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Balancing ER dynamics: shaping, bending, severing, and mending membranes

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Abstract

The endoplasmic reticulum is a multifunctional organelle composed of functionally and morphologically distinct domains. These include the relatively planar nuclear envelope and the peripheral ER, a network of sheet-like cisternae interconnected with tubules that spread throughout the cytoplasm. The ER is highly dynamic and the shape of its domains as well as their relative content are in constant flux. The multiple forces driving these morphological changes depend on the interaction between the ER and microtubules, membrane fusion and fission events and the action of proteins capable of actively shaping membranes. The interplay between these forces is ultimately responsible for the dynamic morphology of the ER, which in turn is crucial for properly executing the varied functions of this organelle.

Introduction

The endoplasmic reticulum (ER) is an intracellular organelle that supports a remarkable number of functions crucial for life. The ER plays a critical role in many aspects of protein compartmentalization. These include membrane translocation, folding, post-translational modification, and transport of both membrane and soluble proteins. Additionally the ER is involved in the synthesis of phospholipids and steroids and in the regulation of Ca^{2+} homeostasis [1,2]. Reflecting this diverse set of functions, the ER is organized in a complex, continuous network of tubules and sheets that includes the nuclear envelope and extends throughout the cytosol into the cell periphery [3–7]. Tubules and sheets are thought to be all interconnected, so that the ER membrane encloses a single continuous luminal space that often occupies more than 10% of the total cell volume [1,3]. Both sheets and tubular domains are present in all eukaryotes that have been examined. Although the proportion of tubules and sheets may vary between different cell types and species, these subcompartments share many common features [8]. ER sheets are flat, extended surfaces with little membrane curvature. The nuclear envelope, although spherical, can be considered as a sheet owing to its large size. ER tubules are long and cylindrical with high membrane curvature and intersect to form an elaborate network that pervades the cytoplasm [9]. The thickness of sheets and diameter of tubules varies depending on the cell type, but typically is around 60–100 nm suggesting that both domains are actively shaped [10]. These distinct

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morphological domains correlate with specialized ER functions. ER sheets are often characterized by the presence of membrane bound ribosomes (rough ER) and perform all functions associated with the biosynthesis of membrane and secreted proteins, including the control of their proper folding and modification. Conversely, ER tubules are mainly formed of smooth ribosome-free ER and are involved in lipid synthesis and delivery. The smooth ER also includes zones of contact with membranes of other organelles. A subdomain morphologically and functionally distinct from the surrounding smooth ER is the transitional ER, where protein and lipids from the ER are exported through COPII coated vesicles towards the secretory pathway [2,9].

The dynamic ER

The ER continuously undergoes significant rearrangements of its structure: vesicles bud from and become incorporated into the ER membrane, new tubules form from existing ones, tubules retract, sheets transition to tubules and tubules to sheets, tubules fuse and likely break apart. Remarkably, despite this constant reorganization the ER maintains luminal continuity and its characteristic structure. These events contribute to making the ER a highly dynamic organelle and the dynamic nature of the ER is conserved through evolution. Although continuous shape changes are seen consistently, the relationship between ER dynamics and ER functions remains still unclear. Different mechanisms underlie the different modalities of ER dynamics. Vesicle budding and fusion pertain to the prominent role of the ER in the secretory pathway and will not be further examined in this review. The outgrowth and retraction of tubules depend on the close association between the ER and the microtubule (MT) cytoskeleton. Shape and shape transitions of the ER membrane are determined by the ability of specific proteins to distort phospholipid bilayers. Finally, ER membranes are remodeled through fusion, a process whereby two separate membranes merge into one, and probably fission that entails the division of a continuous membrane into two separate ones. We will describe here current information regarding these distinct mechanisms of ER dynamics.

