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Peripheral ER Structure to Function

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Summary

The endoplasmic reticulum (ER) is a single continuous membrane-enclosed organelle made up of functionally and structurally distinct domains. The ER domains include the nuclear envelope (NE) and the peripheral ER, which is a network of tubules and sheets spread throughout the cytoplasm. The structural organization of the ER is related to its many different cellular functions. Here we will discuss how the various functional domains of the peripheral ER are organized into structurally distinct domains that exist within the continuous membrane bilayer throughout the cell cycle. In addition, we will summarize our current knowledge on how peripheral ER membranes contact various other regions of the cytoplasm including the cytoskeleton, mitochondria, Golgi, and the plasma membrane and what is known about the functions of these interactions.

Introduction

The ER has a complex structure with three main morphologically distinct regions that can be easily discriminated by fluorescence microscopy: 1) the sheets of the nuclear envelope 2) an extensive network of interconnected peripheral ER tubules and 3) peripheral ER sheets (see Figure 1a). All three of these structural regions exist within the continuous membrane bilayer and therefore must be maintained by proteins that partition as they generate these ER domains [1]. Many mechanisms go into shaping the nuclear envelope (NE) around nuclear contents and the details of this process have been previously reviewed [2]. This review will instead focus on the current knowledge of the structural and functional organization of the peripheral ER thought the cell cycle. Recent work has revealed new factors that contribute to peripheral ER structure by directly shaping the membrane bilayer. This structure is highly conserved and contributes to ER functions [3]. The cytoskeleton interacts with the peripheral ER membrane to spread it throughout the cytoplasm and make the ER an incredibly dynamic organelle. By spreading the peripheral ER membrane throughout the cytoplasm into a complex and continuous network, the ER can physically and functionally associate with other membrane bound compartments. Some of the proteins involved in these contact sites between the ER and other membranes have now been identified and their disruption affects both ER structure and function.

Factors that generate distinct ER shapes in different ER domains

The peripheral ER includes all regions of the ER other than the membrane sheets of the nuclear envelope. The peripheral ER branches out of the NE as an extensive network of interconnected

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tubules and sheets that share a single lumen (see Figure 1A). The elaborate network of ER tubules is perhaps the most distinguishing feature of the peripheral ER (see Figure 1B). It has a much more complex structure than the flat sheets of the NE and not surprisingly is enriched in proteins that shape membranes into tubules while being depleted of some of the inner nuclear membrane proteins known to shape the membrane sheets of the NE around chromatin and nuclear lamins (e.g. the LINC complex, [2]). The reticulon (Rtn) and DP1/Yop1 proteins are two classes of highly conserved, integral ER membrane proteins that shape the network of ER tubules both *in vitro* and *in vivo* (in yeast, plants, and animal cells) [4–6]. Their depletion in yeast and mammalian cells converts the peripheral ER tubules into sheets while their overexpression converts peripheral ER sheets into tubules [6,7]. These are the only proteins known to partition into tubular ER regions and are somehow excluded from the membranes of both the NE and peripheral ER sheets [6] (illustrated in Figure 2).

Current studies are aimed at understanding how Rtn and DP1/Yop1 proteins shape membranes into ER tubules. Reconstitution of yeast Rtn1 and DP1/Yop1 into proteoliposomes generates tubules of about 20 nm diameter suggesting that high concentrations of yeast Rtn1 or DP1/Yop1 are alone sufficient to structure membrane bilayers into tubules [8]. Both the topology and oligomerization features of these proteins are proposed to contribute to their membrane shaping activities [6,9]. Their topology is unusual with two long transmembrane (TM) domains, which sit in the membrane bilayer in a proposed hairpin with the N- and C- terminus as well as the soluble region between the two TM hairpins all facing the cytoplasm (as demonstrated by maleimide peg labeling) [6]. The TM domains are short and could potentially form a “wedge” in the outer leaflet of the membrane bilayer – this could generate membrane curvature by increasing the area of the outer leaflet relative to the inner leaflet. To generate the shape of a tubule rather than a vesicle, this membrane curvature would have to be ordered and only in one dimension. Interestingly, the Rtn and DP1/Yop1 proteins can homo-oligomerize and are immobilized by fluorescence recovery after photobleaching (FRAP) assays in the membrane bilayer [9]. The immobility of the Rtn or DP1/Yop1 complex is similar to that of known scaffolding proteins (like lamin B receptor and CLIMP63) [9]. The ability of the proteins to form tubular ER correlates with their ability to oligomerize as seen by FRAP, sucrose gradients, and cross linking studies; mutants that cannot oligomerize and are not immobilized by FRAP do not rescue tubular ER morphology in Rtn/Yop1 deletion yeast [9]. Other FRAP studies have shown that Rtn over-expression displaces and limits the mobility of luminal ER proteins in both mammalian cells and plants [5,8]. Together, these data demonstrate that the Rtn and DP1/Yop1 proteins are organized structurally to shape the membrane into tubules, but how they function is still unclear.

