicit in this dynamic

variability is the general concept that many different types of cellular signals are able to trigger shape changes across different ER domains.

2. Mechanisms generating high membrane curvature in the ER

The different morphologies of the ER derive in part from variations in membrane curvature across different domains, enabling them to maintain distinct shapes while remaining physically continuous. In fact, such mechanisms are broadly conserved among eukaryotic cells. The luminal diameters of ER tubules are generally similar across a range of organisms and cell types, ranging from about 30-100 nm (for reference, the thickness of the phospholipid bilayer is ~4 nm). Likewise, ER sheets across most species are similar in luminal thickness (50-100 nm) to the diameter of tubules but can extend several micrometers and only curve prominently at the edges [22]. Finally, although the nuclear envelope appears spherical in cells, its large diameter of about 6-10 μm means that the inner and outer nuclear membranes take on a sheet-like appearance, with areas of high curvature mostly restricted to the edges of nuclear pores [3, 10, 23].

Although asymmetric distribution of lipids between the two leaflets of the lipid bilayer could introduce high membrane curvature, this mechanism seems not to be used in the ER. Instead, proteins are widely implicated in generating ER curvature [4, 6, 23, 24]. Generally speaking, proteins can generate and stabilize high-degree curvature in cellular membranes through a variety of means: membrane deformation by force-generating proteins (e.g., molecular motors); bending tubules through highly-curved protein scaffolds that interact with the phospholipid bilayer; protein-protein crowding; and hydrophobic insertion of proteins into the outer leaflet of the bilayer (hydrophobic wedging) [23, 25, 26]. ER tubules can be formed by membranes sliding along microtubules or attached to polymerizing microtubules. In this context, specialized tip attachment complexes (TACs) associated with polymerizing microtubules cooperate to pull the nascent tubules in the plus-end direction, while molecular motors mediate tubular extension along established, acetylated microtubules (Fig. 2B) [27, 28].

Under these and related scenarios, it is imperative to establish how ER tubules are stabilized. Several classes of proteins appear necessary and sufficient for the generation and maintenance of ER tubules in eukaryotic cells and lipid tubules *in vitro* [29-35]. These "ER-shaping," curvature-stabilizing proteins comprise two main families, the DP1/REEPs/Yop1p proteins and the reticulons. Though these families show little overall sequence homology, they share an important structural signature—elongated hydrophobic segments that are predicted to form partially membrane-spanning hairpin (or wedge) domains (Fig. 2C) [29]. Within these extended hydrophobic domains are often charged amino acids or proline residues, and the composition and length may be important for both targeting to different ER domains and shaping them [29, 32, 33, 36]. The bulk of the hydrophobic portions of these ER-shaping proteins appear to occupy the outer leaflet of the phospholipid bilayer, possibly generating curvature via hydrophobic wedging. The reticulons form large, immobile oligomers [31, 33], so scaffolding, protein-protein crowding, or both might also play a role in curvature generation. Last, although the hydrophobic hairpins play key roles in mediating intramembrane interactions required for the formation of these large structures [29, 31], specificity may be also provided by interactions among cytoplasmic domains [3].

Multiple types of evidence indicate that these ER-shaping proteins are able to generate highly-curved ER morphologies. Overexpression of reticulon-4a (Rtn4a) in mammalian cells leads to more prominent tubules and the depletion of ER sheets [23, 29]. Similarly, in plant cells overexpression of reticulons dramatically reduces the luminal diameter of ER tubules, most likely by increasing curvature [34]. Conversely, depleting ER-shaping proteins

of the DP1/REEP/Yop1p and reticulon families from mammalian or yeast cells drives an increase in sheets at the expense of tubules [29, 37]. A seminal demonstration of the tubule-shaping properties of these proteins was provided by Hu et al. [30], who showed that recombinant yeast Yop1p and Rtn1p are sufficient to deform proteoliposomes *in vitro* into tubules with a diameter of 15-17 nm. On the other hand, simultaneous depletion of reticulons and Yop1p in yeast does not prevent ER tubules from being pulled from a cisterna into the bud, emphasizing a primary *in vivo* role in curvature stabilization and maintenance of peripheral ER tubules [35].

