Form Follows Function: The Importance of Endoplasmic Reticulum Shape

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Abstract

The endoplasmic reticulum (ER) has a remarkably complex structure, composed of a single bilayer that forms the nuclear envelope, along with a network of sheets and dynamic tubules. Our understanding of the biological significance of the complex architecture of the ER has improved dramatically in the last few years. The identification of proteins and forces required for maintaining ER shape, as well as more advanced imaging techniques, has allowed the relationship between ER shape and function to come into focus. These studies have also revealed unexpected new functions of the ER and novel ER domains regulating alterations in ER dynamics. The importance of ER structure has become evident as recent research has identified diseases linked to mutations in ER-shaping proteins. In this review, we discuss what is known about the maintenance of ER architecture, the relationship between ER structure and function, and diseases associated with defects in ER structure.

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INTRODUCTION

The endoplasmic reticulum (ER), despite being one of the largest organelles in the cell, was one of the last to be discovered. Although the ER was originally described in 1902 by Emilio Veratti (1), it took the scientific community another 50 years and the introduction of the electron microscope to "rediscover" the ER, when George Palade (3), along with Keith Porter (4, 5), captured the structural complexities of a fine tubular network residing within the cytoplasm (1–6). Since then, more advanced techniques, including three-dimensional electron tomography and confocal fluorescence microscopy, have revealed that the ER is composed of a single continuous membrane that forms a network containing multiple domains with different structures and functions (**Figure 1**).

The two major domains of the ER are the nuclear envelope (NE) and the peripheral ER. At the center of the cell, the NE is made up of two flat ER membrane bilayers, which stack to form the inner and outer nuclear membranes (INM and ONM). The NE shape is maintained by multiple forces, including INM proteins that bind to chromatin and lamin, linker proteins between the INM and the ONM, nuclear pores, and the cytoskeleton (7–9). Expanding from the NE, the peripheral ER branches out from the ONM into the cytosol and forms a series of cisternal sheets and dynamic tubules (**Figure 1*a***). This review does not discuss NE structure in detail but instead focuses on how the peripheral ER is shaped and how ER morphology affects function.

For many years, the relationship between the elaborate structure of the ER and the numerous functions of the ER was largely mysterious. Also unknown was whether defects in ER shape might be relevant to human disease. This situation has changed in the last few years, which have witnessed a tremendous increase in our understanding of how ER shape is determined, how morphology is related to function, and how these processes go awry in some diseases. We are also beginning to understand how the relative ratio of the different ER structural domains in cells varies as ER architecture is modified to meet specific cellular demands. This understanding has been driven primarily by the identification of the proteins and forces that maintain ER morphology.

This review focuses on how the peripheral ER is shaped and how ER morphology affects function. The formation and maintenance of peripheral ER structure are largely regulated by

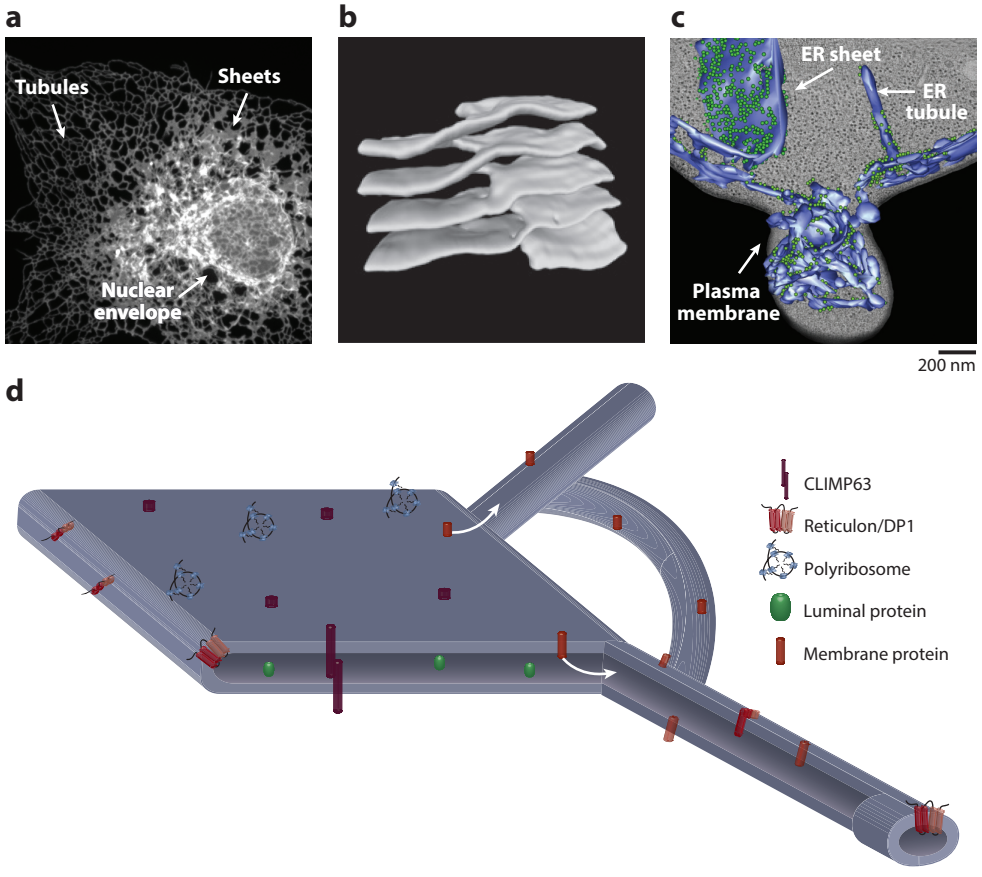

Figure 1

Structural domains of the endoplasmic reticulum (ER). (*a*) A fluorescent image of a Cos-7 cell expressing a green fluorescent protein (GFP)-tagged ER protein (Sec61B) reveals the major structural domains of the ER, including the nuclear envelope and peripheral tubules and sheets. (*b*) A three-dimensional (3D) reconstruction of a mouse acinar secretory cell reveals a complex architecture of stacked sheets that resembles a parking garage. (*c*) A 3D reconstruction by electron tomography of the ER (*blue*) in a budding yeast cell contains interconnected sheets, tubules, and cortical ER. Bound ribosomes are shown in green. Note that the ribosome density is high on cytoplasmic sheets and on the cytoplasmic face of the cortical ER but low on tubules and absent from the face of the cortical ER facing the plasma membrane (*gray*). (*d*) A cartoon model shows the differences (in membrane curvature) and similarities (in luminal spacing) between sheets and tubules. Thus, tubules have a higher surface-to-volume ratio than do sheets, rendering them better suited for surface-dependent functions. Conversely, sheets are a good location for luminal processes. White arrows indicate the directed flow of proteins from ER sheets out into the tubules. Panel *b* modified from Reference 11 with permission from Elsevier; panel *c* modified with permission from Reference 16.