Peripheral ER sheets are enriched for polyribosomes and translocation complexes and contain little or no Rtn or DP1/Yop1 proteins (illustrated in Figure 2). It has been suggested that enrichment of polyribosomes on the membrane generates flat ER membrane sheets in the peripheral ER. Indeed, large protein complexes that flatten the membrane can generate sheet-like ER. Using electron tomography, Puhka et al. showed that cells treated with puromycin, which strips the ER of ribosomes, have more tubules than untreated cells. In contrast, treating cells with cyclohexamide, which only inhibits translation but allows ribosomes to still bind the ER membrane did not have the same effect [10]. Furthermore, overexpression of the ribosome binding protein, p180, which is an ER integral membrane protein, leads to an increase in stacked rough ER sheets and an increase in the cell's secretory capacity. This paper also showed that depletion of p180 in mammalian cells by shRNA had less rough ER, less ER sheets, and lowered secretory capacity [11]. Peripheral smooth ER sheets devoid of polyribosomes can also be artificially propagated by overexpression of proteins that oligomerize in the ER membrane [12,13].

The ratio of sheets to tubules in the peripheral ER could be maintained by cell-type specific levels of polyribosome-bound translocation complexes flattening membranes into rough ER sheets versus Rtn and DP1/Yop1 proteins curving membranes into tubules, since overexpression of either can alter the balance. Presumably cells with high secretory capacity and large arrays of rough ER sheets (like B cells) have high levels of polyribosome-bound translocation complexes and/or low levels of Rtn/DP1, while for cells with mostly tubular ER the converse could be true. But experiments demonstrating this pattern have not been done.

Peripheral ER structure during mitosis

The ER/NE membranes undergo large structural and functional changes during mitosis to allow redistribution of this organelle and its associated proteins to daughter cells. In yeast, the NE does not disassemble to the degree that it does in animal cells. In animal cells, the NE membrane fragments and the membrane and its associated proteins are absorbed into the peripheral ER, which does not disassemble to a significant degree in most cells [7,10,14,15]. Elegant experiments in mammalian cells have now characterized some of the fine structural changes that the peripheral ER undergoes as the cell transitions through mitosis [10]. While the peripheral ER remains continuous during transitions between interphase and mitosis, the shape does change from a mixture of sheets and tubules in interphase to a highly reticulated tubular ER structure devoid of sheets during mitosis [10]. This change in peripheral ER structure during mitosis is accompanied by some measured changes in ER function. It has been shown that both ER exit site numbers and ribosome density are reduced [10,16], suggesting that ER dependent translation and protein transport are also presumably reduced if not halted.

A highly reticulated tubular ER may be more evenly redistributed than sheets to daughter cells at the end of mitosis in animal cells. In addition, recent evidence suggests that the structure of the peripheral ER network during mitosis can affect the rate of nuclear envelope reformation around chromatin [7,17,18]. Using an *in vitro* system derived from *Xenopus* egg extracts, live-imaging showed that an intact tubular network first binds to chromatin to initiate nuclear envelope formation [17]. When tubular ER formation was inhibited (by preincubation with inhibitory antibodies to Rtn 4a) nuclear envelope formation was also inhibited [7,18]. The rate of NE formation *in vivo* has also been shown to be sensitive to the levels of Rtn proteins. Overexpression of Rtn proteins to generate more tubular ER delayed NE assembly, while siRNA depletion of Rtn proteins to deplete tubular ER resulted in faster NE formation than wild type cells [7]. Taken together, these data suggest a role for reticulons and the regulation of peripheral ER shape in NE assembly.

The relationship between ER dynamics and the microtubule cytoskeleton

The peripheral ER is extremely dynamic and tracks along microtubules (MT) in animal cells [19–21]. Even during interphase, the network of tubules and sheets is constantly rearranging and it is quite clear by live-cell imaging that tubular ER networks move along MT. The co-alignment between growing ER tubules and MTs is visually perfect (see Figure 1C). In contrast, regions of the peripheral ER that are not dynamic do not co-align with MTs. These data, as well as *in vitro* systems that show that MTs are not required for proper ER formation [22], demonstrate that MTs distribute and move ER but are not necessary for the inherent shape of the membrane bilayer.

