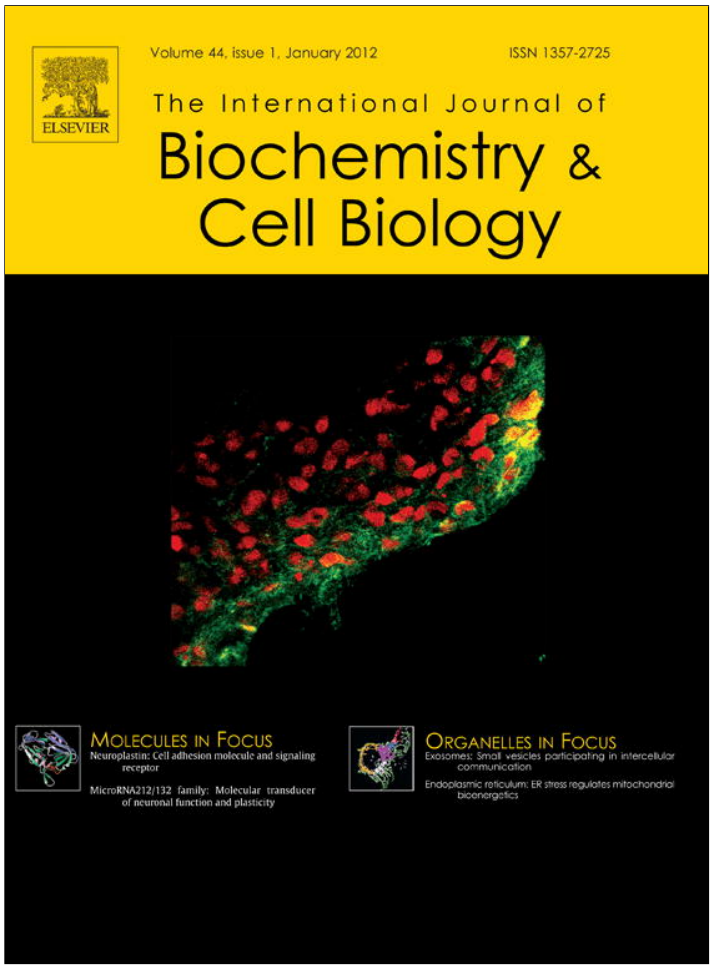

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Review

Molecular basis for sculpting the endoplasmic reticulum membrane

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ABSTRACT

The endoplasmic reticulum (ER) is involved in many critical processes, including protein and lipid synthesis and calcium storage. Morphologically, the ER can be divided into two subdomains: a network of interconnected tubules and interspersed sheets. Until recently, how these different compartments form in a continuous membrane system was unclear. Several classes of integral membrane proteins have been identified in the ER; the reticulons and DP1/Yop1p play roles in the generation of ER tubules, and possibly in stabilizing ER sheets, atlastins and Sey1p are dynamin-like GTPases that facilitate tubular network formation by mediating ER membrane fusion, and Climp63, p180, and kinectin are enriched in ER sheets and influence their formation. In this review, we summarize recent advances in our understanding of how these proteins participate in ER shaping. We also discuss possible mechanisms for regulating ER morphology *via* the cytoskeleton. Lessons learned about sculpting the ER membrane may be applicable to other organelles.

Contents

1. Introduction

The endoplasmic reticulum (ER) is a eukaryotic organelle with distinct morphology and key intracellular functions, such as the synthesis of secretory and membrane proteins. The continuous ER membrane system can be divided into sheet-like structures that include the nuclear envelope (NE) and most of the perinuclear ER, and a network of tubules that often extends throughout the periphery of the cell (Baumann and Walz, 2001; Shibata et al., 2006, 2009; Voeltz et al., 2002). ER sheets are formed by two relatively flat and closely spaced membranes, whereas ER tubules are enclosed single-membrane structures with a diameter of roughly 30 nm in yeast cells (Bernales et al., 2006) or ~50 nm in mammalian cells (Fawcett, 1981). The ER is very dynamic, with sheets rearranging and tubules moving and fusing to form "three-way junctions" (Du et al., 2004; Lee and Chen, 1988).

The amounts of ER sheets and tubules are well balanced and tightly regulated in the cell. For example, recent work has shown that the cytoplasmic tubular ER represents approximately 1/3 of the total peripheral ER in wild-type yeast cells (West et al., 2011). The percentage of each morphological subdomain of the ER varies among different cell types, at different growth stages, and under

Abbreviations: ER, endoplasmic reticulum; NE, nuclear envelope; OSER, organized smooth ER; ATL, atlastin; RHD3, Root Hair Defective 3; HSP, hereditary spastic paraplegia; TM, transmembrane; CT, C-terminal tail; ONM, outer nuclear membrane; INM, inner nuclear membrane; LINC, linker of nucleoskeleton and cytoskeleton; TAC, tip attachment complex; SUN, Sad1 and UNC-84 homology; KASH, klarsicht, ANC-1 and syne/nesprin homology; ALPS, ArfGAP1 lipid packing sensor; FRAP, fluorescence recovery after photobleaching; MVa, myosin-Va.