membrane-associated proteins that compete in a tug-of-war to determine the ratio of ER membrane sheets to tubules. The functionalization of these integral membrane proteins has begun to provide insight into how the characteristic structure of the ER is generated. Furthermore, several diseases that are linked to dysfunctional alterations of ER shape highlight the importance of ER morphology to cellular function.

PERIPHERAL ENDOPLASMIC RETICULUM DOMAINS AND FUNCTIONS

The peripheral ER is an interconnected network of two very differently shaped membrane domains: flat cisternal sheets and reticulated tubules (**Figure 1*a***). An ER sheet resembles a pancake in shape, with a lumen located between the two flat, opposing membrane bilayers. The luminal spacing between the two membrane bilayers of the sheet is well maintained at ~30 nm in yeast and 50 nm in animal cells (10, 11). The flat ER sheets exhibit low membrane curvature, except along the edge, where the lipid bilayer folds upon itself. ER tubules radiate from the NE and from ER sheets to form a reticulated structure that interconnects all domains of the ER. ER tubules are highly dynamic, continually forming and rearranging. They interconnect mostly at three-way junctions, resulting in a loosely polygonal array spread throughout the cytoplasm. The classic reticular structure enables ER membranes to reach various parts of the cell while still allowing room for other organelles to traffic around it (12). ER tubules share some features with sheets, as they also have a luminal diameter of ~30 nm in yeast and 50 nm in animal cells (10, 11, 13). The primary difference between sheets and tubules, therefore, is that the surface of tubules is highly curved (except in the plane of the tubule), whereas that of sheets is largely flat (except at the edges). This means that tubules have a higher surface-to-volume ratio than do sheets, rendering them better suited for surface-dependent functions. Conversely, sheets would be a good location for luminal processes.

The concept that ER sheets and tubules may have different functions was suggested by early electron microscopy (EM) studies, which found that sheets tended to be "rough" (i.e., studded with ribosomes), whereas tubules were largely devoid of ribosomes and therefore "smooth" (14, 15). We now know that ER sheets are the primary location for the translation, translocation, posttranslational modification, and folding of integral membrane proteins and secreted proteins (14, 15). ER sheets are often stacked, and a recent study showed that stacked sheets are connected by twisted membrane surfaces, allowing them to be packed with maximum efficiency (**Figure 1*b***) (16). The functions of ER tubules are less well understood, but they may be the primary regions where lipid synthesis and signaling between the ER and other organelles occur.

The ratio of ER sheets to tubules varies in different cell types and reflects the cells' requirement for processes that occur in these two types of structures. This relationship is exemplified in cells with a high capacity to secrete proteins, such as pancreatic cells, which exhibit layer upon layer of ribosome-studded sheets (15, 16), and cell types that secrete few proteins, such as neurons, muscle cells, and epithelial cells, which contain an abundant tubular network (16, 17). Imaging performed in cells that exhibit a mix of tubules and sheets yields data supporting the idea that ER sheets and tubules have different functions. EM studies of ER domains in yeast cells reveal that sheets have a higher ribosome density than tubules (**Figure 1*c***) (11). Complementary experiments analyzing ER domains in animal cells using immunofluorescence microscopy also indicate that the translocation machinery is more enriched on the ER sheets than in tubules (12). It may be that the relatively flat membranes of sheets are more stable platforms than tubules, which would allow sheets to better support the bulky membrane-bound polyribosomes that are required for protein synthesis.

Differences in the shapes and curvatures of the ER sheets and tubules may also contribute to the distribution of proteins in the ER. The different geometries of sheets and tubules could generate bulk flow movement of membrane proteins from ER sheets into the ER tubules and favor retention of luminal proteins within the sheets. This idea is borrowed from the bulk flow hypothesis, which states that membrane-bound cargo is sorted into the tubular budding domains of an endosome and that luminal cargo should be retained in the spherical vacuolar domain (**Figure 1*d***) (18–20).

Cortical Endoplasmic Reticulum

Cortical ER refers to regions of the peripheral ER that are closely apposed and tethered to the plasma membrane (PM). Contact sites between the ER and PM allow small molecules such as lipids and signals to be exchanged. In some cell types, these contacts are quite extensive. In yeast, for example, the ER is closely opposed to ~40% of the PM in both budding (11) and fission (11, 21) yeast (**Figure 1*c***). The shape of cortical ER is a hybrid between the properties of sheets and tubules. It forms highly fenestrated, flat ER sheets that resemble a slice of Swiss cheese. Thus, it has regions that are flat and many regions that have high curvature. The cortical ER also has a hybrid ribosome density (**Figure 1*c***). It has no bound ribosomes on the plane that faces the PM, probably because it is so tightly tethered there that ribosomes are excluded (11). In contrast, the other side of the cortical ER that faces the cytosol has the same high ribosome density as ER sheets that are not at the cortex (11).

Muscle cells are another cell type in which a significant portion of the PM has closely apposed the ER (22, 23). Indeed, these cells have invaginations of the PM, called T-tubules, which are associated with the ER and thus enable extensive ER–PM contact. These contacts are necessary for the Ca^{2+} signaling that occurs during muscle contraction (22, 24, 25). The ER is one of the major reservoirs of intracellular Ca^{2+} and can release or take up Ca^{2+} from neighboring compartments at contact sites (23, 26, 27).

