Review

Special Issue – 3D Cell Biology

The ER in 3D: a multifunctional dynamic membrane network

Jonathan R. Friedman and Gia K. Voeltz

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA

The endoplasmic reticulum (ER) is a large, singular, membrane-bound organelle that has an elaborate 3D structure with a diversity of structural domains. It contains regions that are flat and cisternal, ones that are highly curved and tubular, and others adapted to form contacts with nearly every other organelle and with the plasma membrane. The 3D structure of the ER is determined by both integral ER membrane proteins and by interactions with the cytoskeleton. In this review, we describe some of the factors that are known to regulate ER structure and discuss how this structural organization and the dynamic nature of the ER membrane network allow it to perform its many different functions.

Overview of ER shape

The ER is the largest membrane-bound organelle in the eukaryotic cell. It is spread throughout the cytoplasm as one continuous membrane-enclosed network that surrounds a single lumen. Domains of the ER have many different shapes and include regions that are flat, tubular, and somewhere in between, and also structures that are adapted to contact other membrane-enclosed compartments. The ER must remain connected while maintaining a shape that will allow other cytoplasmic components to diffuse around it. Only a handful of factors have been discovered that regulate ER structure. In this review, we discuss recent advances in our understanding of how ER membrane proteins, the cytoskeleton, and factors that mediate inter-organelle contact together generate the 3D structure of this elaborate, dynamic, and functionally complex organelle.

How ER shape contributes to function

The ER performs a variety of functions in cells, including but not limited to lipid synthesis, Ca^{2+} handling, and protein translocation and secretion [1]. To perform its many functions, the eukaryotic ER has perhaps the most complicated structure of any organelle. Some ER domains are obvious and can be distinguished by their shapes using fluorescence microscopy. These include the nuclear envelope (NE), and cytoplasmic cisternae and tubules that form the interconnected peripheral ER (Figure 1).

The NE is a distinct domain of the ER comprised of two large, flat membrane bilayers, the inner and outer nuclear membranes (INM and ONM). The INM and ONM are separated by the perinuclear space (PNS), but are connected to each other at nuclear pores [2]. The peripheral ER branches out of the ONM as an extensive network of cisternae and tubules and extends into the cytoplasm all the way to the plasma membrane (PM). The lumen of the peripheral ER is also continuous with the lumen of the PNS. What discriminates ER tubules from cisternae is their very different shapes. ER tubules have high membrane curvature at their cross-section, whereas cisternae are comprised of extended regions of parallel, flat membrane bilayers that are stacked over each other with regions of membrane curvature found only at their edges. There are, however, similarities between ER tubules and cisternae; specifically, the diameter of an ER tubule is similar to the thickness of an ER cisterna (~38 nm vs ~36 nm, respectively, in yeast) [3,4].

Multiple regions of high membrane curvature in the ER are stabilized by the reticulon and DP1/Yop1 (also called REEP) family of proteins; these regions include tubules and the edges of cisternae and fenestra (Box 1) [4,5]. The abundance of reticulon/DP1/Yop1 proteins regulates the abundance of high curvature regions within the peripheral ER [4–9]. However, it is not currently known how the expression level or activity of individual members of this family of membrane-shaping proteins is regulated to fine-tune ER shape. In mammalian cells, there are multiple spliced isoforms of four reticulon family members; each contains a reticulon homology domain, but the members have unique domains, interactions, and expression patterns that likely allow for particular ER tubule arrangements and might confer additional functions (for review, see [10]). Additionally, the prevalence of cisternae versus tubules can vary in such a way that suggests that ER shape can be adapted to a cell's ER functional requirements [11]. For example, secretory cells, such as those in the pancreas, must translocate and secrete a large number of proteins and these cells contain abundant cisternae that are densely studded with ribosomes. In contrast, the ER in muscle cells must rapidly regulate Ca^{2+} levels during contraction and these cells are enriched in ER tubules that are devoid of ribosomes. In yeast cells, which contain both ER cisternae and tubules, cisternae have a higher ribosome density than tubules (~600–1100/μm^2 vs ~250–400/μm^2, respectively) [4]. These correlations suggest that cisternae are better suited for ribosome binding and/or ribosome binding stabilizes cisternal ER structure. In animal cells, proteins associated with the translocation machinery are also enriched in cisternae [8]. Furthermore, changing ER ribosome density can alter ER shape, as treatment of animal cells with

Corresponding author: Voeltz, G.K. (gia.voeltz@colorado.edu).