3. Formation and stabilization of ER sheets

Flat ER sheets do not require stabilization and can form spontaneously, but they have a degree of curvature along their edges similar to that of ER tubules, possibly due to the localized enrichment of curvature-stabilizing reticulons [23, 38]. The relatively constant luminal thickness of extensive ER sheets appears to be maintained through a number of means, with proteins including CLIMP-63, p180, and kinectin playing important roles. Each of these proteins is enriched in ER sheets, and their overexpression induces sheet proliferation. Simultaneous depletion of all of these proteins does not eliminate ER sheets, though those that remain exhibit a decrease in luminal width [38]. Additional players such as polyribosome complexes may also play a role in sheet stabilization [8, 38].

The mechanisms underlying sheet stabilization are becoming increasingly clear. CLIMP-63, kinectin, and p180 each contain coiled-coil domains, and the large domains of CLIMP-63 within the ER lumen form intraluminal bridges that can stabilize a constant sheet width [39]. By contrast, the coiled-coil domains of p180 and kinectin are cytoplasmic, and may form long rods which stabilize the flatness of the sheets (Fig. 2D) [8]. Stacks of sheets may be further stabilized through protein interactions at the cytoplasmic face (Fig. 1D) [40].

4. Enveloping the nucleus

The nuclear envelope consists of the outer and inner nuclear membranes that connect at the sites of nuclear pores but are otherwise separated from one another in sheet-like structures [10, 41]. This separation is about 40-50 nm, thinner than the distance separating the two membranes of a typical peripheral ER sheet. Proteins of the reticulon and DP1/REEP/Yop1p families are required for nuclear pore formation [42], likely because of their membrane-curving and stabilizing functions, and the nucleoporin core complex is also likely involved (Fig. 3) [23]. Flatter areas of the nuclear envelope are stabilized by interactions of nuclear membrane proteins with chromatin and the nuclear lamina [10]. In particular, the linker of nucleoskeleton and cytoskeleton (LINC) complex plays a key role in holding the distance between the outer and inner nuclear membranes constant. This complex consists of Sad1 and UNC-84 (SUN) in the inner nuclear membrane and KASH (klarsicht, ANC-1, and syne/nesprin homology) domain proteins that are inserted into the outer nuclear membrane. SUN proteins (e.g., Sun 1 and Sun 2) interact with the KASH domain proteins (e.g., Nesprins), generating a protein bridge that connects the outer and inner nuclear membranes and may link the nuclear lamina to the cytoplasmic cytoskeleton (Fig. 3) [8, 43].

5. Fusing the network

Previous sections of this review have emphasized how the formation and maintenance of ER morphology depends on the proper segregation of various types of shaping proteins. But producing highly-curved ER tubules is only one part of shaping the peripheral ER network, and the formation of three-way junctions to generate the characteristic polygonal array requires the fusion of tubules. Abundant evidence suggests that the atlastin family of dynamin-related GTPases plays a critical role in this process. The atlastins are large,

multimeric, integral membrane GTPases that localize predominantly to highly-curved ER membranes, including tubular ER and edges of ER sheets [44-48]. In mammals, there are three closely related atlastins, each of which harbors an N-terminal GTP-binding domain, a middle assembly domain, two very closely spaced hydrophobic segments near the C-terminus, and a C-terminal tail [44, 46]. Atlastin-related GTPases appear to be ubiquitous and include the functional orthologs Sey1p in *S. cerevisiae* and RHD3 in *Arabidopsis* [47]. These large GTPases interact with ER-shaping proteins of both the DP1/REEP/Yop1p and reticulon families and mediate the formation of three-way junctions in the ER (Fig. 2C) [46, 47, 49].

Concordant with their role in ER network formation, the atlastins and Sey1p are distributed in puncta along ER tubules including at three-way junctions [3, 46, 47], where they mediate homotypic fusion of ER membranes [48, 50]. Recent structural and biochemical studies support a model whereby atlastin oligomers in one membrane interact in *trans* with atlastin oligomers in an apposing membrane through GTP binding, forming a tethered complex. Upon GTP hydrolysis, conformational changes pull the membranes into closer proximity, with curvature generated by the atlastin membrane domains and the C-terminal domain, destabilizing the membrane and allowing fusion to occur. In the post-fusion state, GDP is released from atlastin, rebooting the fusion machinery [8, 51-53].