Cortical ER also exists in other cell types, but the fraction of the peripheral ER at the cortex is much smaller than in muscle cells or yeast. In these cells, Ca^{2+} levels in the ER lumen regulate close contacts between the ER and the PM; when the luminal Ca^{2+} concentration decreases, the amount of ER contacting the PM increases. These contact sites are then the position of store-operated Ca^{2+} entry (SOCE) from the PM, which in turn restores Ca^{2+} concentration in the ER lumen (28, 29). Recent evidence indicates that cytosolic Ca^{2+} levels also regulate ER–PM contacts by proteins that are not part of the SOCE pathway (30, 31).

MECHANISMS SHAPING THE ENDOPLASMIC RETICULUM

The membrane of the peripheral ER network is organized into a complex shape. In addition, it is constantly rearranging its position along the cytoskeleton. Thus, generating and maintaining the dynamic architecture of the ER require contributions from the cytoskeleton, motor proteins, proteins that mediate ER–ER fusion, and membrane-bending proteins.

Endoplasmic Reticulum Dynamics on the Cytoskeleton

The association between ER and the cytoskeleton plays a critical role in the formation of new ER tubules, ER dynamics, and the overall maintenance of the ER network. In mammalian cells, the association between the ER and microtubules (MTs) drives most of these processes (32, 33). ER tubules are extended along MTs by both retrograde and anterograde machinery (**Figure 2**) (34–37). When MTs are depolymerized, the dynamics of ER tubules come to a halt. Although the reticular network persists for some time without MTs in some cells, the integrity of its tubular shape is eventually lost, and ER membranes retract from the periphery into perinuclear sheets (38). Thus, interactions between the ER membrane and MTs are just as important to maintaining ER shape as the membrane-shaping proteins, which are discussed below.

Research in both the plant and yeast fields has demonstrated that the ER can also be transported throughout the cell through interactions with the actin cytoskeleton, rather than MTs (35, 37, 39, 40). Actin may play a significant role in ER structure in mammalian cells as well; several

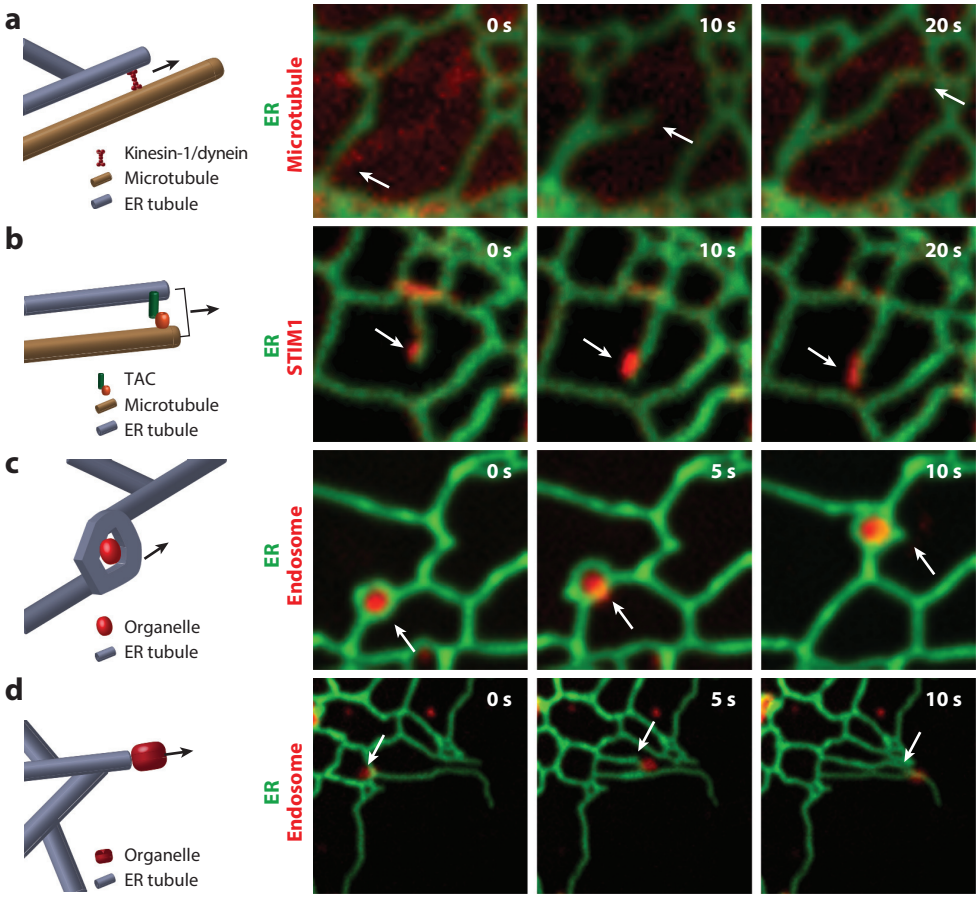

Figure 2

Four types of endoplasmic reticulum (ER) dynamics. (*a*) ER tubules undergo motor-dependent "sliding" along the length of a microtubule. (*b*) During tip attachment, the ER protein stroma-interacting molecule 1 (STIM1) (*red*) localizes to the tip of an ER tubule (*green*) and couples ER dynamics with the growth and movement of the plus end of a microtubule. (*c*) ER ring rearrangements (*green*) are positions where ER tubules circumscribe other organelles, here an early endosome (*red*). (*d*) ER tubules can also be pulled behind organelles trafficking on the cytoskeleton. The tip of an ER tubule is linked to a trafficking endosome (*red*). Abbreviation: TAC, tip attachment complex. Panel *a* modified with permission from Reference 46; panel *c* modified with permission from Reference 57; panels *b* and *d* modified from images provided by A. Rowland and P. Chitwood.

reports have demonstrated that ER sheets interact with the actin cytoskeleton (41–45). Treatment of animal cells with the actin depolymerizer latrunculin A resulted in disrupted ER organization characterized by a more reticular structure that was unevenly spread throughout the cytoplasm. Consistent with the increased reticular structure, the peripheral ER demonstrated a significant decrease in the prevalence of ER sheets. These data suggest that actin dynamics could regulate the shape, dynamics, position, and possibly functions of ER sheets (45). Here, we focus on the role of ER association with MTs in determining ER structure and function in mammalian cells, because these interactions are the best understood. Live-cell imaging has revealed three main types of movement by ER tubules, all of which depend on MTs: (*a*) sliding, whereby a new tubule is pulled

out of an existing tubule along the shaft of a stable MT (**Figure 2*a***), (*b*) the movement of the tip of a tubule along the cytoskeleton via a protein complex called TAC (tip attachment complex) (**Figure 2*b***), and (*c*) ring rearrangements, a variation of ER sliding in which rings of ER move along the side of MTs and occasionally close (**Figure 2*c***).