  doi:10.1016/j.tcb.2011.07.004

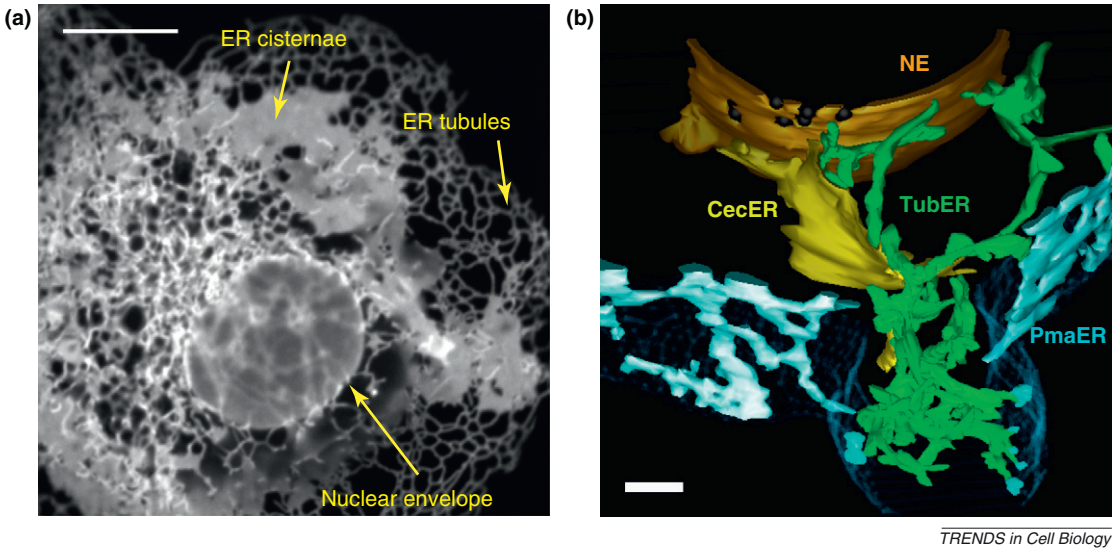

Figure 1. Endoplasmic reticulum (ER) morphological domains in mammalian cells and yeast. (a) Cos-7 cell expressing the ER membrane marker GFP-Sec61β. The ER in mammalian cells is a continuous membrane network that shares a lumen. This network is comprised of the nuclear envelope (NE) and peripheral ER cisternae and tubules. **(b)** Electron microscope tomography reveals the 3D organization of the ER in budding yeast cells at nanometer resolution. The NE, central cisternal ER (CecER), tubular ER (TubER), and PM-associated ER (PmaER) are shown. Note that the NE is continuous with the PmaER through domains that are both tubular and cisternal. Scale bars: **(a)** 10 μm; **(b)** 100 nm. Image in **(b)** adapted with permission from *J. Cell Biol.* [4].

puromycin causes ribosome displacement followed by loss of ER cisternae and gain of ER tubules [12]. These data suggest that ER cisternae may be the preferred site of protein translocation.

A recent paper showed that an additional function of ER cisternae is to aid in the unfolded protein response (UPR). This study demonstrated that in yeast, ER stress-induced UPR leads to membrane expansion of the ER into cisternae [13]. However, the cisternal shape is not what allows the stress response because conversion of sheets to tubules does not inhibit stress alleviation [13]. The authors proposed a model in which the important feature of membrane

Box 1. How endoplasmic reticulum tubules and cisternae get their shape

The peripheral endoplasmic reticulum (ER) in most cells contains a mixture of interconnected membrane tubules and cisternae. The relative amount of tubules versus cisternae depends to a large extent on the proteins that regulate ER membrane curvature, the reticulons and DP1/Yop1. These proteins are conserved integral membrane proteins that can be found in all eukaryotes. They partition exclusively into regions of the peripheral ER that have high membrane curvature, which includes the edges of cisternae as well as tubules [5,8,9,13,63]. Initial work both *in vitro* and *in vivo* have identified these proteins as the major factors necessary for organizing the ER membrane bilayer into the shape of a tubule [5,64], but they also organize membrane curvature at the edges of cisternae and fenestrations [4] (Figure Ib).

Reticulons contain a reticulon homology domain consisting of two long (30–35 amino acids) transmembrane domains, separated by a soluble linker. These transmembrane domains insert as hairpins in the cytoplasmic leaflet of the ER membrane bilayer [5] (Figure Ia). The short length of these hairpin domains is required in animal cells for: i) partitioning into regions of membrane curvature; and ii) generating membrane curvature [65,66]. Reticulon proteins also oligomerize into immobile higher-ordered structures in the ER membrane, a requirement for proper tubule formation [67]. Therefore, a reasonable model to explain how reticulons function is that they form hairpin structures that oligomerize in the outer leaflet of the ER membrane to increase outer leaflet area relative to inner leaflet area, thus generating membrane curvature by altering bilayer symmetry.

In contrast, relatively little is understood about ER cisternae. These domains are comprised of flat areas of ER membrane that are evenly spaced around the ER lumen and are connected at highly curved edges. Climp63 partitions into ER cisternae and its overexpression propagates the formation of cisternal ER at the expense of tubules [8]. When Climp63 is depleted, cisternae persist, but their intraluminal spacing is altered [8]. These data suggest that, although Climp63 is not required for cisternae formation, it may form intraluminal linker complexes that regulate cisternal dimensions.