Microtubule-dependent dynamics

ER tubules track along microtubules [11–13] and in animal cells alteration of ER dynamics can be achieved by inducing MTs depolymerization [12,13]. There are two distinct mechanisms whereby ER tubules move along MTs. One mechanism is defined as tip attachment complex (TAC) dynamics and involves the attachment of the tip of the ER tubule to the tip of the MT plus end. During TAC dynamics, the ER tubule grows or retracts as its MT partner grows or retracts. TAC dynamics are dependent on the physical interaction between STIM1, an ER resident transmembrane protein, and the MT plus end–binding protein, EB1[14]. The second mechanism is defined as sliding and involves binding of the tip of the ER tubule to an existing MT shaft followed by sliding of the ER tubule along the MT [11,13]. ER tubule sliding is faster and more frequent than TAC dynamics but the proteins that mediate this process have not been identified. Recently, it has been shown that ER sliding occurs on stable MTs post-translationally modified by acetylation. Furthermore, mitochondria but not other organelles contact the ER preferentially at acetylated MTs suggesting that different ER dynamics occur on distinct MT populations to make contacts with different organelles [15]. CLIMP63, a resident ER membrane protein that contains a MT binding domain and is excluded from the nuclear envelope, has also been proposed to provide a direct link between MTs and the ER thus helping to distribute the ER in the cytoplasm [16,17].

Membrane shape and shape transitions

The ER is composed of different structural and functional domains. The nuclear envelope and sheet-like cisternae are characterized by relatively flat, planar membranes on most surfaces while tubules exhibit high membrane curvature. ER domains and their membrane shape are generally fluid and often change during processes such as cell division, growth, and metabolic state. Indeed, live-cell imaging clearly shows that even during interphase the network of tubules and sheets undergoes constant rearrangements.

In aqueous solution phospholipid bilayers spontaneously form either spherical or laminar shaped structures. The different domains within the ER membrane exhibit notable morphological variation which depends on the spatial arrangement of the lipid bilayers in low curvature sheets or high curvature tubules indicating that membrane shaping relates to the generation of membrane curvature [18,19]. How are these energetically unfavorable membrane shapes of ER domains generated and maintained? A complex interplay of factors is likely to ultimately determine membrane morphology, however, one important way to shape membranes involves the use of proteins endowed with the ability to deform lipid bilayers [4,19]. Proteins can shape membranes in a variety of ways. Mechanical force can be applied to a lipid bilayer by molecular motors pulling on membrane proteins. Peripheral membrane proteins with an intrinsic curvature can conform membranes to their shape, and integral membrane proteins with specialized hydrophobic domains can selectively insert or wedge into the outer monolayer to physically generate curvature [4].

All these mechanisms could synergistically contribute to conferring the typical shape of ER domains, however, recent work has indicated that the reticulon (Rtn) and DP1/REEP/Yop1 proteins are two classes of highly conserved, integral ER membrane proteins prominently involved in the morphogenesis of peripheral ER tubules [6,20–22]. Their topology is thought to contribute to their ability to deform the membrane [5,22]. Rtn and DP1/REEP/Yop1 contain two large hydrophobic segments, in some cases subdivided into smaller transmembrane (TM) domains, resulting in a number of possible transmembrane topologies [23], including a ‘W’ topology in which both the N- and C-termini protrude in the cytosol [22]. The TM domains are proposed to insert only in the outer leaflet of the membrane bilayer forming a ‘wedge’ and thereby generating membrane curvature by increasing the surface area of the outer leaflet relative to the inner leaflet. The length of the TM domains has been shown to be a crucial determinant of the capacity of Rtns to shape the ER into tubules [24,25]. The ability of Rtn and DP1/REEP/Yop1 to form tubular ER correlates with their ability to oligomerize and the demonstrated immobility of these complexes is reminiscent of scaffolding proteins like lamin B receptor and CLIMP63 [5], potentially suggesting a contribution of scaffolding mechanisms to the membrane shaping activity of reticulons. Importantly, in vivo depletion of Rtn and DP1/REEP/Yop1 in several systems converts the peripheral ER tubules into sheets while their overexpression converts peripheral ER sheets into tubules [22,24,26]. A direct demonstration of the membrane shaping activity of these protein has been provided in vitro by showing that reconstitution into liposomes generates proteolipid tubules of 15–17 nm diameter indicating that Rtn1 or DP1/REEP/Yop1 are sufficient to remodel the membrane bilayer into tubules [27]. It is likely that the relative proportion of ER tubules and sheets in cells is determined by modulating the levels of these ER-shaping proteins and differentially partitioning them.