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different conditions (Shibata et al., 2006). For example, during the yeast budding process, the bud contains mainly tubular ER at early stages (West et al., 2011); the ER in plant root cells is initially more cisternal but becomes predominantly tubular after maturation (Ridge et al., 1999). In contrast, professional secretory cells, such as pancreatic or plasma cells, are filled with layers of rough ER sheets densely studded with translating ribosomes. Retinal pigment epithelial cells contain stacked ER sheets that lack ribosomes, termed organized smooth ER (OSER) (Porter and Yamada, 1960), a structure that can also be induced by the over-expression of certain ER proteins (Snapp et al., 2003).

The intracellular distribution of ER membranes also varies among different species. In yeast and plant cells, the ER is commonly gathered in the cortex, a region found immediately beneath the plasma membrane. Some ER tubules or sheet-like cisternae traverse the cytosol to connect the cortical ER with the NE. As for the ER in mammalian cells, the sheets are condensed in the perinuclear region; the peripheral area of the cell is reached by a polygonal network of ER tubules.

Morphological ER domains have drawn the attention of researchers not only because of their morphology, but also because of their critical functions. The wide distribution of the tubular ER network make it a popular site for interactions with other intracellular structures, such as mitochondria and the cytoskeleton, and possibly for lipid synthesis and Ca^{2+} signaling. The high-curvature in the cross-section of the tubules would also allow a quick start for vesicle budding. Recently, the ER exit sites where COPII vesicles are generated to mediate ER-to-Golgi transport were shown to reside mainly in the high-curvature regions of the ER (Okamoto et al., 2012). On the other hand, the relatively flat surface of the ER sheets is preferred by polysomes, and is a hotspot for protein synthesis. Defects in ER morphology, particularly the loss of tubular ER, can cause retarded growth in budding yeast and decreased embryonic survival in *Caenorhabditis elegans* (Audhya et al., 2007; Voeltz et al., 2006), as well as a neurodegenerative disease known as hereditary spastic paraplegia (HSP) in humans (Hu et al., 2009). These facts strongly suggest that vital functions of the ER rely on its morphological integrity.

How are characteristic features generated and maintained in the ER? How are ER dynamics achieved and regulated? In studies addressing these questions, the reticulons and DP1/Yop1p were found to induce high-curvature in ER tubules (Voeltz et al., 2006), and a class of membrane-bound, dynamin-like GTPases called atlastins (ATLs) were implicated in the fusion of ER membranes (Hu et al., 2009; Orso et al., 2009) (Fig. 1). Several mechanisms for generating the peripheral ER sheets, which involve the reticulons and proteins that are enriched in sheets, have been explored (Shibata et al., 2010). In addition, ER shape is regulated through external forces generated by the cytoskeleton. Based on these findings, we will discuss possible mechanisms and research directions for ER shaping and remodeling.

2. Formation of the tubular ER network

ER tubules differ from sheets in that they have high curvature in the membrane cross-section. ER tubules are likely generated by a mechanism of unidirectional membrane curving. Once the tubules are generated, they connect with each other through homotypic fusion to form a reticular network.

2.1. Generation of high-curvature in ER tubules

The reticulons and DP1/Yop1p were identified as tubule-forming proteins that induce high-curvature in ER tubules (Voeltz et al., 2006). These proteins preferentially localize in the tubular region of the ER; sulfuric modification or antibodies against reticulons block the formation of the ER network *in vitro*, and over-expression or deletion of these proteins affects the amount of ER tubules (Voeltz et al., 2006). The ability of these integral membrane proteins to form tubules is best demonstrated in an *in vitro* reconstitution assay, in which reconstituted Yop1p or Rtn1p deform proteoliposomes into a tubular shape instead of a conventional spherical shape (Hu et al., 2008).

The primary structures of the reticulons and DP1/Yop1p are not conserved, but they both contain two hydrophobic segments that are too long for a single passage of the membrane bilayer. Thus, the transmembrane (TM) segments may form a hairpin in the membrane, allowing the protein to occupy more space in the outer leaflet than the inner leaflet, and inducing local curvature in the bilayer as a wedge insertion (Voeltz et al., 2006). This hypothesis is supported by the topology of the proteins, i.e. the N-terminus, C-terminus, and intervening loop of the two hydrophobic segments are all exposed to the cytosol (Voeltz et al., 2006). Recent studies have shown that both mammalian and plant reticulons suffer mislocalization and dysfunction when the hydrophobic segments are manipulated to disrupt the potential hairpins (Sparkes et al., 2010; Tolley et al., 2010; Zurek et al., 2011). The hairpin may be a common feature of integral membrane proteins that preferentially localize to curved membranes. Examples of such proteins include ATL, spastin, a microtubule-severing ATPase on the ER, and caveolin-1, one of the major structural components of the caveoli (Hu et al., 2009; Le Lan et al., 2006; Park et al., 2010; Parton et al., 2006; Shibata et al., 2009). The existence of hairpin-like transmembrane regions could be used as a criterion for predicting membrane proteins that may be involved in curvature generation or sensing.