Sliding. The most frequently observed type of ER movement is ER tubules "sliding" along MTs (34, 46, 47). This motion is driven by molecular motor proteins traveling along MTs and requires kinesin-1 or dynein, depending on whether the tubule is moving toward the plus or minus end of an MT (**Figure 2*a***) (34, 35, 48, 49). Inhibition of kinesin-1 function reduces ER tubule movement and extension both in vitro and in vivo (48–51). The morphological outcome of depleting kinesin-1 is a reduction in tubular ER (49). Inhibition of dynein has a similar outcome: a reduction in the number of tubules moving toward the cell center and an increase in peripheral sheets at the expense of tubules (49). Thus, the ratio of ER sheet to tubules is dependent on motor-driven sliding of ER tubules.

The function of ER sliding is not yet known, but it must serve an important purpose. Not only does ER sliding occur constitutively; it also must require a great deal of energy because it depends on molecular motors. ER sliding events track preferentially along MTs that are modified by acetylation (46). Acetylated MTs have unusually curved structures and prolonged stability to nocodazole treatment. Nocodazole treatment quickly (within minutes) depolymerizes the bulk of MTs in the cytoplasm that are not modified (~80% of the MTs), yet robust ER sliding continues along the nocodazole-resistant remaining acetylated MTs for up to an hour (46). It is curious that ER dynamics occur mostly on a small subset of MTs. This finding suggests that the purpose of ER sliding could be to traffic smooth ER material to a destination that is also present on modified MTs.

TAC and the role of STIM1. TAC represents another type of ER movement in which the tip of an ER tubule is linked to the plus end of an MT and the ER tubule grows and shrinks together with the MT to which it is attached (**Figure 2*b***) (34, 52). The TAC machinery includes EB1, an MT plus end–binding protein, which is linked to an integral ER membrane protein, stroma-interacting molecule 1 (STIM1) (34, 35). Because these dynamics are localized to MT plus ends, they occur in the cell periphery, just under the PM. STIM1 is an interesting protein because it has also been shown to play a pivotal role in regulating Ca^{2+} homeostasis through activation of SOCE (47, 53, 54). Depletion of ER Ca^{2+} stores results in a rapid relocation of STIM1 from TACs to ER–PM contact sites, where it hetero-oligomerizes with Orai Ca^{2+} channels to form a Ca^{2+} release–activated channel (CRAC), giving the ER lumen direct access to the extracellular Ca^{2+} during SOCE (47). Surprisingly, a functional connection between TAC and SOCE has not been found, even though both processes require STIM1.

Ring rearrangements: the contact site connection. Ring structures of tubular ER have been observed to move around the cell in an MT-dependent manner at speeds consistent with molecular motors (36, 46). These rings sometimes become smaller and smaller, leading to complete closure (33, 36, 55). Time-lapse imaging of the formation of ring structures and their closure provides insight into ring structure organization and dynamics. Rings consist of two three-way junctions, one of which appears static or anchored with respect to the reticular ER network, whereas the second is dynamic with the ability to slide along the reticular ER. It is the sliding of the dynamic junction toward the anchored junction that mediates ring closure (36, 46).

Until recently, the functions of these rings remained mysterious. Simultaneous imaging of the ER and other organelles has finally revealed a clue. Numerous ER rings are positions where the ER circumscribes endosomes and mitochondria (**Figure 2*c***) (46, 57, 58). These organelles are

constricted where they are wrapped by ER, and subsequent studies showed that these are sites where mitochondrial fission and endosomal fission occur (56, 59). Thus, many ring closure events are the result of ER cinching at the positions where other organelles undergo fission.

The effect of interorganelle contact sites on ER shape and dynamics must be massive, given that, for example, a typical animal cell could have more than 100 endosomes and mitochondria and most are attached to the tubular ER network (12, 56–59). Also, many ER sliding events may occur at positions where an ER tubule is pulled behind a trafficking organelle (**Figure 2*d***). Therefore, it is possible that most ER dynamics in the cell are indirectly caused by tethered organelles dragging the ER around with them.

Formation of Peripheral Endoplasmic Reticulum Tubules

Several integral membrane proteins have been implicated in the generation and maintenance of the various structural domains within the ER. Unraveling the mechanisms of these proteins has revealed some answers to how the continuous membrane bilayer of the ER can be molded into complex shapes. Specifically, the significant curvature that is characteristic of peripheral ER tubules is maintained by a series of wedge-shaped proteins that are embedded within the ER membrane and thereby maintain tubular curvature. These proteins localize exclusively to ER tubules and the edges of ER sheets, which, like tubules, are highly curved.

Reticulons. The reticulon proteins are a highly conserved and abundant family of integral membrane proteins that structurally shape ER tubules (60–62). In mammals, there are four reticulon genes (*RTN1*, *RTN2*, *RTN3*, and *RTN4/Nogo*), which encode several protein isoforms (RTN1A–C, RTN2A–C, RTN3A and -B, and RTN4/NogoA–C) that are generated through alternative splicing events or the use of different promoters (60, 63–68). Comparatively, *Saccharomyces cerevisiae* contain two reticulon genes (*RTN1* and *RTN2*), each of which encodes a single protein (Rtn1p and Rtn2p), whereas *Caenorhabditis elegans* has only one reticulon gene (*ret-1*), which encodes three different reticulon protein isoforms (nRTN-A, nRTN-B, and nRTN-C) (69). Overexpression of some reticulon isoforms drives the formation of elongated ER tubules and disruption of peripheral ER sheets (38). In animal, yeast, and plant cells, depletion of reticulon proteins reduces ER tubules and increases peripheral ER sheets (62, 70, 71). Thus, reticulons are necessary and sufficient to control ER tubule levels. Consistent with their function is the finding that reticulon proteins localize exclusively to regions of high membrane curvature, including ER tubules and the edges of ER sheets, and are excluded from the flat membrane domains of the NE and peripheral ER sheets (70).