The means for dynamically regulating atlastin-mediated fusion have remained less clear, as has the functional role of atlastin interactions with the ER shaping proteins. However, a recent study in *S. cerevisiae* by Chen et al. [54] has indicated that Lnp1p, a member of the conserved Lunapark family defined by several hydrophobic domains and a Zn^{2+} finger motif, also plays a role in ER network formation. Lnp1p binds to the reticulons and Yop1p and resides at ER tubule three-way junctions in both yeast and mammalian cells. In yeast, the interaction of Lnp1p with the reticulon Rtn1p as well as the localization of Lnp1p to ER junctions are regulated by Sey1p. The authors proposed that Lnp1p might counterbalance Sey1p-directed polygon formation by promoting polygon loss through ring closure [54]. Last, it remains unclear whether atlastins account for all fusion of ER tubules, or whether other protein classes are involved. In fact, *sey1Δ* yeast exhibit some residual ER-ER fusion that requires the ER SNARE [50], indicating that other mechanisms may be uncovered in the future.

6. Cytoskeletal and organellar interactions

Another means by 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). Organelle interactions with the ER are a focus of another review in this volume as well as several other recent reviews, and won't be discussed further here [4, 18]. Instead, we will focus our discussion on the roles of plasma membrane and cytoskeletal interactions in shaping the ER network.

Though the ER appears intimately associated with the cytoskeleton in all eukaryotic cells, there are important differences among cell types and across species. In animal cells, there is a particularly close association of the ER with the microtubule cytoskeleton, and the ER is formed along microtubules through a number of distinct mechanisms [27, 28, 55]. In yeast, movements of nuclear ER during the cell cycle are dependent on microtubules, while cortical ER movement and morphology depend on actin fibers [56]. Actin-based mechanisms also predominate in plants [6, 12, 57]. Notably, the cytoskeleton is not absolutely required for ER tubule formation, and an interconnected tubular network can be generated from a *Xenopus* microsomal membrane fraction in a GTP-dependent manner in the absence of microtubules [58]. Even so, cytoskeletal interactions are important for the

characteristic appearance of ER in cells, since disruption of the microtubule cytoskeleton with nocodazole causes collapse of the ER by retraction from the cell periphery and conversion of peripheral ER tubules to extended sheet-like structures [14, 59].

Links between cytoskeletal elements and ER proteins provide important insights into how the ER is dynamically regulated. In addition to interacting with the ER shaping proteins, atlastins also bind the microtubule-severing, AAA (ATPases associated with a variety of cellular activities) protein spastin [60, 61], physically linking membrane and cytoskeletal remodeling proteins. This interaction occurs between the paired hydrophobic membrane domain of the atlastins and a hydrophobic hairpin domain present near the N-terminus only in the larger M1 spastin isoform; the M87 spastin isoform lacks this domain. Atlastins and M1 spastin also interact with DP1/REEPs within the tubular ER [62]. These latter proteins comprise 6 members in humans: REEP1-6—REEP5 is also known as DP1. Both structurally and phylogenetically there are clear distinctions between two main clades, comprising REEP1-4 and REEP5-6. The yeast ER-shaping protein Yop1p is most similar to REEP5-6, while REEP1-4 proteins also interact with microtubules through an extended C-terminal cytoplasmic domain enriched in basic amino acids [62]. Though coordinated interactions among REEPs, atlastins, and M1 spastin via hydrophobic segments provide a compelling mechanism for coupling ER membrane remodeling to cytoskeletal dynamics, it remains unclear whether these proteins are components of structures such as TACs. In a complementary manner, CLIMP-63 may mediate the attachment of ER sheets to microtubules [63]. In yeast, Estrada et al. [64] demonstrated that cortical ER relies on actin, the myosin V motor Myo4p, and the adaptor protein She3p, a component of the exocyst tethering complex. During bud growth, ER tubules are anchored to the bud tip, and they extend from there in a manner regulated by the cell wall integrity MAP kinase pathway [56].