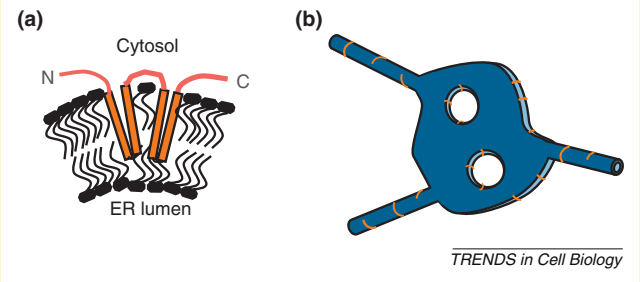

Figure I. Models of how reticulon proteins shape regions of high membrane curvature in the peripheral endoplasmic reticulum (ER). (a) Schematic of reticulon topology in the outer leaflet of the ER. Long transmembrane domains increase outer leaflet area relative to inner leaflet area, generating membrane curvature. **(b)** Schematic of ER cisternae and tubules (blue) indicating regions where reticulons (orange) have been observed to localize and shown to regulate membrane curvature, including ER tubules and the edges of cisternae and fenestra. Model in **(a)** adapted with permission from [5]. Model in **(b)** updated from [8].

expansion is increased ER volume to allow for additional handling of misfolded proteins. Indeed, recent work has shown that the volume-to-surface area ratio of such cisternae in wild-type cells exceeds that of ER tubules [4], indicating that the cisternae formed upon ER stress are the most favorable conformation to meet the need for increased ER volume.

Whether ER tubules are enriched for other ER functions remains to be determined. For a long time, it was thought that because ER cisternae are the preferred site of ER protein translocation, ER tubules must perform the lipid synthesis and Ca^{2+} handling functions of the ER. However, there is no evidence that either process localizes preferentially to ER tubules. ER tubules might be the preferred site for ER vesicle budding because the high curvature of ER tubules could be better suited for the formation of ER-to-Golgi transport vesicles (COPII) at ER exit sites (ERES). Markers for ERES are found at ER tubules [14], although it is not known if these markers are excluded from ER cisternae. It has also been difficult to demonstrate whether other specific ER functions preferentially localize to either cisternae or tubules. Tubules in the ER might simply provide a useful architecture to allow it to distribute throughout the cytoplasm as a continuous network without disrupting general diffusion and trafficking. In addition to cisternae and tubules, other peripheral ER subdomains that can be defined by their unique functions and locations include the ERES as well as regions that contact the PM, organelles, and the cytoskeleton. What makes these domains difficult to study is that they can be visualized only by electron microscopy (EM) or by using multiple fluorescent markers and confocal microscopy in well-resolved cells. We discuss ER domains that form contact sites with other organelles later in this review.

Maintenance of ER continuity through membrane fusion

Unlike every other organelle, the ER does not appear to undergo regulated fragmentation or division. Even during cell division, the ER remains continuous [15,16]. Components of the ER fusion machinery have recently been identified. The atlastin proteins (and their yeast homolog Sey1) belong to a large, ER membrane-integral GTPase family that stimulates homotypic ER fusion. Atlastin localizes preferentially to the tubular ER and interacts with reticulons [17]. Atlastin mutations or its depletion leads to unbranched ER tubules in mammalian cells [17] and ER fragmentation in *Drosophila* neurons [18], and its overexpression leads to ER membrane expansion [17,18]. Mutations in atlastin and interacting proteins have been linked to hereditary spastic paraplegia [19], and such atlastin mutants are defective in ER fusion [20], indicating that ER continuity and shape is crucial for cell function (for a review of atlastin, see [21]).

There is no evidence from live-cell imaging that the ER undergoes fission. This begs the question of why the ER must maintain its continuity. The most logical explanation is that cross-talk occurs between ER domains that are located in distal regions of the cytoplasm. For example, it might be that ER Ca^{2+} signals or stress responses cannot be handled locally, but require the global ER. Sharing a continuous lumen could help ensure that the entire ER appropriately and rapidly responds to such signals.

The ER depends on the cytoskeleton for its structure and dynamics

The ER is a dynamic organelle that continuously rearranges its structure on the cytoskeleton while maintaining continuity as a singular organelle. The ER fusion machinery and the reticulon proteins play a stabilizing role in maintaining overall ER structure during these dynamics. In metazoans, organization of the cytoplasmic ER network is highly dependent on its ability to bind and move on microtubules (MTs) (for discussion of yeast and plants, see Box 2). Treatment of animal cells with drugs that depolymerize MTs causes the ER to slowly retract from the cell periphery towards the NE [22].

ER tubules grow along MTs by two distinct mechanisms: via tip attachment complex (TAC) and ER sliding dynamics (Figure 2). During TAC movements, the tip of the ER tubule is bound to the tip of a dynamic MT, and the ER tubule grows and shrinks in concert with the dynamics of the plus-end of the MT (in a motor-independent fashion). TAC events occur through a complex between the integral ER membrane protein STIM1 and a protein that localizes to the tip of a dynamic MT, EB1 [23]. During ER sliding events, tubules are pulled out of the ER membrane by the motor proteins kinesin-1 and cytoplasmic dynein along MTs that are marked by acetylation [24,25]. ER sliding is much more common than TAC and is the predominant mechanism responsible for dynamic ER rearrangements in interphase cells [25,26]. No ER proteins have been shown to regulate ER sliding. Kinectin is one candidate, being an integral ER membrane protein that binds to kinesin-1 [27]. However, recent work suggests that kinectin localizes preferentially to ER cisternae [8] and reduced frequency of ER sliding in the absence of kinectin has not been shown.