All reticulons have a core reticulon homology domain (RHD) located at their C termini. The RHD consists of two hairpin transmembrane (TM) domains separated by an intervening hydrophilic loop (72). The topology of the reticulons has been determined experimentally and reveals that the N- and C-terminal domains, along with the hydrophilic loop within the RHD, all face the cytosol (70). The hairpin TM domains within the RHD adopt an unusual topology that suggests how these proteins regulate membrane shape. The TM hairpins are unusually short and occupy more space in the outer leaflet of the ER membrane than in the inner leaflet, causing the membrane bending that is necessary to generate regions of high curvature in the ER (70, 73). The reticulon proteins also form immobile oligomers on the ER membrane (38). The structure of these oligomers probably determines the diameter of the tubules. The RHD alone can oligomerize and partition to ER tubules. These membrane-shaping properties are lost in a mutant in which the TM domains are lengthened to resemble the membrane-spanning α-helices found in most TM domains (74).

The N termini of reticulon paralogs and their isoforms are highly variable (72, 75), suggesting that different reticulons have specialized functions. Some evidence supports this hypothesis. The overexpression of RTN3 in HeLa cells, for example, significantly inhibited retrograde transport of proteins from the Golgi apparatus to the ER (76). RTN2B has been linked to regulation of the trafficking of a neuronal glutamate transporter, EAAC1, from the ER to the cell surface (77). RTN1 interacts with SNARE (SNAP receptor) complex proteins and regulates vesicular trafficking, specifically exocytosis, as overexpression of RTN1C enhanced secretion of human growth factors (78). A careful comparison between the membrane-shaping activities for each reticulon isoform is required to dissect reticulon functions and provide insight into their specialization.

DP1/REEP5/Yop1. A ubiquitous membrane protein, defective in polyposis 1 [DP1; also known as TB2 or receptor expression enhancing protein 5 (REEP5) in animal cells], was first characterized as a regulator of peripheral ER tubules after it was identified as an interacting protein of RTN4A, along with RTN3 (70). DP1 is a member of the REEP family of proteins (REEP1–6). Yeast DP1 (Yop1) is an abundant ER protein that, together with Rtn1, maintains ER structure. In cells lacking both proteins, the peripheral ER loses nearly all regions of membrane curvature and is converted into massive ER sheets. Because ER structure is relatively normal in cells lacking only one of these proteins, Yop1 and Rtn1 seem to have overlapping functions in regulating tubule formation and maintenance in *S. cerevisiae* (70). Like reticulons, DP1/Yop1 localizes to the peripheral ER tubules and is excluded from both the NE and peripheral sheets. It also shares the membrane topology of reticulons, with two short hairpin TM domains that are predicted to stabilize high membrane curvature. DP1/Yop1, like reticulons, forms homo-oligomers that are less mobile compared with other ER membrane proteins. These oligomers are predicted to form structures that surround the ER membrane in an arclike pattern (38, 79). Computational modeling supports the idea that cylindrical tubules can be generated and maintained with a small percentage of tubular inducing proteins, compared with total membrane surface (79). In fact, the model estimates that only ~10% of the surface of peripheral tubular ER in *S. cerevisiae* is occupied by tubular proteins such as Yop1 and Rtn1p/Rtn2p (79).

Formation of Peripheral Endoplasmic Reticulum Sheets

Peripheral ER sheets, or cisternae, are found in all eukaryotic cells (10). Several different machineries regulate the abundance and shape of ER sheets. A sheet has several physical features. It consists of two flat membranes stacked together with an intervening lumen, connected at the edges by high-curvature membrane. Several mechanisms have been identified so far that are likely to work together to (*a*) stabilize flat membranes, (*b*) stack opposing membranes together to make a sheet, and (*c*) curve the edges.

Ribosomes and sheet stabilization. EM studies have shown that sheets have a higher ribosome density than do tubules (~1,000 ribosomes/μm^2) and are likely to be the primary location of protein translocation (11). This correlation suggests that bound ribosomes and polyribosomes might regulate ER shape (13, 15). Elegant experiments have shown that when ribosomes are released from the translocation machinery on the ER by drug treatment, the ER sheets are disrupted (80). Additionally, polyribosomes are associated with other sheet-enriching proteins and are required for the segregation of these proteins into the ER sheets (13, 81). These data show that ribosome binding, probably as polyribosomes, plays some role in stabilizing the low-curvature membrane of the sheets and in regulating the retention of sheet-enriching proteins in the cisternae.

CLIMP63. EM studies have also revealed that ER sheets have a constrained luminal spacing of ~30 nm in yeast and 50 nm in mammalian cells (10, 13). This finding suggests that an intraluminal tether holds the two membranes that form an ER sheet at a constant distance. CLIMP63 (formally p63) is a 63-kDa, nonglycosylated, type II integral membrane protein of the ER that localizes to the ER sheets and may function to maintain the distance between the two membranes in an ER sheet (82–84). Depletion of CLIMP63 does not significantly alter the ratio of ER sheets to tubules; however, overexpression of CLIMP63 results in a dramatic increase in the number of ER sheets and fewer tubules. Thus, CLIMP63 appears to work in opposition to the reticulons, which increase tubules and decrease sheets when overexpressed (13). Structurally, CLIMP63 is composed of an extended coiled-coil domain, a TM domain, and an N-terminal cytoplasmic segment that can bind MTs both in vivo and in vitro (82, 85). CLIMP63 has a luminal coiled-coil domain that regulates its localization to sheets. Interactions between these charged coiled coils are predicted to drive CLIMP63 oligomerization, and these could bridge the luminal spacing of sheets (83). Indeed, CLIMP63 depletion reduces the luminal spacing from 45–50 nm to 25–30 nm (13).