Stabilization and proper distribution of the ER network also depends on interactions with the plasma membrane, and in fact ER-plasma membrane junctions are highly-conserved structures across species. A recent study by Emr and colleagues [17] found that wholesale depletion of three classes of ER-plasma membrane tethering proteins in yeast -- Ist2 (related to mammalian TMEM16 channels), the tricalbins (orthologs of the extended synaptotagmins), and the vesicle-associated membrane protein-associated proteins Scs2 and Scs22 [65-67] -- caused untethering of the ER from the plasma membrane and the aberrant accumulation of ER in the cytoplasm. Furthermore, phosphoinositide signaling was misregulated at the plasma membrane, and the ER unfolded protein response was constitutively activated [17].

7. Dynamic alterations in ER shape

Thus far, we have emphasized how proteins establish and maintain the different morphologies of ER domains. However, the ER is highly dynamic and undergoes continuous fusion reaction, interactions with the cytoskeleton and other organelles, and transitions between tubules and sheets. Numerous regulatory schemes have been described that move ER, often in conjunction with molecular motors (e.g., kinesin-1 and myosin V) and actin-and microtubule-based cytoskeletal elements.

One regulatory protein is the cytoplasmic protein p22, an EF-hand Ca^{2+}-binding protein that binds microtubules in a manner dependent on *N*-myristoylation and forms Ca^{2+}-dependent interactions with microsomes [68]. Another example of dynamic ER regulation is provided by the stromal interaction molecule (STIM) proteins, which act as Ca^{2+} sensors in the ER, oligomerizing in response to Ca^{2+} depletion and translocating to the plasma membrane via a polybasic C-terminal domain, then activating highly Ca^{2+}-selective Orai channels to trigger Ca2+dependent signaling events [69]. In addition, STIM1 also functions in ER

morphogenesis through interactions with the microtubule +TIP protein end binding 1 (EB1). Protein phosphorylation of STIM1 triggers its dissociation from EB1 during mitosis and represents a major regulatory mechanism that results in the exclusion of concentric ER sheets from the mitotic spindle [70].

Even more dramatic morphological changes occur during cellular events such as fertilization and cell division. Within minutes after fertilization, the ER in starfish eggs fragments, accompanied by a release of Ca^{2+} from internal stores [71]. In fact, Ca^{2+}-induced reversible ER fragmentation has also been reported in cell lines and neurons [72, 73], prefiguring another role for signaling pathways in the modification of ER morphology. A prominent change in ER morphology also occurs during cell division, though the character of these changes remains a matter of debate. Puhka et al. [13] have reported that in mitotic cells the ER undergoes a spatial reorganization and transformation of sheets toward more fenestrated and tubular forms, while Lu et al. [14] have countered that from prometaphase to telophase in various mammalian cell types, most ER is organized as extended cisternae.

Though ER dynamics in animal cells rely heavily on microtubules, short-range movements utilize the myosin V motor. For instance, myosin-Va acts as an organelle transporter to pull ER as cargo into the dendritic spines of cerebellar Purkinje neurons. The myosin-Va accumulates at ER tips as the ER moves into spines, and ATP hydrolysis by myosin-Va is required for this spine ER targeting. Thus, an actin-based motor moves ER within animal cells to regulate synaptic plasticity [20].

Another example of prominent ER dynamics in dendrites was recently reported by Cui-Wang et al. [21]. These authors demonstrated that membrane proteins including AMPA-type glutamate receptors rapidly diffuse within the continuous network of dendritic ER, but are confined by zones of increased ER complexity at dendritic branch points and near spines. The spatial range of receptor mobility was restricted by metabotropic glutamate receptors in a protein kinase C- and CLIMP-63-dependent manner. Moreover, these local zones of ER complexity compartmentalized ER export and were associated with sites of new dendritic branch formation, thus spatially scaling secretory trafficking within complex dendritic arbors [21].