The differences between TAC and ER sliding mechanisms suggest that they might contribute to very different ER functions. The TAC protein STIM1 also functions in store-operated Ca^{2+} entry (SOCE) at the PM, and TAC might be specific for this (see below). In contrast, the function of ER sliding dynamics is not known even though

Box 2. The role of actin in shaping yeast endoplasmic reticulum

The endoplasmic reticulum (ER) network in yeast cells relies on the actin cytoskeleton network as opposed to microtubules. The yeast ER network is dynamic, and both ER tubule growth and ring closure can be observed, although less frequently than in animal cells [68]. Some ER colocalization with actin filaments is observed, and actin depolymerization halts ER dynamics [68]. In much the same way, ER tubule movement in plants is also dependent on myosin and the actin cytoskeleton [69,70].

In yeast cells, the cytoskeleton might not play the same integral role in shaping the ER network that it does in mammalian cells. Depolymerization of actin does not dramatically affect ER structure in the mother cell, but it is required for ER inheritance into the bud [68,71,72]. In contrast, depletion of reticulons and DP1/Yop1 alone causes loss of ER domain organization in the mother cell but does not prevent ER domains from being inherited into the bud [4]. Together, these data suggest that actin is required in yeast to generate ER domain structure, whereas reticulons and DP1/Yop1 are required to maintain it.

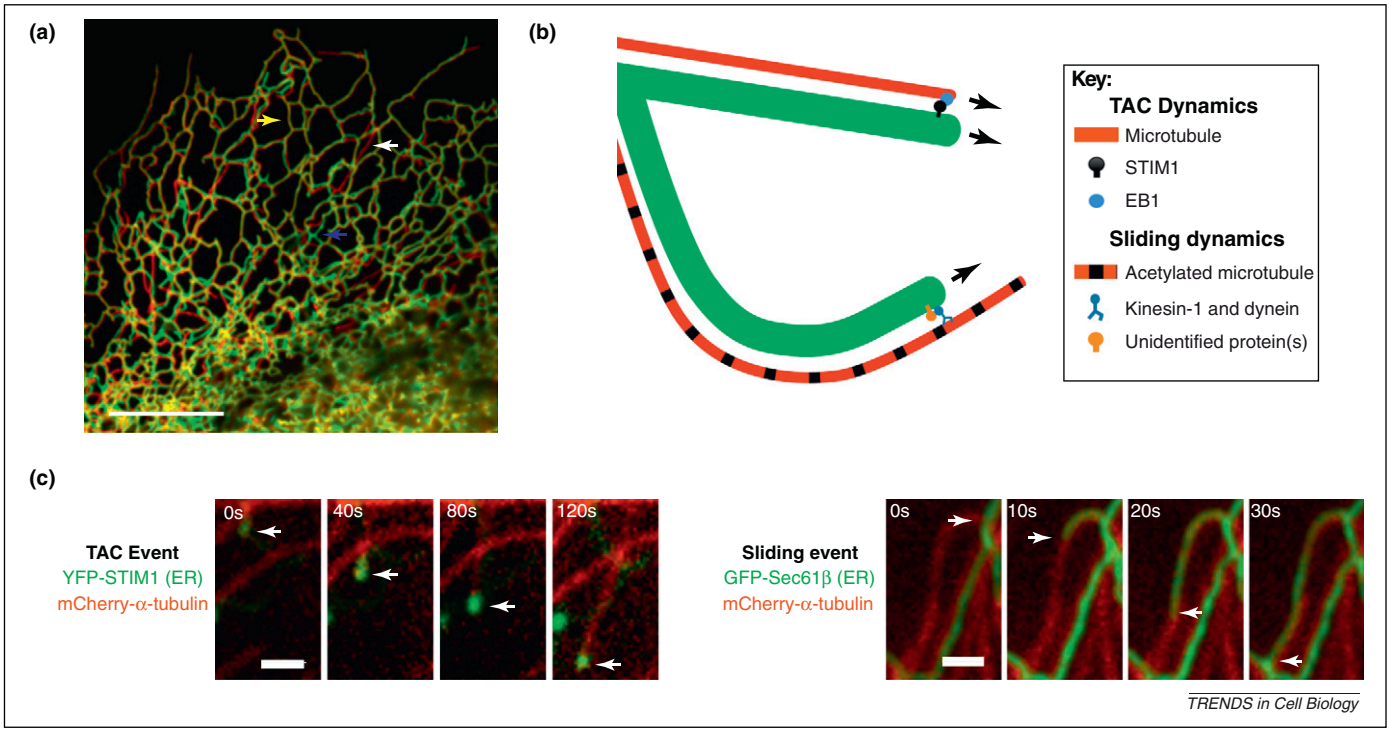

Figure 2. Two types of microtubule (MT)-dependent endoplasmic reticulum (ER) dynamics occur in mammalian cells. (a) Cos-7 cell images obtained at 30 s intervals and merged to demonstrate peripheral ER dynamics (GFP-Sec61β expression shown at t = 0 s in green and t = 30 s in red). Arrows indicate unchanged ER (yellow), new ER tubule growth (white), and ER rearrangement (blue). **(b)** Model illustrating tip attachment complex (TAC) and sliding dynamics. TAC occurs on dynamic MTs; the ER extends along with the plus-end of a growing MT via an interaction between the ER protein STIM1 and the MT plus-end protein EB1. Sliding occurs on acetylated, curved, nocodazole-resistant MTs in a MT motor-dependent manner. The proteins that attach the ER to these motors are unknown. **(c)** Examples of a TAC event (left) and an ER sliding event (right). Cos-7 cells are expressing either GFP-Sec61β or YFP-STIM1 to visualize ER dynamics (green) and mCherry-α-tubulin to visualize microtubules (red) at the times indicated. Arrows indicate the position of the ER tubule. Scale bars: **(a)** 10 μm; **(c)** 1 μm. Images in **(a)** and **(c)** adapted with permission from *J. Cell Biol.* [25].