Reticulons localize to the edges of ER sheets in yeast and animal cells (13). Thus, reticulons may also guide the curvature at the edges of sheets. Electron tomography of yeast mutants in which Rtn1 and Yop1 are deleted shows a massive expansion of ER sheets, accompanied by a small change in mean luminal spacing compared with wild-type cells (11). These data suggest that the curvature induced by Rtn1 and Yop1 at the edges of sheets may also regulate the organization of ER sheets.

Homotypic Fusion

A characteristic of the ER that becomes readily apparent upon live-cell imaging is that, even though it is very dynamic, it always remains continuous. Maintaining the continuity of the ER network requires that new ER tubules undergo constitutive and efficient homotypic (ER–ER) fusion whenever they contact other ER membranes. Several recent studies have revealed that a series of ER-localized GTPases mediate the fusion of ER tubules.

Atlastins. Atlastins are a family of conserved dynamin-related GTPases that localize to the ER and regulate ER fusion in multiple eukaryotes (86, 87). Mammals encode three atlastin paralogs (ATL1, ATL2, and ATL3), which are differentially expressed in multiple tissues. Atlastins are most homologous to members of the dynamin/Ms/guanylate-binding protein family of GTPases (GBPs) and share the GTPase, "middle," and other domains common to this family. Unlike those of many dynamins, the C termini of atlastins contain two putative TM domains, which are thought to form a hairpin, and an amphipathic helix–containing domain, which exposes most of the protein to the cytosol (88). In this way, the structure of the atlastins resembles that of another family of large GTPases, the mitofusins, which promote homotypic fusion of neighboring mitochondria through formation of hetero/homo-oligomeric complexes via heptad repeat regions in the C terminus that are similarly exposed to the cytoplasm (88, 89). However, unlike the mitofusins, atlastins have a catalytic arginine finger (R77), which is exposed in the GDP-bound state to stimulate GTP hydrolysis upon rearrangements within the G domain (90).

In vitro experiments have demonstrated that *Drosophila* atlastin catalyzes fusion of synthetic liposomes (86). The mechanism of fusion has been probed by structure–function studies. The crystal structures of the GTPase domain revealed two dimeric confirmations corresponding to a prefusion state and a postfusion state. These structures suggest a fusion mechanism in which the atlastins in two apposing membranes dimerize through interactions between their GTPases, which are bound to GDP. After the GDP is exchanged for GTP, the energy of hydrolysis drives

conformational changes in the atlastins that pulls the ER membranes close enough together that they fuse (91, 92). Increasing the length of the linker between the N-terminal GTPase domain and the TM domain significantly impairs ER fusion, thereby indicating the distance by which the ER membranes are brought together upon atlastin dimerization and implicating GTP hydrolysis as a critical step for atlastin-mediated ER fusion (90, 93). Subsequent destabilization of the membrane and induced curvature by the atlastin membrane domains could be the mechanism that drives fusion of the ER membrane (90, 94–96).

Similar to reticulons and DP1/Yop1, atlastin proteins partition into peripheral ER tubules and are excluded from the NE and peripheral sheets. However, atlastins also have the fascinating property that, when overexpressed, they accumulate at three-way junctions within the ER network (97). This localization is perfectly consistent with the observation that overexpression of a GTP-binding mutant of atlastin generates elongated ER tubules and reduces the frequency of three-way junctions (87). Although no sequence-related protein has been detected in yeast, a putative ortholog, Sey1p, was identified and found to be enriched in cortical ER and to mediate homotypic ER fusion in vitro and in cells (87, 98). Yeast Sey1p localizes to three-way junctions (99) and regulates ER morphology in a fashion similar to metazoan atlastin (87, 98). Together, these data demonstrate that atlastins regulate ER shape in a GTP-dependent manner.

The highly conserved Lunapark family of proteins (Lnps) is a recent addition to what remains a short list of proteins that regulate peripheral ER shape. Lnp1 was identified through an *S. cerevisiae* screen for ER proteins that disrupt the shape of the cortical ER network. Cells that lack Lnp1 contain a highly reticulated cortical ER. This phenotype is the opposite of that observed for atlastin depletion. Indeed, fluorescently labeled Lnp1p localizes to the ER, and like Sey1p/atlastin, it can also concentrate at three-way junctions (99). Mechanistically, Lnp1p works synergistically with reticulons and DP1/Yop1p but antagonistically to the atlastin homolog in yeast, Sey1p (99, 100). These data suggest that Lunapark and atlastin proteins may play opposing roles in regulating reticulation of the peripheral ER network. How Lunapark proteins shape ER membranes remains an important and unanswered question.

Rab GTPases. Rab GTPases regulate membrane trafficking and fusion events at multiple distinct steps throughout the secretory and endocytic pathways. Recently, two ER-localized Rab GTPases, Rab10 and Rab18, were found to determine ER shape by regulating ER dynamics and fusion. An in vitro assay for ER vesicle fusion and tubule formation using membranes derived from *Xenopus* egg extracts was used to identify the highly conserved Rab10 as an ER-enriched GTP-binding protein (101). In cells, Rab10 depletion or expression of a GDP-locked Rab10 mutant (T23N) causes proliferation of peripheral ER sheets at the expense of tubules (101, 102). In these studies, fluorescently labeled Rab10 localized to the ER and, remarkably, was enriched at the leading edge of more than 40% of ER sliding events. Moreover, ER–ER fusion usually occurred when the Rab10-enriched tip of an ER tubule encountered another ER membrane. In contrast, in the absence of Rab10 or upon Rab10 T23N expression, ER dynamics and fusion were dramatically reduced. Thus, the defect in ER dynamics and fusion after Rab10 inactivation explains the increase in ER sheets upon loss of functional Rab10.