Since ER tubules can be observed to fuse during interphase in a variety of cell types, mechanisms must exist to regulate this process. Very recently, English and Voeltz [15] identified Rab10 as a modulator of ER morphology at dynamic ER-associated structures that track along microtubules and marks areas of nascent ER tubule growth. This was unexpected, since Rab10 has been studied mainly for its roles in endocytic recycling, especially the basolateral recycling pathways in polarized epithelial cells, as well as for GLUT4 storage vesicle transport to the plasma membrane and dense core vesicle secretion [74]. However, Rab10 depletion or expression of a Rab10 GDP-locked mutant clearly altered ER morphology [15], resulting in fewer ER tubules due to a reduced ability of dynamic ER tubules to grow out and successfully fuse with adjacent ER. This Rab10 domain is enriched in at least two ER enzymes that regulate phospholipid synthesis, phosphatidylinositol synthase (PIS) and choline/ethanolamine phosphotransferase 1 (CEPT1). Since the formation and function of this Rab10/PIS/CEPT1 dynamic domain could be inhibited by expression of a GDP-locked Rab10 mutant or by depletion of Rab10, these dynamic morphology changes may be coupled to phospholipid synthesis at these leading edge domains. However, a luminal ER marker did not distribute to these domains, raising the possibility that this constitutes a distinct membrane compartment. In fact, photoactivation experiments performed by Kim et al. [75] had previously indicated that a dynamic domain similarly enriched in PIS is ER-derived, but distinct and highly mobile. They described this structure as a PIPERosome (PI-producing ER-derived organelle) which

makes contact with a variety of organelles. Thus, the mechanism by which Rab10 influences ER structure remains unclear.

Interestingly, the mostly early-endosomal-associated Rab5 has also been implicated in ER shaping [76], and an intriguing link between Rab5 and Rab10 in another context has been described in *C. elegans*, where RAB-5 and RAB-10 recruit one another's Tre2/Bub2/Cdc16 domain-containing GAP molecules in a reciprocal exclusion cascade at a Golgi apparatus-endosomal interface to regulate neuropeptide release [77]. Also, a *C. elegans rab-10* mutant exhibited increased endosomal phosphatidylinositol-4,5-bisphosphate levels, with subsequent alterations in the distribution of the membrane-bending proteins RME-1/Ehd and SDPN-1/Syndapin/Pacsin. This likely occurs via RAB-10 interactions with the Arf6GAP CNT-1 because Arf6 activates type I phophatidylinositol-4-phosphate 5 kinase, which is involved in the synthesis of phosphatidylinositol-4,5-bisphosphate [78]. Together, these endosomal studies prefigure a general involvement of Rab10 within specialized domains linked to phospholipid synthesis and protein recruitment.

The involvement of Rabs in modulating ER morphology is an emerging area of research, and some effects may not be direct or conserved. At a fundamental level, the involvement of Rab GTPases may not be surprising, since they provide directionality and specificity to tubule formation and budding in a variety of cellular contexts, and Balch and colleagues implicated Rabs in ER membrane fusion years ago [79]. Even so, there are over 60 human Rabs known, yet only 11 in *S. cerevisiae*, and most of the dozen or so human Rabs associated with various ER functions lack clear yeast orthologs, including Rab10 [80]. *Arabidopsis* also does not have a clear Rab10 ortholog, though the tubular ER network in plant cells is also highly dynamic. Thus, it is not clear how phylogenetically conserved the Rab10-dependent process is for regulating ER dynamics. This stands in contrast to the atlastin/RHD3/Sey1p GTPases and most ER-shaping reticulons/REEPs, which have orthologs in all eukaryotes. One possible explanation is that although ER movement and remodeling in animal cells is mostly microtubule-based, in yeast and plants actinomyosin dependence predominates [6]; thus different organisms may have evolved different means for the regulation of ER distribution and dynamics.