There are two mechanistically distinct ways that ER tubules “travel” along microtubules (MT): by the tip attachment complex (TAC) vs. sliding. During TAC movement, the tip of the ER tubule appears (by fluorescence microscopy) to be attached to the (+) tip of a dynamic MT. As the (+) end of the MT grows or shrinks, so does the ER tubule. Two proteins have been identified that affect TAC movement of ER tubules, STIM1 and EB1. STIM1 is an integral ER membrane protein with a luminal N-terminal EF-hand Ca²⁺ binding domain, a single TM domain, and

a C-terminal MT-binding domain. It has a proposed role in both ER Ca^{2+} signaling and in TAC movement of ER tubules - two functions that are not necessarily mutually exclusive [23–26]. Several lines of evidence demonstrate a role for STIM1 in TAC ER remodeling: 1) STIM1 concentrates at the tips of ER tubules where they are attached to growing MTs by the TAC, 2) STIM1 depletion by siRNA reduces movements of ER tubules through the TAC, and 3) STIM1 interacts directly with EB1 (a MT (+) tip binding protein). Consistent with these results, depletion of EB1 by siRNA also reduces the movements of ER tubules by the TAC on MTs [24]. Collectively, these data suggest that STIM1 on the ER can bind to EB1 on the (+) tip of a MT to allow an ER tubule to grow and shrink with the tips of dynamic microtubules.

Interestingly, STIM1 and EB1 are not involved in the rapid sliding movements of the ER along microtubules [24]. During “sliding”, the ER tubules appear to jump onto the shaft, rather than the tip, of the microtubule and slide. No factors have been identified that are responsible for ER sliding even though they far outnumber the TAC movements, depending on the cell type 70–95 % of events are sliding [21,24]. The rate of ER sliding has also been measured to be much faster than TAC-mediated growth. Having both TAC and sliding mechanisms on the ER indicates that these processes may have different functions.

Other proteins have been identified that may link the ER to microtubules either directly or indirectly. One of these, CLIMP63, is a resident ER membrane protein that also has an MT binding domain. CLIMP63 is enriched in the peripheral ER sheets and tubules and is excluded from the nuclear envelope [27]. The C-terminus of CLIMP63 homo-oligomerizes in the ER lumen, which causes CLIMP63 to diffuse slowly in the membrane (demonstrated by FRAP assays) and is necessary for its exclusion from the nuclear envelope [27]. The N-terminus of CLIMP-63 has been shown to bind to MTs *in vitro* [28]. Overexpression of CLIMP-63 protein in COS cells causes an increase in co-localization between ER tubules and microtubules suggesting that CLIMP63 may link the ER directly to microtubules to help spread it into the cytoplasm. Conversely, overexpression of a CLIMP63 mutant protein, which lacks the MT binding domain, causes the mutant protein to accumulate in sheets close to the nucleus and results in retraction of peripheral ER towards the nucleus [28]. Recently, it was also shown that depolymerization of MTs or siRNA depletion of CLIMP-63 protein in mammalian cells both increase the lateral mobility of the ER translocation complex (by FRAP) [29]. These data collectively suggest that CLIMP63 and microtubules may contribute to the generation of rough ER domains, probably peripheral ER sheets, where translocation complexes are partitioned.

Functional interactions between the ER and other membrane systems

The ER has regions that appear in tight association with nearly every other membrane-bound compartment in the cell including the mitochondria, peroxisomes, golgi, vacuole, chloroplasts, and plasma membrane. These interactions have been shown in many cases to be functionally important and explain why the ER is organized into an extensive structure spread throughout the cytoplasm. The two main reasons for the ER to contact the membranes of other organelles are 1) nonvesicular transport of ER synthesized lipids and sterols between the two adjacent membranes and 2) calcium signaling between organelles.

Mitochondria and ER linkage is important for proper calcium signaling, apoptotic regulation and the synthesis of cytochrome c oxidase, phospholipids and glycosphingolipids [30]. Proper spacing of the ER and mitochondria is essential for cell function and survival; physical bridges between these two organelles have been visualized by electron microscopy. These protein bridges are likely to determine the distance between the two membrane systems, which average at about 10nm for smooth ER and 25nm for rough ER [30]. Clever experiments have shown that limited proteolysis or the expression of shortened artificial linkers between the ER and mitochondria leads to disruption of mitochondrial functions including Ca^{2+} signaling and

apoptosis signaling [30]. It is likely that the spacing between the ER and mitochondria must also be precise to allow lipid flipping between the two membrane systems during phospholipid synthesis, which is coordinated between biosynthetic proteins located on both the ER and the apposing mitochondrial membrane [31,32].