In contrast to tubule formation, the mechanisms utilized to produce and stabilize the sheet-like morphology of ER cisternae are less well understood. It has been shown that Rtn and DP1/REEP/Yop1 segregate into the tubular ER regions but are essentially excluded from the NE and scarce in peripheral ER sheets, suggesting that their absence may prevent them from assuming a tubular morphology [22]. Among the mechanisms believed to be responsible for

maintenance of sheet morphology are the presence of polyribosomes complexes. Stripping the ER of ribosomes with puromycin results in cells with a greater proportion of tubules compared to untreated cells [28] and overexpression of the membrane ribosome binding protein p180 leads to an increase in rough ER sheets as well as in the cell's secretory capacity while its depletion leads to less rough ER sheets and lowered secretory capacity [29]. Another hypothesis postulates that the flat shape of ER sheets depends on scaffolding within the ER lumen by proteins like the coiled-coil protein CLIMP63. Oligomers of CLIMP63 bound to the membrane and spanning the ER lumen may determine the thickness ER sheets [17]. This dual role of CLIMP63 as an internal shape manager and its link to cytoplasmic MTs, suggest that CLIMP63 could be involved in MT-mediated dynamics and ER shape changes.

Recent work, however, suggests that neither polysomes nor coiled-coil proteins are essential for sheet formation which instead appears to involve the reticulons and DP1/REEP/Yop1p proteins [30]. Reticulons and DP1/Yop1p localize to both tubules and sheet edges, their depletion leads to increased sheet areas, and their overexpression converts sheets into tubules. Because these proteins can localize to sheet edges their oligomerization may generate scaffolds around curved membranes, which may be shaped as open arcs, whose function would be to stabilize the high membrane curvature at the edges, bringing the two membranes of the sheet into close apposition [30]. In this context, a theoretical model has been developed supporting the view that the reticulons and DP1/Yop1p alone can generate both tubules and sheets, and suggesting that their abundance determines the ratio of these domains [30].

Fusion and fission

Another critical aspect of ER dynamics is membrane fusion. When observed in vivo by GFP labeling, the ER undergoes obvious fusion events that are visible as the merging of separate tubules. Membrane fusion activity is essential for preserving the typical structure of the ER [7]. In fact, it has been demonstrated that a fusion reaction is absolutely required for ER network formation using an in vitro system derived from Xenopus egg extracts [31,32]. Furthermore, the ER undergoes major structural rearrangements to permit proper redistribution of the organelle to daughter cells during cell division. The nuclear envelope disassembles and its associated proteins enter the peripheral ER. The peripheral ER also changes morphology from a mixture of sheets and tubules in interphase to a highly reticulated tubular ER structure without sheets during mitosis [28]. It is likely that membrane fusion plays an important role in the reformation of a morphologically and functionally normal ER in the daughter cells.

Fusion of ER membranes has been shown to depend on GTP [31,32] and recently the atlastins have been implicated in this process. The atlastins are distantly related to the superfamily of large dynamin-related GTPases that associate with different intracellular membrane compartments [33]. Dynamin-1, the prototypical member of this family, functions in vesicle budding from the plasma membrane during clathrin-mediated endocytosis. Other members of this family are necessary for the fusion and fission of mitochondria membranes and for the scission of vesicles such as caveolae and phagosomes [33,34].

Atlastins are integral membrane proteins that contain an N-terminal GTPase domain and two transmembrane spans whose insertion in the lipid bilayer results in the N- and C-termini protruding into the cytoplasm [35–37]. Vertebrates have three closely related atlastins (1,2 and 3) while invertebrates possess only one ortholog. The single Drosophila atlastin has been demonstrated to be the GTPase responsible for the homotypic fusion of ER membranes

[38]. In vivo depletion of Drosophila atlastin causes ER fragmentation and conversely its overexpression induces excessive ER fusion. Drosophila atlastin mediates tethering through trans-oligomer formation between molecules located on adjacent membranes as well as the ensuing bilayer merger and atlastin reconstitution into liposomes promotes their GTP-dependent fusion [38]. Consistent with its requirement for homotypic fusion of ER membranes, fly atlastin is restricted to the ER. In contrast, human atlastins localize predominantly to the tubular ER but also to the ERGIC and cis-Golgi [36]. Human atlastins have been implicated in ER network formation and membrane remodeling [35,39,40], yet a direct demonstration of their fusogenic ability is still lacking. Moreover, the three human atlastins exhibit differential tissue enrichment and/or distribution [35,36] raising the possibility that hetero-oligomer formation might underlie ER fusion in mammals.