8. Insights from neurological disease

Highly-polarized cells such as neurons present special challenges for shaping and distributing the ER network because of their large size and complexity [81]. Acompelling example of the importance of ER morphology and distribution in neurons is provided by a class of human diseases known as the hereditary spastic paraplegias, whose cardinal feature is a length-dependent axonopathy of the corticospinal motor neurons, the axons of which can reach up to one meter in length [82]. Though these disorders comprise over 50 distinct genetic loci (*SPG1-55*), over half of all affected individuals, including those with the three most common forms, harbor autosomal dominant mutations in one of four proteins already discussed for their roles in ER network formation: spastin (SPG4), atlastin-1 (SPG3A), and REEP1 (SPG31) and reticulon-2 (SPG12) [68]. These proteins interact with one another and mediate ER shaping and interactions of the tubular ER network with the microtubule cytoskeleton [3, 82].

Additional studies have suggested a role for ER shaping mechanisms in the pathogenesis of related neurologic disorders such as amyotrophic lateral sclerosis (ALS). In the superoxide dismutase 1 (SOD1) G93A transgenic mouse model for ALS, which involves both corticospinal and lower motor neurons, reticulon-4A selectively redistributed the ER chaperone protein disulfide isomerase, and reticulon-4A overexpression protected against neurodegeneration. Conversely, knock out of reticulon-4A,B on this SOD1 G93A

background worsened disease symptoms in mice [83]. Further supporting a role for ER morphogenesis in ALS disease pathogenesis, the VAP-B mutant P56S that underlies ALS8 is associated with the production of a novel form of organized smooth ER [84] as well as a nuclear envelope defect associated with impaired transport of nucleoporins and emerin to the nuclear envelope [85]. Taken together, these studies identify tantalizing links that may support a general neurologic disease mechanism centered on aberrant alterations in ER shape.

9. Conclusion

A unifying model for ER organization and dynamics posits that the ER network can be formed and maintained by ubiquitous proteins such as atlastin GTPases and reticulons/REEPs, with interactions between the ER and the cytoskeleton, other organelles, and the plasma membrane also playing a role. Highly-selective Rab GTPases, Lunaparks, SNAREs, and other protein classes may also be involved. Seemingly, the distinct morphologies of the ER network mask an even more complex heterogeneity of lipid- and protein-defined functional domains that will continue to illuminate how ER form follows function.

With an expanding number of proteins now identified that mediate the generation and shaping of the ER network, the stage is now set for investigations into the mechanisms regulating ER morphology and distribution within the cell. Signaling pathways linked to modifications of these proteins seem likely to play key roles in modulating the structure of the ER within cells and dynamically positioning functional ER domains. Finally, since ER morphology defects appear to be the prominent mechanism underlying the hereditary spastic paraplegias and related disorders, the identification of additional disease genes may provide new insights to untangle the form and elucidate the functions of the ER.