Scaffolding is another mechanism commonly used by curvature-generating proteins. Scaffolds are usually created through protein oligomerization that leads to a curved shape (McMahon and Gallop, 2005; Praefcke and McMahon, 2004). Both the reticulons and DP1/Yop1p form oligomers in membranes, as shown by cross-linking, gradient centrifugation, and FRAP experiments (Shibata et al., 2008). The oligomerization of tubule-forming proteins could directly influence the diameter and abundance of ER tubules. Reticulon mutants with a lower tendency to oligomerize are not excluded from the NE and are unable to generate tubules *in vitro* and *in vivo* (Hu et al., 2008; Shibata et al., 2008). In addition, the over-expression of tubule-forming proteins in cells results in the constriction of ER tubules (Shibata et al., 2008). Reticulons and DP1/Yop1p oligomers probably form arc-like scaffolds around the tubules. Arc structures would allow efficient shaping with relatively low membrane occupancy and free diffusion of other membrane proteins in tubular ER membranes (Shibata et al., 2008). The assembly and disassembly of tubule-forming proteins are likely to be tightly regulated, possibly in an energy-dependent manner (Shibata et al., 2008).

The reticulons and DP1/Yop1p conceivably combine the "wedge insertion" and "scaffolding" mechanisms to shape ER tubules (Fig. 1). These tubule-forming proteins are necessary for ER tubules *in vivo* and sufficient for forming tubules in membranes *in vitro*. Nevertheless, tubular ER does not completely vanish after the deletion of all known tubule-forming proteins in budding yeast (Voeltz et al., 2006; West et al., 2011), suggesting that additional mechanisms for ER tubule formation are yet to be identified.

2.2. Generation of the tubular ER network by homotypic fusion

After the tubules are formed and stabilized in the ER, they are connected into a reticular pattern. A class of membrane-bound GTPases, atlastins (ATLs), has recently entered center stage for homotypic ER fusion (Hu et al., 2009; Orso et al., 2009). ATLs mostly localize to the tubular ER and interact with the tubule-forming

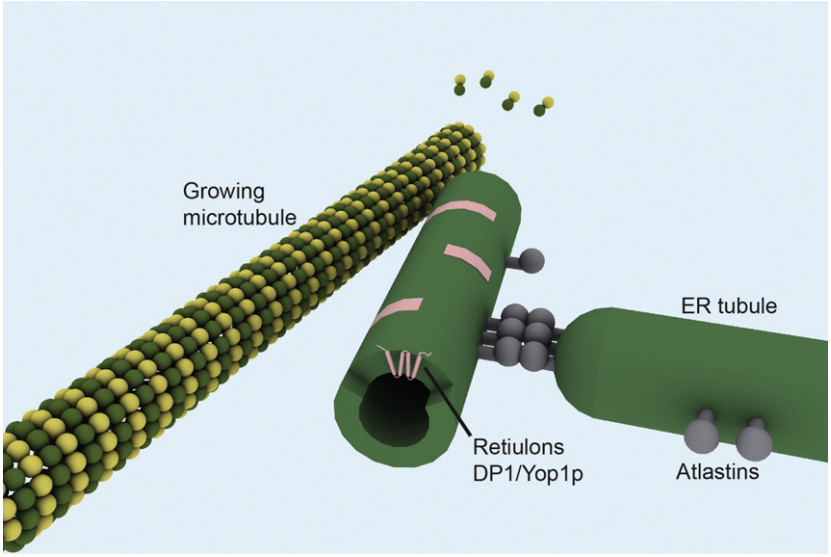

Fig. 1. Mechanisms for generating the tubular ER network. Microtubules pull the tubules (green) out of ER membranes. Tubulins are showin yellow and lime. The reticulons and DP1/Yop1 (pink) induce and stabilize high curvature in ER tubules by inserting into the membrane as a "wedge" and forming an arc-like "scaffold". The atlastins (gray) connect the tubules to generate a reticular network.

proteins, mainly through their TM domains. Depletion of ATL leads to non-branched ER, suggesting compromised fusion between ER tubules. The same ER defects are observed when cells are transfected with ATL mutants with defective GTPase activity, or the N-terminal cytosolic domain or TM region only. Antibodies against ATL inhibit ER network formation in *in vitro* experiments using *Xenopus* egg extracts (Hu et al., 2009). More convincingly, purified *Drosophila* ATL mediates liposome fusion in a GTP-dependent manner *in vitro* (Orso et al., 2009). Taken together, these data support the notion that ATL mediates homotypic ER fusion.