the cell likely consumes a great deal of energy to constantly reorganize the structure of the ER on MTs using motor proteins. One possibility is that the ER could use different dynamic mechanisms to establish or maintain contact with other membrane-bound organelles and the PM [25]. Evidence for this idea comes from work showing that ER sliding and mitochondria colocalize over acetylated MTs (see discussion below), whereas ER and early endosomes, which are also tightly coupled, do not [25].

The importance of maintaining ER–organelle contacts

The ER contacts almost every membrane-bound organelle in the cell, including mitochondria, endosomes, Golgi, and peroxisomes, as well as the plasma membrane [28,29]. Here, we discuss what is known about the extent and function of these interactions in order to understand why ER dynamics are so highly coupled to other organelles.

ER–mitochondrial contact

The ER and the mitochondria interact to form contacts that have been studied both biochemically and functionally. Two functions that occur at contact sites between the ER and mitochondria are lipid synthesis and Ca^{2+} signaling, the latter of which is crucial for apoptotic regulation (for review, see [30]). The best evidence for the role of ER-mitochondrial contact in mediating proper Ca^{2+} handling comes from experiments in live cells that use artificial tethers to alter the distance at contact sites between the ER and mitochondria; these experiments find that Ca^{2+} signaling is altered when the distance between ER and mitochondria is disrupted [31,32].

Improvements in live-cell fluorescence microscopy have enabled the study of ER–mitochondria interactions over time. Whereas previous work has focused on the dynamics of either the ER (discussed above) or mitochondria [33], recent visualization of both organelles during movies shows that they maintain contact despite the movement of each organelle [25] (Figure 3). This raises the question of how organelles rearrange their structure around each other as they move. Interestingly, these contacts colocalize on the subset of MTs that are marked by acetylation [25]. These data suggest an elaborate interplay between proteins that regulate ER–mitochondrial contact and proteins that regulate the contact of these organelles with MTs during their movement.

Recent discoveries in yeast and mammalian cells have uncovered some of the proteins involved in linking these two organelles. In yeast, ERMES is a complex of four proteins (MMM1, MDM10, MDM12, and MDM34) that tethers the ER to mitochondria [34]. Mutations in the ERMES complex lead to growth defects and defects in lipid synthesis that can be rescued by the addition of a synthetic ER-mitochondrial linker [34]. Mammalian homologs of the ERMES complex have not yet been identified. In mammalian cells, Mitofusin 2 (Mfn2) has been shown to regulate ER-mitochondrial contact and Ca^{2+} handling [35]. The name of this protein is derived from its role in homotypic fusion of mitochondria [36]. Mfn2 is proposed to tether the ER to mitochondria by also localizing to the ER where it can interact with either Mfn1 or Mfn2 on the mitochondrial outer membrane [35].

There are likely to be additional tethers that link the mitochondria to the ER because these contact domains