Acknowledgments

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Highlights

1. The ER is a continuous organelle comprising sheets, tubules and the nuclear envelope.

2. Protein families including reticulons and REEPs/DP1/Yop1p shape high-curvature ER tubules.

3. Atlastin GTPases mediate the fusion of ER tubules to form a polygonal network.

4. Multiple ER network proteins are mutated in hereditary spastic paraplegias.
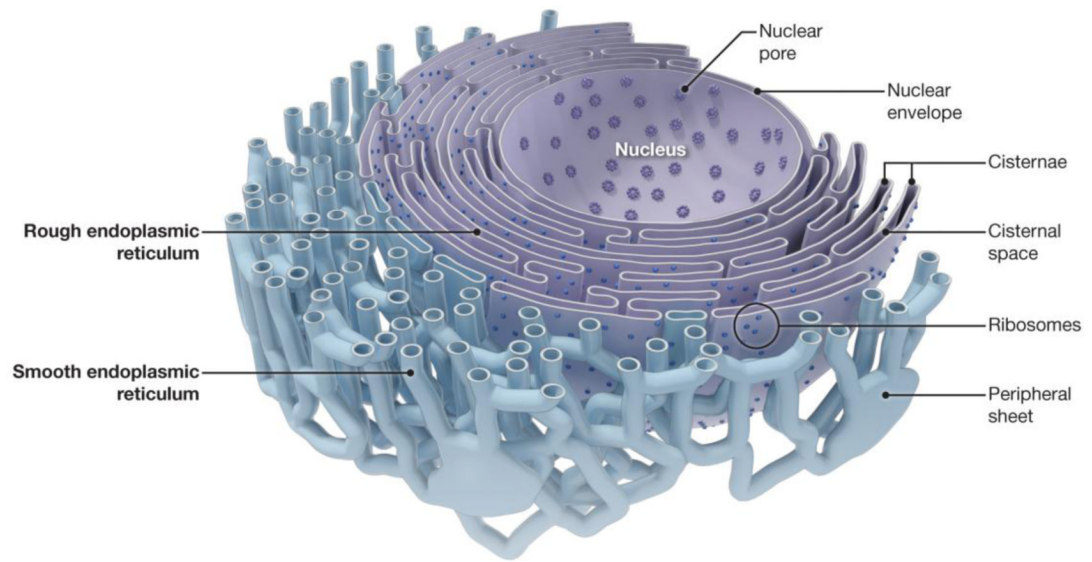
Fig 1.
The ER network in cells. Schematic diagram showing the different structural features and morphologies of the continuous ER in animal cells.
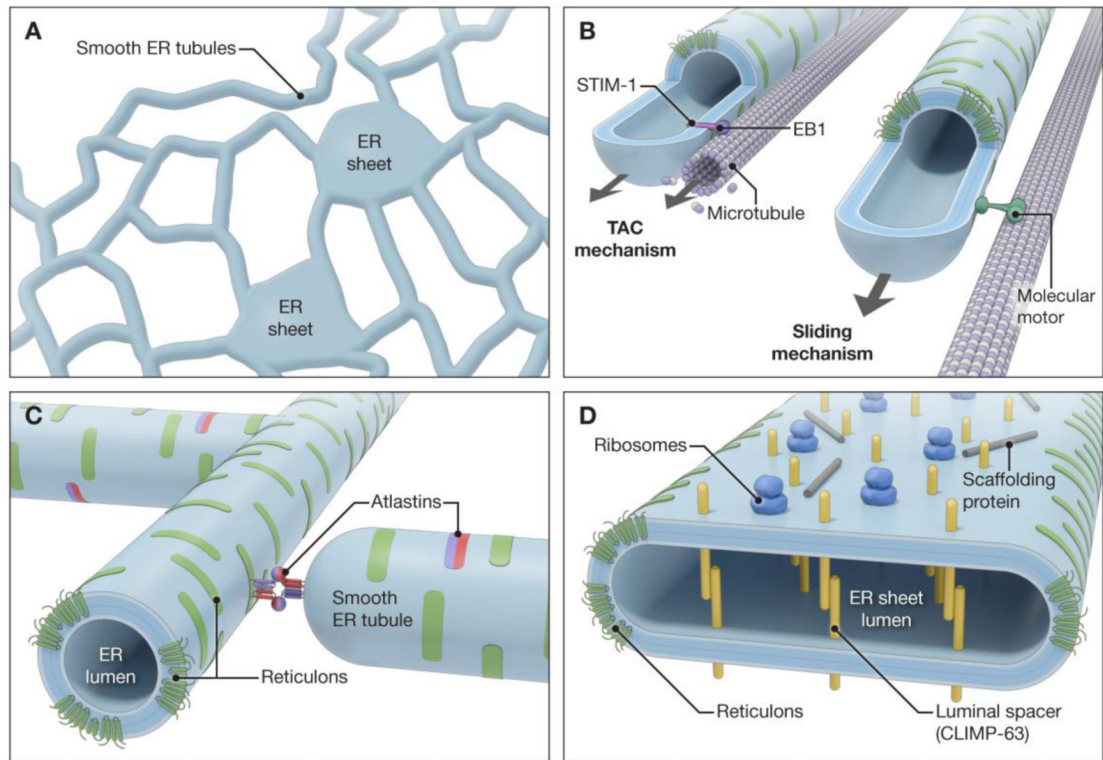
Fig 2.
Mechanisms involved in shaping the ER network. (A) Schematic diagram showing interconnected smooth ER tubules and peripheral sheets. (B) Sliding and TAC mechanisms involved in the formation and extension