Mutations in human ATL1 cause HSP (Zhao et al., 2001), a neurodegenerative disease caused by the shortening of the axons in corticospinal motor neurons, which leads to progressive spasticity and weakness of the lower limbs (Salinas et al., 2008). HSP is likely a result of a defect in ER morphology. Consistent with this hypothesis, spastin, another commonly mutated gene in HSP, may affect ER morphology indirectly by severing microtubules. In addition, REEP1, a homolog of tubule-forming protein DP1 (also known as REEP5) (Park et al., 2010), and reticulon 2 (Montenegro et al., 2012) were recently added to the list of HSP-causing genes, further suggesting morphological ER defects as the pathogenic mechanism for HSP. ATL knockout mice have not yet been reported, but knocking down ATL1 in primary neuron cultures leads to axon shortening, mimicking the pathological conditions of HSP (Zhu et al., 2006). Neuronal defects have been observed in both drosophila and zebrafish when ATL is depleted (Fassier et al., 2010; Lee et al., 2009). Taken together, these observations indicate that ER coherence and integrity is physiologically important.

Little is known about how homotypic fusion is achieved. The crystal structures of the N-terminal cytosolic domain of human ATL1 (cytATL1) provided new insight into ATL-mediated fusion (Bian et al., 2011; Byrnes and Sondermann, 2011). cytATL1 is composed of a GTPase domain and a three-helix bundle connected by a linker region. Two different conformations of cytATL1 have been observed. In the first conformation, two GTPase domains form a dimer with the nucleotide binding pockets facing each other, and the helical bundles associate with their own GTPase domains, pointing in opposite directions. The TM domains following the helical bundles are likely in two apposing membranes. This conformation would resemble a pre-fusion state. In the second conformation, the GTPase domains adopt similar positions, but the helical bundles interact with the GTPase domains in the pairing molecules. The C-termini point in the same direction and are within close proximity. This conformation would represent a post-fusion state in which two ATL molecules reside in the same membrane. Biochemical data confirmed that GTP binding induces dimerization, and GTP hydrolysis and Pi release trigger conformational changes in both cytATL1 and the reconstituted full-length protein (Bian et al., 2011).

Notably, the interaction surfaces between the GTPase domain and three-helix bundle are very similar in pre-fusion and post-fusion states; thus, the energy gain from the conformational change in the N-terminal cytosolic domain of ATL seems to be relatively small. Therefore, additional mechanisms are likely needed to complete ATL-mediated fusion. The conserved region of the C-terminal tail (CT) in ATL is predicted to be an amphipathic helix and to interact with the membrane. This region of ATL likely inserts into the lipid bilayer, a known feature of amphipathic helices, and destabilizes membranes to facilitate fusion. Consistent with this potential ability, truncation of the CT of ATL1 causes HSP. ATL has also been shown to have elevated GTPase activity in lipids than in detergents (Pendin et al., 2011). The stimulated GTPase activity may be a result of cooperativity mediated by the interactions between TM segments. Thus, the two TM segments and the CT of ATL may play important roles in the fusion process.

A simple model has been deduced (Fig. 2): ATL molecules form oligomers in the membrane and then interact from apposing ER membranes through GTP binding, tethering the membrane; upon coordinated GTP hydrolysis and Pi release, conformational changes occur to pull the two membranes into close proximity; the CT destabilizes the membrane to promote lipid-mixing and allow fusion; after ATL molecules reach the post-fusion state, GDP is released to reset the fusion machinery. In summary, this model suggests that GTP-mediated dimerization and helical bundle relocation are important for ATL-mediated fusion, and the TM and CT cooperate with the N-terminal cytosolic domain to achieve fusion. The model also provides some explanations regarding how ATL1 mutations cause HSP.

Not all eukaryotic species contain an ATL homolog, but analogous proteins, including Sey1p from *S. cerevisiae* and Root Hair Defective 3 (RHD3) from *A. thaliana*, have been found in species lacking ATL (Brands and Ho, 2002; Zheng et al., 2004). Sey1p and