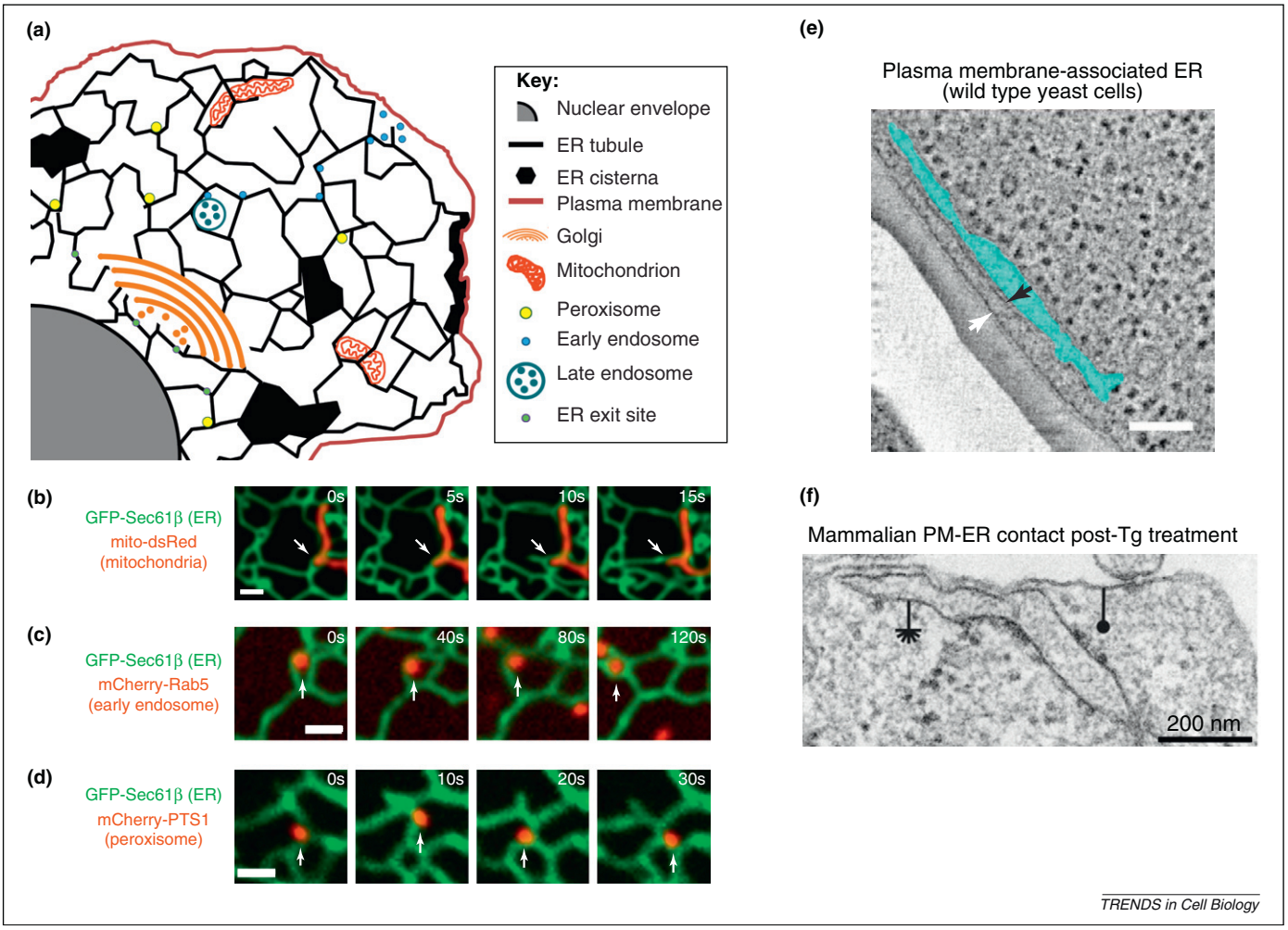

Figure 3. The endoplasmic reticulum (ER) has many different subdomains that can interact with other membrane-bound compartments. (a) Model depicting known ER domains and organelle contact sites in mammalian cells. Many of these contacts are maintained despite the dynamics of both membrane-bound compartments. **(b–d)** Coupling of ER dynamics with other organelles. Cos-7 cells expressing GFP-Sec61β (ER) and **(b)** mito-dsRed (mitochondria), **(c)** mCherry-Rab5 (early endosomes), and **(d)** mCherry-PTS1 (peroxisomes) at the times indicated. Arrows indicate the position of the ER–organelle contact. **(e)** Electron microscope (EM) tomography of wild-type yeast cells reveals close apposition between the plasma membrane-associated ER (PmaER) and plasma membrane (PM) (black and white arrows mark ER and PM membrane bilayer, respectively). The distance between the two opposing membranes is maintained and ribosomes are excluded from the intervening region. **(f)** EM tomograph of HeLa cells treated with thapsigargin (Tg, 1 μM) to deplete ER Ca^{2+} stores. Note induction of extensive ER–PM ribosome-excluded contact sites. Asterisk marks ER and closed circle marks PM. Scale bars: **(b, c, d)**: 1 μm; **(e)** 50 nm. Images in (b), (c), and (e) adapted with permission from *J. Cell Biol.* [4,25]. Image in **(f)** adapted with permission from [52].

are responsible for multiple functions. For example, there are reported interactions between ER and mitochondrial proteins that, although not believed to tether the organelles, are implicated in processes such as apoptosis and Ca^{2+} signaling [37,38]. To provide intricate regulation of each of these processes, different tethers may exist; presumably, we have only begun to uncover the complexes involved in these important linkages.

ER–Golgi contact

Traditionally, the relationship between ER and Golgi is discussed in terms of their roles in anterograde (COPII vesicle) and retrograde (COPI vesicle) transport as part of the secretory pathway [39]. However, the ER also makes close contacts with the Golgi as part of other processes. High-resolution EM shows very close contacts between the ER membrane and the trans-Golgi, which have been proposed to be involved in direct lipid transport (non-vesicular trafficking) [29,40]. Ceramide, which is synthesized at the ER, is transferred to the Golgi membrane for its conversion to sphingomyelin, a process that is performed by the ceramide transferase CERT [41]. Additionally, phosphatidylinositol is transferred directly from the ER to the Golgi by Nir2 [42]. It is hypothesized that, in both cases, shuttling of these lipids from the ER to the Golgi occurs at ER–Golgi contact sites where these membranes are as little as 10 nm apart. However, the factors linking these two membranes together have not been identified.