Very recently two structures corresponding to the N-terminal cytoplasmic domain of human atlastin-1 have been solved (Figure 2A) [41,42]. The atlastin-1 GTPase domain bears notable similarity with that of GBP1, another distant member of the dynamin superfamily of GTPases implicated in immune response [43,44]. The middle domain that separates the GTPase domain from the membrane spans, was shown to fold into a three helical bundle. In both studies, two different crystal forms were identified that differ with respect to the location of the middle domain three helix bundle relative to the GTPase domain (Figure 2A, crystal form1 and crystal form 2). Similar to GBP1, within the GTPase domain lies a proposed atlastin dimerization interface that was observed as part of a crystallographic dimer. The solved structures contain bound GDP, however, there is a strict requirement for the binding of GTP to induce atlastin-1 dimerization in solution, making it uncertain which functional states these GDP-bound crystals represent.

Nevertheless, these recent atlastin snapshots provide insight into a potential fusion mechanism by this class of fusogen (Figure 2B). It is possible that monomeric atlastin in a nucleotide-free state is primed for membrane docking by GTP binding. Crystal form 2 (Figure 2A, right) may represent this “pre-fusion” state of atlastin given the predicted relationship between the GTPase domain, the three helix bundles, and the transmembrane segments. The initial interface between atlasins in adjacent membranes may be the GTPase domain; however, this interaction likely matures or is stabilized by rearrangements between the three helix bundle and the GTPase domain. This conformational change has been proposed to be driven by nucleotide hydrolysis. The energy derived from this domain rearrangement may be used to promote lipid mixing and membrane fusion. Crystal form 1 probably represents a “post-fusion” conformer.

An outstanding question concerning ER dynamics is whether maintenance of the ER structure requires that membrane fusion be counterbalanced by fission events. An illuminating example is provided by mitochondria whose shape is maintained by balancing the rates of fusion and fission [45]. Interestingly, in mitochondria these fusion and fission reactions are mediated by the large GTPases of the dynamin superfamily mitofusins, OPA1 and DRP1 [46,47]. Although live-cell imaging has not furnished compelling evidence for the occurrence of ER fission, other indications suggest that this may be the case. For example, the disassembly of the ER during mitosis implies that the cell has the ability to break down ER membranes and this ability could depend on membrane fission. Additionally, the frequent fusion events that take place in the ER membrane would potentially lead to an increasingly reticulated ER in the absence of a complementary fission reaction, yet experimental observation shows that ER morphology is generally preserved. For these reasons we believe that fission is likely to play a role in maintaining normal ER shape. Therefore, one important direction for futures studies will be to establish the existence of fission, its role in ER morphogenesis and maintenance as well as the identification of the protein machinery involved in this process.

Interplay of proteins involved in ER dynamics

A network of physical interactions among ER-shaping proteins and MTs is beginning to unfold suggesting that these proteins may work in concert to establish ER shape and control its dynamics. Atlastins interact with both the DP1/REEP/Yop1 and the reticulon families [39,48]. Interestingly, mutation of atlastin-1 and REEP1 cause two different forms of hereditary spastic paraplegia, a group of neurological disorders characterized by a length-dependent axonopathy of the corticospinal motor neurons [49]. In addition, REEP1 has been proposed to interact directly with microtubules as well as with a membrane-bound form of spastin, a AAA ATPase responsible for microtubule severing [48], mutated in another form of spastic paraplegia. Spastin has also been reported to physically interact with atlastin [35,48,50,51]. However, one report claims that spastin binds to the C-terminal cytoplasmic tail of atlastin explicitly excluding any involvement of the N-terminal cytoplasmic domain [50], another shows that the interaction with spastin occurs specifically through the N-terminal cytoplasmic domain of atlastin [51], and yet a third one reveals that the region of atlastin crucial for binding spastin is the intramembrane segment [48]. These discrepancies are difficult to reconcile and cast serious doubts on the specificity of the physical interaction between spastin and atlastin.