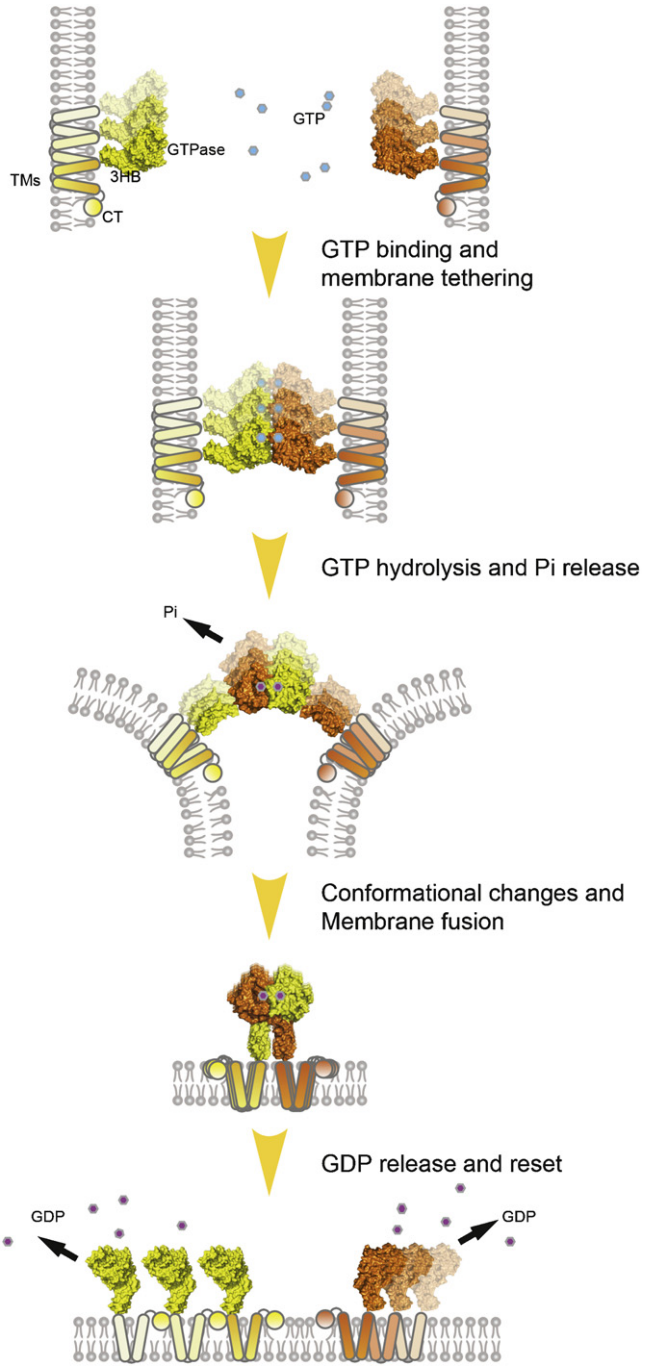

Fig. 2. A model for ATL-mediated homotypic fusion of ER membranes. ATL oligomerizes through the TM segments, tethers apposing membranes upon GTP binding, hydrolizes GTP to induce conformational changes that pull membranes together, allow lipid mixing by perturbation of the CT, and fuses the ER membranes. After fusion, GDP is released to reset the machinery for a new cycle. ATL regions and the nucleotide states are indicated.

RHD3 maintain the same molecular architecture and membrane topology as ATL; both the N-terminal GTPase and CT face the cytosol and are connected by two closely spaced TM segments. More importantly, this class of dynamin-like GTPases shares a unique GTPase signature motif with GBP proteins, which distinguishes them from other large GTPases. This conservation suggests that Sey1p and RHD3 are functional analogs of ATL.

The possibility of Sey1p mediating homotypic ER fusion is further supported by recent studies (Anwar et al., 2012). Sey1p has been shown to interact with tubule-forming proteins Rtn1p and Yop1p in yeast; deletion of *SEY1* and either *RTN1* or *YOP1* causes defects in the tubular ER network. The fusion ability of Sey1p was confirmed *in vitro* with purified protein and *in vivo* during mating, and human ATL1 can partly rescue the loss of Sey1p, suggesting a functional similarity (Anwar et al., 2012). However, the deletion of only *SEY1* in yeast does not yield prominent non-branched ER tubules as in mammalian cells, or the ER fragmentation seen in *Drosophila*. These findings suggest that yeast cells fuse ER membranes *via* an additional mechanism. One candidate for this purpose is ER SNARE Ufe1p (Patel et al., 1998), because *ufe1–1sey1Δ* displays a severe growth phenotype and disrupted ER shape. The overlapping ER fusogens found in yeast may reflect its unusual demands due to the existence of a very large cortical ER network.

The plant analog RHD3 (Root Hair Defective 3) is named after its mutant phenotype, short and wavy root hairs (Schiefelbein and Somerville, 1990). Similar to ATL proteins, RHD3 is ubiquitously expressed, and its mutations mostly affect root hair cells, which resembles ATL1 mutations in which the long axon-containing neuronal cells are most affected. RHD3 mutants also exhibit long, cable-like ER tubules in plant cells (Zheng et al., 2004). Surprisingly, Sey1p is not able to replace RHD3 in plants (Chen et al., 2011). Though these results need to be confirmed and the fusion ability of RHD3 remains to be tested, it is possible that RHD3 shares some features of ATLs and Sey1p in the generation of the tubular ER network, but it may have additional roles in plant cells.