Interestingly, Rab10 colocalizes with at least two phospholipid biosynthetic enzymes, phosphoinositol synthase (PIS) and choline/ethanolamine phosphotransferase (CEPT1), which also concentrate at the leading edge of ER sliding events (101). Thus, Rab10-enriched domains at the tips of sliding ER tubules may promote lipid synthesis and/or delivery to various parts of the cell. Whether lipid synthesis by PIS and CEPT1 occurs at Rab10-dynamic domains, and whether PIS or CEPT1 is required for regulating Rab10-dependent dynamics of the peripheral ER, remains to be determined.

Rab18 is a highly conserved Rab that is targeted to the ER by a Rab3–GAP complex (102). Loss-of-function mutations in Rab18 or Rab3–GAP subunits cause a similar phenotype: an increase in peripheral ER sheets. This phenotype is reported to be even more dramatic than the phenotype observed upon Rab10 depletion. Interestingly, Rab10 depletion redistributes Rab18 to peripheral ER sheets (102). It is not known whether these two Rabs are redundant, are synergistic, or regulate different steps during ER dynamics and fusion.

It seems clear that the Rabs and the atlastins facilitate ER fusion by entirely different mechanisms because the disruption of Rab10 or Rab18 function increases ER sheets, whereas atlastin disruption gives the opposite phenotype: an increase in unbranched tubules (87, 101, 102). The atlastins can directly fuse ER membranes and are likely to play a role in maintaining general ER continuity (86), whereas the Rabs might regulate the interaction between the ER and molecular motors during ER sliding and the rapid fusion that is occurring during these dynamics (43). Indeed, ER Rab depletion (or dominant negative expression) mimics the disruptions in ER sliding observed in kinesin-1 and dynein disruption experiments (49, 101, 102). Thus, when Rab10 or Rab18 is depleted, the inability to stabilize successful tubule fusion events ultimately deteriorates ER tubule maintenance and increases the size and abundance of sheets.

FUNCTIONAL CONSEQUENCES OF ENDOPLASMIC RETICULUM STRUCTURE: IMPLICATIONS FOR DISEASE PATHOLOGY

The organization of the ER has critical implications for maintaining not only ER function but also overall cellular health. Recently, ER morphology defects caused by mutations in many ER-shaping proteins, described above, have been linked to the pathology of several human diseases, including neurological disorders and viral infections. These links show the importance of ER morphology in ER function and organismal and cellular homeostasis.

Neurological Disease

ER morphology appears to play an important role in generating neuronal architecture (103). Neurons are highly specialized cells with an extensive peripheral ER network that packs the soma, axons, and dendrites (104–106). Functionally, axonal ER is especially interesting because it does not have bound ribosomes and therefore is not the site of protein translocation into the ER (106, 107). Axons (and dendrites) may rely on the ER for Ca^{2+} stores required in pre- and postsynaptic signaling, as well as the synthesis and trafficking of lipids required to support the development and extension of these neuronal processes. Not surprisingly, the peripheral ER is elongated and tubular in neuronal processes. It is also very dynamic. A mechanism must exist to extend peripheral ER tubules from the soma into the dendrite and axon along MTs all the way to their ends, and keep it there. Recently, multiple links have been found between mutations in genes encoding ER structural proteins and two common neurodegenerative diseases (103).

Alzheimer's disease. The reticulon proteins have been linked to Alzheimer's disease (AD). AD is one of the most common neurodegenerative diseases and affects more than 30 million people worldwide (108). The neuropathological features of a patient with AD include the presence of amyloid plaques and neurofibrillary tangles within the brain, and these correlate with neuronal degeneration and dementia (109). All four of the reticulon family members (RTN1–4) have been implicated in binding and modulating the activity of the type I TM aspartyl protease BACE1, which reduces the production of amyloid-β (110). Overexpression of the reticulon proteins significantly reduces the levels of amyloid-β. Given that BACE1 is initially expressed in the ER, interactions

with reticulons may prevent BACE1 from localizing to the Golgi network, preventing processing to the mature form and thereby inhibiting the generation of amyloid-β peptide (110).

In addition to the accumulation of amyloid plaques, other pathological features of AD include altered phospholipid and calcium metabolism and impaired mitochondrial function (111–115). These changes might be the result of altered ER–mitochondrial contacts in the cells of AD patients (56, 111, 116), as three major functions occur at ER–mitochondrial contact sites: Ca^{2+} transfer, lipid biosynthesis, and mitochondrial fission. Indeed, the number of ER–mitochondrial contacts is higher in brain samples from postmortem AD patients and AD mouse models (116, 117). Thus, the increase in ER–mitochondrial contacts might cause the mitochondrial fragmentation and dysfunction and changes in metabolism that occur in AD. These links suggest how alterations in ER structure affect the pathological features of AD (111, 117–120).

Hereditary spastic paraplegia. Hereditary spastic paraplegia (HSP) is a genetic neurological disorder characterized by progressive lower-limb spasticity and weakness resulting from axonal degeneration within motor neurons. HSP comprises a large family of genetic neurologic disorders (nearly 50 distinct loci) (121–123). ATL1 is the second most commonly mutated protein in HSP and represents the most common cause of early onset HSP (121, 124). Although ATL1 is ubiquitously expressed, it is expressed at very high levels in the brain (88, 125). Loss of ATL1 in cultured cortical neurons significantly inhibits axonal elongation, highlighting a functional requirement for ER fusion (126). Interestingly, Rab10 depletion also attenuates axonal elongation, although disruption of Rab10 has not yet been linked to a genetic neurological disorder (127).