of ER tubules along stable, acetylated microtubule tracks and polymerizing microtubules, respectively. (C) ER shaping reticulons and atlastins on ER tubules, with a schematic depiction of atlastin-dependent tubule fusion. (D) ER proteins involved in formation and stabilization of ER sheets. Scaffolding proteins include p180 and kinectin. Adapted and modified from Lin et al. [8] and Pendin et al. [86].
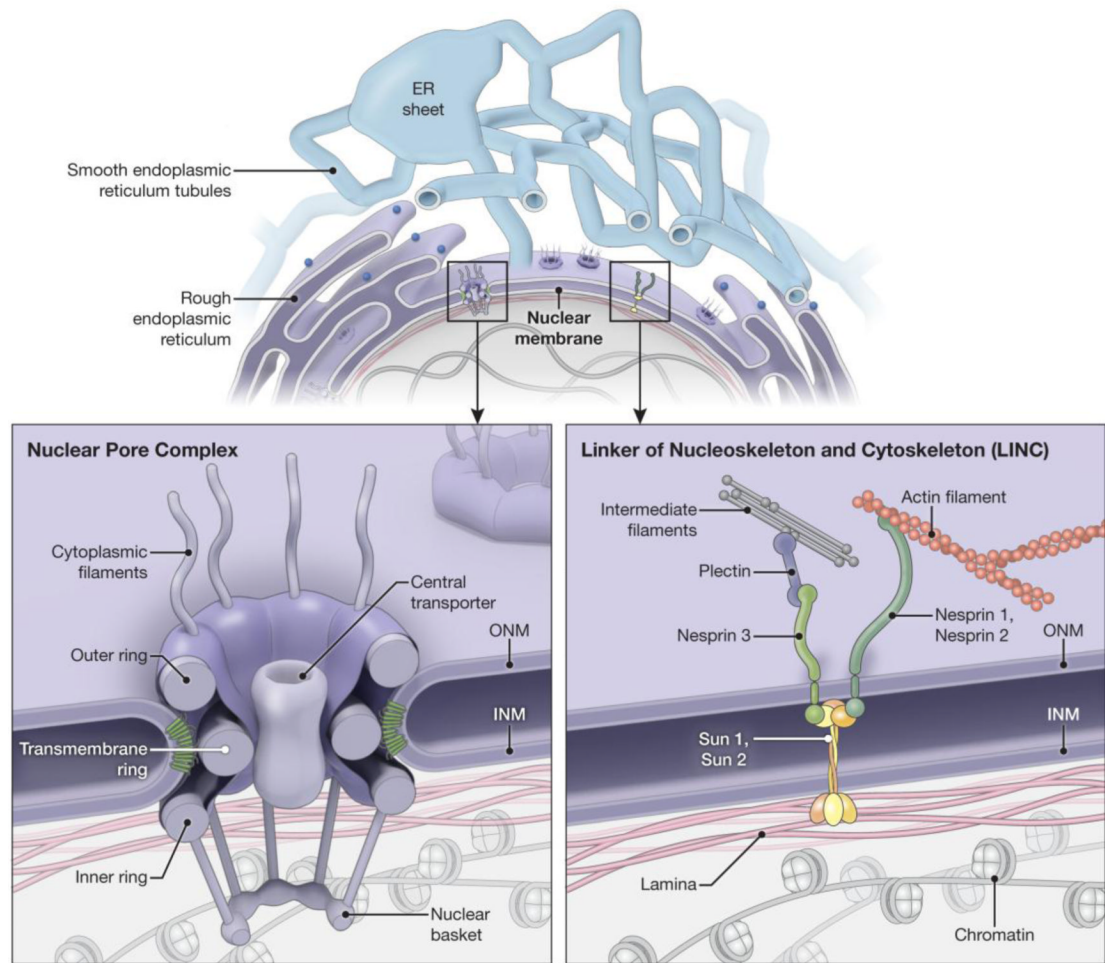
Fig 3.
Specialized nuclear envelope protein complexes. Top, Schematic diagram showing the continuous lumen between the nuclear membrane and the ER network. Bottom, Enlargements of the boxed areas in the top panel, showing the organization of the nuclear pore complex (left) and LINC complex (right). ER-shaping proteins are shown in green in the left panel. INM, inner nuclear membrane; ONM, outer nuclear membrane.