3. Formation of ER sheets

Both the peripheral ER sheets and the NE consist of two closely apposed membranes. To generate these cisternal structures, the distance between the two apposed membranes has to be maintained and the membrane curvature at the edge of the sheets has to be stabilized.

3.1. Sheet-enriched membrane proteins

Candidates for shaping ER sheets were screened from among proteins that are enriched in ER sheets, particularly in professional secretory cells with proliferated ER sheets, or are significantly upregulated during the differentiation of these cells. Climp63, p180, and kinectin are among the proteins that fit the search criteria (Shibata et al., 2010). All of these proteins are enriched in peripheral ER sheets but largely missing from the ER tubules and NE. The role of sheet-enriched proteins was confirmed by the observation that the over-expression of each of them results in drastic proliferation of ER sheets. In addition, the relative expression of these proteins and tubule-forming proteins determines the balance between ER tubules and sheets. However, even the depletion of all three proteins does not completely eliminate ER sheets, suggesting the existence of additional sheet-forming factors (Shibata et al., 2010).

A common feature of Climp63, p180, and kinectin is that they contain coiled-coil domains. Climp63 has a large coiled-coil domain in the ER lumen that bridges the two membranes, controlling the width of ER sheets (Klopfenstein et al., 2001) (Fig. 3). The coiled-coil domains of kinectin and p180 are cytosolic. These domains have been speculated to attach to membranes as long rods, maintaining the flatness of the sheets (Fig. 3). Interestingly, when one or several of these proteins are depleted from cells, the width of the ER sheets decreases (Shibata et al., 2010). This observation suggests that the default state of the cisternal structure in the ER has a concave surface with a narrower width. The main action of Climp63 is thought to be maintenance of a larger distance between apposing ER membranes, whereas p180 and kinectin flatten the surface. A well-controlled luminal space generated by these, and

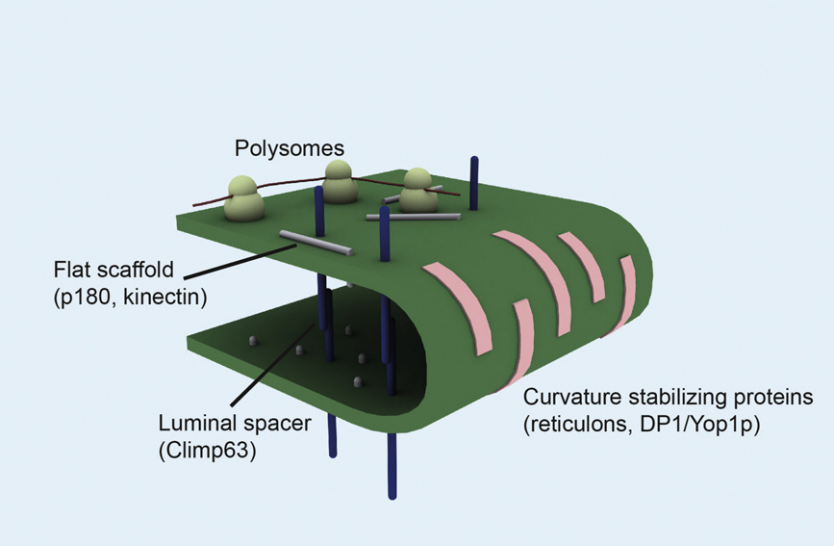

Fig. 3. Mechanisms for generating ER sheets. Proteins like Climp63 (blue) form a luminal bridge *via* the coiled-coil domain. Proteins like p180 and kinectin (gray) are also enriched in sheets and form a flat scaffold with their cytosolic coiled-coil domains. Curvature-stabilizing proteins (pink) localize at the edge and maintain the high curvature. Polysomes prefer sheets and may also facilitate sheet formation.

possibly other, mechanisms may guarantee tight ER sheet stacking, if necessary, and accommodate sufficient luminal components, such as chaperones.

Additional players in ER sheet formation may be polysomes. The existence of polysomes on the surface of the ER may influence membrane shape. Disassembly of translating ribosomes converts some ER sheets into tubules (Puhka et al., 2007) and redistributes some sheet-preferring proteins into the tubular region (Shibata et al., 2010). The association between sheet formation and polysomes is consistent with the idea that a relatively flat sheet surface provides a more suitable platform for polysomes, and rough ER corresponds to ER sheets in general. The association also explains why components of the translocon complex, which mediates the translocation of nascent polypeptides across ER membranes, are enriched in ER sheets.