The proteins involved in translocation of lipids and sterols between mitochondria and ER are not known, however, recent work has strongly suggests that Mitofusin2 (MFN2) may regulate the formation and stabilization of the bridge between the ER and mitochondria. MFN2 localizes to the mitochondria and ER junctions. At these junctions MFN2 participates in homo- or hetero-complexes composed of MFN2 at the ER and MFN1 or MFN2 at the mitochondria [33] (see Figure 2). *In vivo* studies showed that when MFN2 is deleted in mouse embryonic fibroblasts the bridge between the ER and mitochondria is lengthened [33]. The MFN2 deletion decreases the integrity of both the ER and the mitochondria; this result could either be because the interaction plays a direct structural role in both organelles or because loss of contact sites could adversely affect phospholipid biosynthesis. These results demonstrate that MFN2 has a role in controlling part of the ER-mitochondria bridge; however, other components of this bridge still need to be identified.

The ER also interacts directly with the Golgi at membrane contact sites, which have been proposed to allow the nonvesicular transport of some lipids from the ER to the Golgi [34,35]. Recent work demonstrates that integral ER membrane proteins VAP-A and VAP-B affect the integrity of contact sites for lipid transfer between the two membranes [36]. The function of VAP-A and VAP-B may be to sense and control lipid transfer by controlling its interactions with the lipid transfer binding protein Nir2, oxysterol-binding protein (OSBP) and ceramide-transfer protein (CERT) which are localized to the Golgi [34,37,38] (see Figure 1D). Depletion of VAP-A and VAP-B by RNAi prevents the Golgi targeting of Nir2, OSBP, and CERT and consequently alters the lipid composition of the Golgi.

The far reaches of the peripheral ER are also closely apposed to the plasma membrane in yeast and mammalian cells (< 50 nm) [39]. The short distance between the two membrane systems suggests a protein complex could link them together. As mentioned earlier, the ER synthesizes cholesterol and many of the lipids that compose membranous compartments, including the PM. The transport of cholesterol to the PM is likely to be non-vesicular because it is Brefeldin A insensitive (BFA inhibits transport from the ER to the Golgi) and is mediated by ATP-dependent carrier proteins [40]. The Osh family of proteins is thought to regulate the transport of cholesterol to the PM. The endoplasmic reticulum membrane is close enough to the plasma membrane that it is conceivable that the Osh protein complex could translocate cholesterol across this gap (see Figure 2). There is some debate as to whether or not Osh proteins directly bind to cholesterol [41] or if they indirectly affect cholesterol transport by affecting the ability of the PM to sequester cholesterol [42]. *In vitro* assays have demonstrated the ability of Osh proteins to transfer sterols between membranes [43], but *in vivo* evidence of Osh proteins regulating transfer between the ER and PM is still missing. Phospholipids are also likely to be directly transferred to the PM from the ER. When the trafficking of phospholipids from the ER is monitored following their synthesis, both Phosphatidylcholine and Phosphatidylethanolamine accumulate more rapidly on the PM than would be predicted if they were transported predominantly through the secretory pathway [44]. The proteins involved in the translocation of phospholipids from the ER to the PM are not known.

Conclusions

There are three main ways ER structure is determined that we have discussed: 1) membrane proteins that partition within the membrane bilayer and directly shape it by forming oligomeric structures, 2) interactions between membrane proteins on the ER and the cytoskeleton, and 3)

interactions with other membrane-bound compartments. Much progress has been made, but still only a handful of the proteins that contribute to each of these three processes have been identified. For those identified, there is still much to learn about how these various interactions are regulated to make the elaborate structure of this large membrane-bound compartment functional.

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*Of special interest

**Of outstanding interest

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Figure 1.

The structure of the ER. A) A mammalian COS cell fluorescently labeled with GFP-tagged Sec61 β outlines morphologically distinct regions of the ER including the nuclear envelope (NE), peripheral ER sheets (S) and tubules (T). Also see close up image of tubules in (B). (C) peripheral ER in COS cells fluorescently labeled with cherry-Tubulin (red) and GFP-Sec61 β (green) shows ER tubule tip (green arrow) tracking with growing MT tip (red arrow) by TAC mechanism. Part A was reproduced with permission from Nature Publishing Group. Images in parts B and C were kindly provided by Brant Webster.

Figure 2.

Resident ER proteins utilized by the cell to shape ER membrane and link it to other regions within the cytoplasm. Reticulon (blue shapes) and DP1/Yop1 proteins shape the tubular ER and p180 (brown circle), polyribosomes (black tracks) and components of the translocon complex (Sec61 β -turquoise circle) shape the sheet-like ER, and the inner and outer membranes of the nuclear envelope are linked by the LINC complex (pink and red rectangles). The peripheral ER has numerous contact points with various membranes. The ER proteins form bridges with the mitochondria through MFN2/MFN1 dimers (pink and purple circles), with Golgi membranes through VAPs (gray circles) interacting with lipid transfer binding proteins (LTBP- bright blue circles), and the plasma membrane potentially through Osh proteins (gold shape, sterols are green circles).