ER–endosome contact

Recent work has established a relationship between the ER and the endocytic pathway. In animal cells, a direct interaction between the ER-localized phosphatase PTP1B and the endocytic cargo EGFR at ER–endosome contact sites has been observed by immuno-EM [43], suggesting that ER proteins might modify endocytosed cargoes. Interestingly, the movements of early endosomes are coordinated with ER dynamics and these two organelles can be tightly linked over time, suggesting that proteins linking the endosomes to the ER membrane are somehow coordinating this contact with

the machinery that moves the organelles along the cytoskeleton [25] (Figure 3). Direct contacts have also been observed between the ER and late endosomes and yeast vacuoles [4,44]. A recent paper showed that, in animal cells, ER contacts regulate the intracellular distribution of late endosomes [44]. The authors propose a complex regulatory pathway for late endosome positioning in which the ER protein VAP interacts with a late endosome-bound cholesterol sensor ORP1L in conditions of low cholesterol to tether the two organelles together [44]. However, there are likely to be other factors mediating ER–endosome contact and the functions of these contacts remain unclear.

ER–peroxisome and ER–lipid droplet contacts share common features

One of the most unique ER–organelle interactions is that between the ER and peroxisomes. Peroxisomes have several functions, including the breakdown of long-chain fatty acids. What makes the ER-peroxisome interface particularly interesting is that in both yeast and mammalian cells, peroxisomes are derived at least in part from the ER membrane [45,46]. Not only does peroxisomal biogenesis occur via the ER, but peroxisomes can also maintain close contact with the ER membrane over time [46] (Figure 3), and non-vesicular transport is common between these organelles in yeast [47]. As yet, however, no factors have been identified that regulate this lipid transfer event. Lipid droplets, like peroxisomes, are also derived in part from factors synthesized on the ER membrane and mature lipid droplets also can be tethered to the ER membrane [48].

ER–PM contact

In addition to binding almost every other membrane-bound organelle in the cell, the ER makes extensive contact with the PM. Recent work elucidating the 3D structure of the ER in yeast by EM and tomography demonstrates that a mixture of interconnected ER tubules and fenestrated cisternae covers 20–45% of the cytoplasmic surface of the PM [4,13] (Figures 1, 3, and 4). Contact between this PM-associated ER and the PM is maintained at a mean spacing of 33 nm, which is close enough to exclude ribosomes from the face of the ER membrane that contacts the PM [4] (Figure 3). ER–PM contacts are important for the regulation of phosphatidyl inositol metabolism and might be sites of direct, non-vesicular sterol transfer [49,50].

In mammalian cells, the ER–PM domain is also important in Ca^{2+} regulation. A complex between the ER protein STIM1 (which is also involved in TAC ER dynamics, see above) and the PM protein Orai1 is the mechanism behind SOCE for the ER. When ER Ca^{2+} stores are depleted, STIM1 forms higher ordered structures that then target to the PM [51]. This Ca^{2+}-depleted ER is induced by STIM1