REEP1 is another commonly mutated gene in HSP; it belongs to a family of six related proteins (REEP1–6), which were first identified through their role in trafficking olfactory receptors to the PM (121, 122, 124, 128, 129). REEP1 knockout mice demonstrate weakness and spasticity of the hind limbs, which recapitulates phenotype characteristics of HSP. Additionally, cortical motor neurons isolated from REEP1 knockout mice have a reduction in peripheral ER complexity, suggesting that REEP1 is necessary to maintain reticular ER structure in these cells. REEP5, also known as DP1, has already been implicated in regulating the formation and maintenance of ER tubules. Similar to REEP5/DP1, REEP1 localizes to the ER and contains two short hairpin TM domains, which are predicted to be capable of generating membrane curvature. Indeed, REEP1 can promote positive curvature on liposomes in vitro (130). Unlike REEP5/DP1, however, REEP1 contains an extended C-terminal domain that binds MTs through its MTBD (MT-binding domain) (121). Interestingly, recent evidence shows that atlastin interacts with REEP1 and may therefore indicate that, whereas REEP5/DP1 plays a direct role in ER morphology, REEP1 may instead be involved in regulating the dynamics and distribution of the peripheral ER (122, 131, 132). Given the close association between the ER and the MTs, REEP1's ability to bind MTs may therefore play a critical role in regulating ER movement and distribution within the neuron. Future studies will surely help elucidate the role REEP1 plays in HSP pathology and determine whether the clinical presentation of the disease is due largely to alteration in ER morphology, ER distribution, or a combination of the two.

Viral Infection

Several recent papers have shown that viruses alter ER structure to facilitate their propagation. These viruses modify ER structure to shelter the viral genome from host cell nucleases and co-opt the ER-bound ribosomes to synthesize their viral protein machineries (133–138). For example, infection by Japanese encephalitis virus (JEV) causes rapid filling of the lumen with viral particles upon cellular infection, which is accompanied by hypertrophy of the ER membranes.

The alteration in ER structure is also characterized by the proliferation of rough ER to support production of viral proteins for replication (139). Positive-strand RNA [(+)RNA] viruses also promote their replication by altering ER structure. These viruses, which include the brome mosaic virus (BMV), are responsible for several mammalian and plant diseases and utilize the ER for RNA replication through the formation of spherule vesicles on the peripheral ER (136–138, 140, 141). Compared with the positive-curving properties of reticulons and DP1/Yop1, infection by (+)RNA viruses induces ER membranes to undergo negative curvature by forming vesicular invaginations within the outer ER membrane to form spherules, which serve as sites of RNA synthesis (137, 142). These structures provide shelter from host cell nucleases and act as scaffolds to concentrate replication factors and catalyze virus-dependent reactions (133–138). Interestingly, loss of reticulons and Yop1 in yeast cells significantly inhibited BMV RNA replication by more than 80% (143). These data suggest that viruses use reticulons and DP1/Yop1 to rearrange the structure of the tubular ER to accommodate their needs (70, 79, 143, 144).

Further evidence of the role of ER structure in promoting viral replication is that some viruses directly interact with proteins required for shaping the ER. For example, enterovirus 71 (EV71), a member of the Picornaviridae family of viruses, forms a mature viral polypeptide, 2C, which binds and interacts with RTN3 (145). Knockdown of RTN3 through targeted small interfering RNA (siRNA) significantly reduced viral replication, and expression of siRNA-resistant RTN3 was sufficient to rescue infectivity, indicating that RTN3 plays an essential role in EV71 replication and infection (145). Although modification of ER morphology is a common theme for several viruses during RNA synthesis, reticulons and DP1/Yop1 are not always associated with the promotion of viral infection. Specifically, Wu et al. (146) recently provided evidence for the opposite phenotype in hepatitis C virus (HCV); RTN3 was found to bind to the HCV NS4B protein and thereby inhibit viral replication. RTN3 interacted specifically with the second amphipathic α-helical domain (AH2), thereby preventing self-oligomerization on the ER and subsequent formation of HCV particles (146–148).

Another way that viruses reshape ER membranes is by promoting the formation of double-membrane vesicles derived from the ER. These vesicles are found in cells following infection with HCV, dengue virus (DV), and severe acute respiratory syndrome (SARS) (136, 140, 141). Three-dimensional electron tomography studies have shown that DV vesicle structures have narrow necks and pore openings similar to those of BMV-induced single-membrane spherules, whereas SARS vesicle structures are sealed off from the cytosol (136, 141). SARS virus utilizes three viral nonstructural proteins (nsps) to induce ER remodeling. SARS nsp3 and nsp4 induce membrane stacking, whereas nsp6 drives membrane invagination and double-membrane vesicle formation (149). In contrast, HCV or DV can induce vesicle formation with the expression of a single viral integral membrane protein, NS4B or NS4A, respectively (136, 141). If this is a fundamental mechanism for double-membrane vesicle formation, then the observation that SARS infection requires three proteins to form double-membrane vesicles suggests that HCV NS4B and DV NS4A might employ host cell factors to stack and invaginate ER membranes. However, more studies focused on how viral factors directly or indirectly manipulate ER structure are needed. A better understanding of how viruses co-opt and rearrange the ER membrane will allow drugs to be developed that can perturb these processes.

CONCLUSIONS AND REMAINING QUESTIONS

Why the peripheral ER has such a complex and dynamic structure remained largely enigmatic for many years. Early EM studies suggested that the various domains of the peripheral ER, for example, the rough and smooth portions, had distinct cellular functions, but the relationship

between the structures of these domains and their functions was not well understood. Clearly, the structure of the ER is modified to meet the particular demands of the cell, as evidenced by the diverging ER morphologies in specialized cells. In general, cells that are primed for protein or sterol secretion (such as pancreatic or adrenal cells) have evolved peripheral ER with increased sheets, whereas cells requiring extensive Ca^{2+} modulation (such as muscle cells) are characterized by peripheral ER made up of a higher percentage of ER tubules. The dynamic nature of the ER also suggests that the transitions between ER sheets and tubules can occur quite rapidly in response to changing cellular environments.

Recent evidence suggests that much of the elaborate, dynamic structure of the ER is driven by the apparent need for the ER to track along MTs and remain closely apposed to other organelles in the cell. This understanding has resulted from improved multicolor live-cell imaging techniques and from the identification of proteins and forces that maintain ER structure. These studies raise important questions for the future. How are contacts between the ER and organelles generated, and what are the functions of these contacts? What cellular processes require these contacts? Why do most cells express a variety of reticulon and reticulon-like membrane-shaping proteins? How do cells sense cellular stresses and modify ER size and structure in response? What role does ER mobility play in ER function? As we answer these questions, we will develop a better understanding of how the ER regulates the activities of other organelles and how defects cause various diseases.

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Errata

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