3.2. Help from the tubule-forming proteins

The similarity between the luminal width of ER sheets and the diameter of ER tubules indicates that the curvature at the cross-section of ER tubules or at the edge of ER sheets is generated by the same mechanism. Thus, the tubule-forming proteins can be speculated to also participate in sheet formation. Reticulons and DP1/Yop1p are proposed to form arc-like oligomers, which could localize to the edge of the sheets and fit the curvature nicely (Fig. 3). Indeed, endogenous or over-expressed reticulons are observed at the edge of ER sheets in mammalian, yeast, and plant cells (Hu et al., 2009; Nziengui et al., 2007; Shibata et al., 2010; Sparkes et al., 2010). Consistent with the proposal, reticulons are up-regulated during ER sheet proliferation (Benyamini et al., 2009). Given their dual roles in ER shaping, reticulons and DP1/Yop1p are better termed as "curvature-stabilizing proteins". These proteins may actively control the relative amounts of sheets and tubules in the ER.

3.3. Generation of the nuclear envelope

The NE consists of two parallel membranes, the outer nuclear membrane (ONM) and the inner nuclear membrane (INM), which connect at the nuclear pore. One determinant of NE shape is the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, which consists of Sad1 and UNC-84 (SUN) protein in the INM and the klarsicht, ANC-1, and syne/nesprin homology (KASH) protein in the ONM (Starr and Fridolfsson, 2010). SUN protein interacts with KASH protein through its C-terminal SUN domain, forming a bridge that spans the perinuclear space (Padmakumar et al., 2005). Depletion of either component causes large bulges in the NE (Crisp et al., 2006). Recent structural data revealed that the SUN domain of SUN2 forms a trimer consisting of a leaf-like domain that provides a potential binding platform for the KASH domain, and a stalk region that connects to the INM. Oligomerization of the SUN domain appears to be important as SUN protein and the KASH domain may form a 3:3 complex (Zhou et al., 2012). The N-termini of SUN proteins attach to nuclear lamin, whereas the N-termini of KASH proteins bind to cytoplasmic cytoskeleton proteins; thus, the LINC complex also functions in nuclear migration and positioning (Zhang et al., 2009), and possibly nuclear shaping, with the help of cytoskeleton proteins.

The distance between the INM and ONM (~28 nm) is shorter than the distance between the two membranes of the peripheral ER sheets (~50 nm). The SUN–KASH interaction and Climp63 likely exert opposite forces on the apposing membranes due to the different lengths of these bridges. A higher degree of membrane bending is also indicated at the edge of the NE sheets, i.e. around the nuclear pore. This curvature is likely stabilized by the core scaffolding nucleoporins Nup84 complex (Shibata et al., 2009). The Nup84 complex contains Sec13p, a component of the COPII coat that is required for ER–Golgi transportation (Siniossoglou et al., 1996). The Sec13–Nup145 complex is structurally reminiscent of the Sec13p–Sec31p complex in the COPII coat (Hsia et al., 2007), which forms a curved lattice in COPII-coated vesicles. In addition, the nucleoporin Nup133, which belongs to the Nup84 complex, has an amphipathic helix called the ALPS (ArfGAP1 lipid packing sensor) motif in its N-terminal region. The ALPS motif is characterized by the enrichment of serine and threonine in the hydrophilic side of the amphipathic helix, rendering it hypersensitive to membrane curvature (Drin et al., 2007). These motifs in nucleoporins may even stabilize the curvature or actively deform membranes around the nuclear pore. Several other nucleoporins, including Nup188, Nup170, Nup85, and Nup120 in yeast and Nup 214 in humans, are predicted to have an ALPS-like domain and may use the same strategy to facilitate nuclear membrane shaping (Drin et al., 2007).

4. Regulation of ER morphology by the cytoskeleton

Like many other organelles, the ER has a close relationship with the cytoskeleton, which has been proposed to provide the driving forces for ER movement and morphological transitions. In animal cells, microtubules play a major role in ER remodeling. Treatment with nocodazole, a microtubule disassembly reagent, causes dramatic changes in ER morphology (Bannai et al., 2004; Shibata et al., 2009; Terasaki et al., 1986). Two types of microtubule-dependent ER remodeling have been documented. First, ER-associated motor protein mediates ER sliding along the existing microtubule. Second, the attachment of ER membranes on the growing tips of microtubules through tip attachment complexes (TACs) allows the extension of ER tubules (Fig. 1) (Waterman-Storer and Salmon, 1998).