Although the microtubule cytoskeleton is not absolutely required for the formation of an interconnected tubular network in vitro [32], ER-microtubule interactions are probably crucial to determine the typical morphology of the ER in the cell. For example, pharmacological disruption of microtubules leads to retraction of the ER from the cell periphery and the formation of extended ER cisternae [52]. The interactions of REEP1and spastin with microtubules could help stabilize the structure of ER tubules, the most dynamic portion of the ER, while scaffolding proteins such as CLIMP63 may mediate the attachment of microtubules to ER sheets. Therefore, the confluence of interactions between ER-shaping proteins and MTs offers a mechanism for coupling ER morphology with cytoskeletal dynamics. The importance of this relationship for maintaining ER function is underscored by the observation that its disruption may cause disease. Despite this progress, however, the functional understanding of these interactions and their implications remains limited and will require further investigation.

Conclusions

There are three general processes known to influence ER dynamics. First, association of the ER with the MT cytoskeleton, second, membrane shape transitions from sheets to tubules and vice versa that are controlled by proteins capable of membrane deformation, and third, the remodeling of membranes that occurs by fusion and fission. The ER is a vital and dynamic organelle that is beginning to reveal how its many shapes and faces are used to provide the varied functions required of this organelle. Work in the last few years has uncovered a number of new players involved in ER dynamics and morphogenesis. Future efforts will focus on identifying more of the players and understanding how the ensemble of these proteins accomplishes the task of ER dynamics and influences ER function.

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Highlights

1. the endoplasmic reticulum is a very dynamic membrane-bound organelle
2. ER dynamics rely on microtubule interactions, fusion and fission and shape transitions
3. proteins controlling these events and their interplay are being revealed

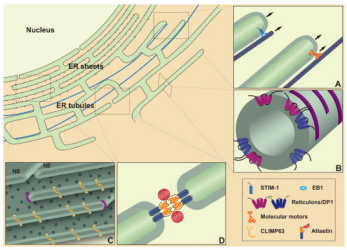

Figure 1.

Domain organization of the endoplasmic reticulum network within the cell. **A**, ER tubules move about the cytoplasm by attaching to microtubules using a TAC mechanism (left) or by a sliding mechanism (right). **B**, the reticulons and DP1/REEP/Yop1 shape flat membranes into tubules using a combined wedging and scaffolding mechanism. Their hydrophobic segments insert like a wedge in the outer lipid layer causing the bilayer to bend and their ability to homo- and hetero-oligomerize may produce arc-like scaffolds around the tubules. **C**, ER sheets, observed here in cross section, can be generated by the presence of the reticulons and DP1/REEP/Yop1 at their edges to stabilize locally the high curvature. In addition, transmembrane scaffolding proteins localized in both membranes interact through their luminal domains to maintain the two membranes flat and at a constant distance. NE, nuclear envelope. **D**, two ER tubules in the process of being merged by the fusogenic activity of the atlastin GTPase.

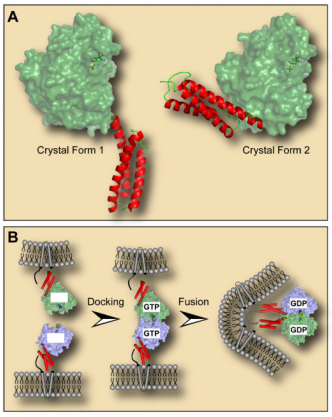

Figure 2.

Model for atlastin-mediated membrane fusion. **A.** Models the N-terminal cytoplasmic domain of human atlastin-1. The two crystal forms that may represent post- and pre-fusion structure are shown. The GTPase domain is cartooned as a surface representation, the middle domain is shown as red cylinders, the transmembrane domain as gray cylinders and GDP as sticks. **B.** Membrane fusion model. Nucleotide-free atlastin monomers are drawn as crystal form 2 in adjacent membranes (left). Nucleotide binding permits an association between GTPase domains (middle). GTP hydrolysis promotes domain rearrangements that drives membrane fusion and results in the post-fusion crystal form 1 (right). The models were rendered in Pymol from 3Q5D and 3Q5E.