Kinesin-1 is a motor protein that transports cargo toward the plus end of the microtubule (Bola and Allan, 2009). Kinesin-1 has been reported to drive the movement of ER tubules, and an ER membrane protein, kinectin, serves as the anchor (Kumar et al., 1995; Toyoshima et al., 1992). Depletion of kinectin results in shrinkage of the ER towards the perinuclear region (Santama et al., 2004). Overexpression of the inhibitory domain of either kinectin or kinesin-1 blocks their interaction (Santama et al., 2004) and results in clustering of the ER network and interfered transportation of ER into the cellular lamella during migration (Santama et al., 2004; Zhang et al., 2010). As kinectin is enriched in ER sheets and over-expression of kinectin causes sheet expansion, the kinesin-1 and kinectin interaction is likely not the major mechanism for motor proteins pulling ER tubules.

Compared with motor protein-driven ER sliding, TAC-mediated ER dynamics contribute to only a minor portion of microtubule-related ER mobility (Grigoriev et al., 2008; Shibata et al., 2009). ER-resident protein STIM1 has been suggested to play a role in this process. STIM1 is an essential component of store-operative Ca^{2+} entry. The growing ER tip and the plus end of the elongating microtubule are often labeled by STIM1 puncta, and STIM1 binds directly to EB1, a microtubule plus end binding protein. Depletion of either STIM1 or EB1 dramatically reduces TAC-dependent ER movement (Grigoriev et al., 2008). The interaction between STIM1 and EB1 allows STIM1-containing ER tubules to reach the plasma membrane and activate Orai, a Ca^{2+} release-activated Ca^{2+} (CRAC) channel residing in the plasma membrane, for Ca^{2+} uptake. STIM1–EB1-dependent ER rearrangement appears to be used for calcium storage regulation, a special function of the ER.

Additional ER membrane proteins, including Climp63, p180, and REEP1, have been found to bind microtubules directly (Klopfenstein et al., 1998; Ogawa-Goto et al., 2007; Park et al., 2010). Overexpression of these proteins leads to microtubule bundling and is correlated with distorted ER morphology, but the physiological significance of these interactions remains to be determined. The role of Climp63 as a luminal ER spacer does not rely on its microtubule-binding ability (Klopfenstein et al., 1998, 2001; Vedrenne et al., 2005). Climp63 has been suggested to facilitate protein translocation by anchoring ER sheets to microtubules and reducing the mobility of the translocon complex (Nikonov et al., 2007). Whether microtubule binding helps the sheet-enriched proteins achieve proper ER dynamics remains to be tested. Recently, REEP1, a family member of DP1/REEP5, was shown to bind microtubules directly through its C-terminal region (Park et al., 2010). The depletion of REEP1 in cells results in only minor defects in the tubular ER network, but deletion of the microtubule-binding region in REEP1 is associated with HSP. Further investigation is needed to understand the microtubule-related influence of these proteins on the ER.

Although the role of actin filaments in ER remodeling is considered less important in mammalian cells, it should not be simply ignored. Recently, myosin-Va (MVa) was reported to transport ER into the dendritic spines of Purkinje neurons (Wagner et al., 2011). ER tips that grow into dendritic spines are MVa-positive and insensitive to nocodazole treatment. The absence of ER in the dendritic spines of MVa-knockout neurons can be rescued by re-introducing wild-type protein, but not its mutant (Wagner et al., 2011). These findings are consistent with that fact that myosin being capable of short-range ER mobility *in vitro* (Tabb et al., 1998; Wollert et al., 2002). The role of actin filaments is even more prominent in plant and yeast cells, in which the actin filaments are the major cytoskeleton. The depolymerization of actin filaments, but not microtubules, in these cells significantly decreases the rate of ER remodeling (Prinz et al., 2000).

5. Conclusion

Great progress has been made over the last few years in understanding how ER morphology is generated, maintained, and regulated. However, many questions still need to be answered. Experimental evidence is needed to prove the wedge shape and arc formation of the curvature-stabilizing proteins. Large-scale proteomic studies have reported that these proteins are the targets of several post-translational modifications, whether they are regulated in such ways during ER remodeling is not clear. Understanding how ATL mediates homotypic fusion is another important goal. How different mechanisms cooperate, and whether any additional ones are involved, is still unknown. Finally, the issues concerning ER sheet formation and proliferation are largely unknown. The NE, which consists of specialized ER sheets, may utilize similar mechanisms but with different components. How the NE is shaped and reassembled during mitosis remains to be determined.

Investigations of ER shaping and remodeling will allow us to pursue the functional significance of the morphological ER domains and its implications in related human diseases. In the case of HSP, whether particular ER function is affected or the disease is a result of general ER malfunction linked to morphological defects needs to be clarified. In addition, the integrity and dynamics of the ER are tightly associated with other organelles, particularly the Golgi apparatus. Whether the indirect impact of ER morphology on the Golgi contributes to pathogenic mechanisms remains to be tested. Finally, models proposed for the generation of ER tubule curvature by integral membrane proteins, GTP-driven membrane fusion, and ER sheet formation could be applied as a general principle to the shaping and remodeling of other organelles.

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