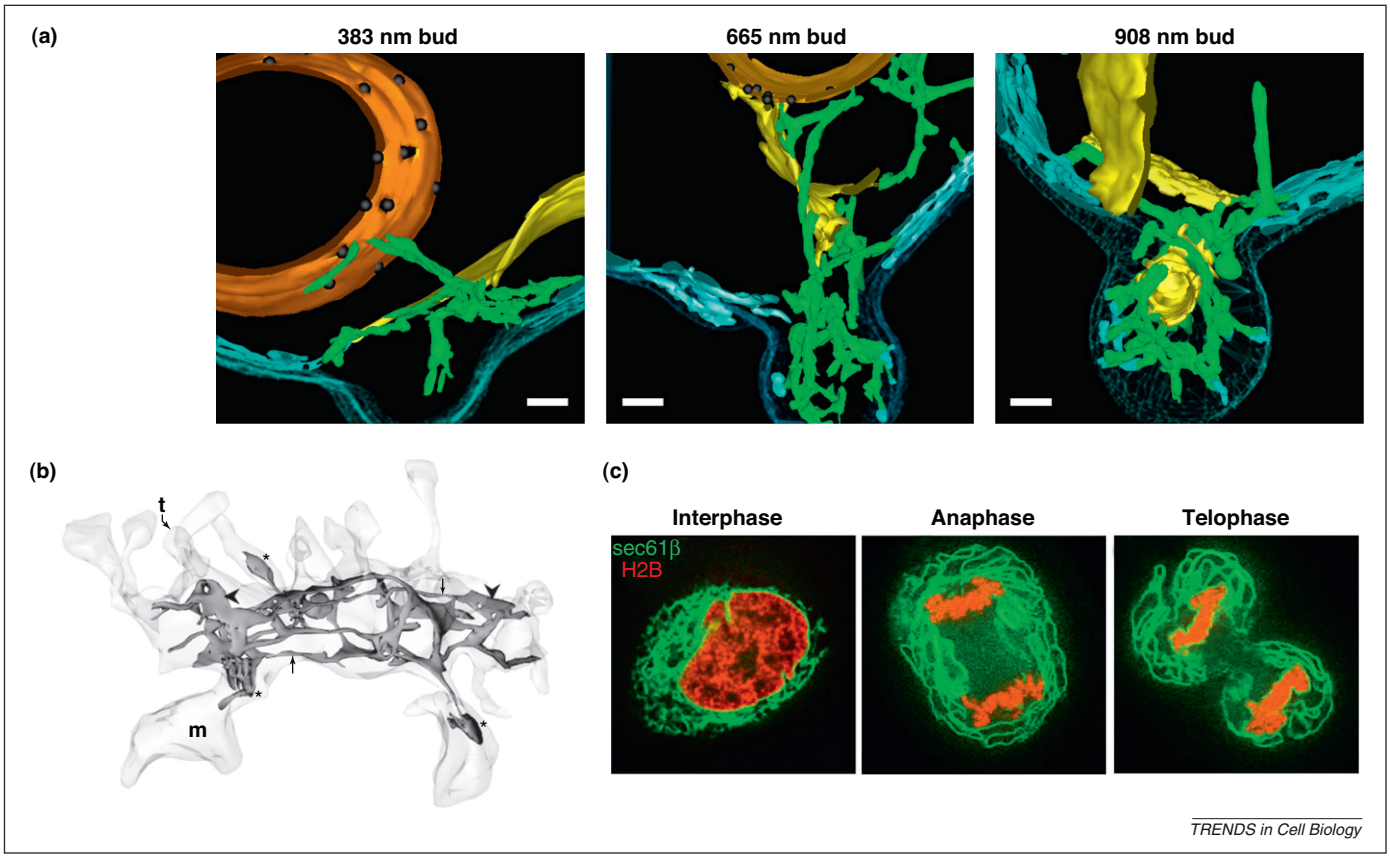

Figure 4. The endoplasmic reticulum (ER) is redistributed as cell shape changes and its morphology changes completely during mammalian mitosis. (a) 3D electron microscope (EM) tomography of wild-type yeast cells reveals how ER domains are inherited into the daughter bud. Models are ordered from left to right with increasing bud size. ER tubules are inherited first into the bud (left and middle panel, tubular ER in green). The inherited tubular ER establishes contacts with the plasma membrane (PM) to form new PM-associated ER (PmaER) domains (middle and right panels, PmaER in blue). CecER in yellow. **(b)** A 3D EM reconstruction of the ER network within the dendrite of a hippocampal neuron. Note the enrichment of tubular ER. **(c)** Fluorescent images of HeLa cells expressing H2B-mRFP (chromatin) and GFP-Sec61β (ER) demonstrate that the 3D ER structure changes dramatically between interphase and the indicated stages of mitosis. The nuclear envelope (NE) breaks down, ER tubules are converted to cisternae, and the ER moves to the periphery of the cell away from the mitotic spindle. Scale bars: **(a)** 200 nm. Images in **(a)** adapted with permission from *J. Cell Biol.* [4]. Images in **(b)** adapted with permission from [73]. Images in **(c)** adapted with permission from [62].

to form ribosome-excluded cisternal contacts with the PM [52]. At the PM, STIM1 interacts with Orai1 to form a CRAC channel that allows Ca^{2+} influx into the ER [53]. Despite the involvement of STIM1 in both TAC dynamics and SOCE, a direct link between these two processes has not been uncovered. It is intriguing to think that movement of STIM1 via TAC allows its translocation to the PM and complex formation with Orai1; however, inhibition of TAC did not appear to affect SOCE under non-physiological conditions [23]. Thus, although a link between TAC and SOCE may exist, it has not been formally proven.

The proteins that tether the ER to the PM are not known but would be expected to be quite abundant, at least in yeast, to attach so much ER to the PM. The contact between the ER and PM is regulated by the reticulon/Yop1 proteins; in the absence of these proteins in yeast, the peripheral ER adopts a flat cisternal shape that dramatically increases ER association with the PM [4]. However, it is likely that this effect is indirectly caused by a loss of ER membrane curvature.

So far, we have discussed how the ER develops and maintains its extremely complicated morphology, which includes distinct domains and multiple shapes. This morphology is highly dependent on ER dynamics along the cytoskeleton. Additionally, the ER contacts almost every other organelle in the cell, performs a variety of functions at these contacts, and even appears to share coordinated movements with these organelles. Therefore, a likely possibility is that the ER must be dynamic to establish and maintain these functional contacts as organelles move around the cell.

A potential role for ER dynamics in cell shape change

The ER might also be dynamic for the same reasons that MTs grow and shrink; such dynamic instability of MTs allows cells to rapidly explore 3D space during cell migration, differentiation, and polarization [54]. In much the same way, ER dynamics might allow the ER to adapt to changes in cell morphology during such processes. ER distribution is likely to be important for cells with complex morphologies; for example, in neurons, the ER must be distributed down the length of narrow axons and dendrites, a process that appears to be coordinated with their development [55] (Figure 4). ER positioning and dynamics might also be important for cell migration [56]. The ER membrane's ability to undergo constant tubule growth and reshaping could allow it continuously to probe the cytoplasm during periods of regulated PM extension so that the ER is present in the new cell boundary. The kinesin-1 binding protein kinectin is implicated in extending the ER into growing cellular lamellae [57]; however, it remains to be determined whether kinectin depletion alters the rate of ER dynamics during migration.

The 3D structure of the ER also changes dramatically in animal cells as they progress through mitosis. In prophase, the NE breaks down and NE protein components are dispersed into the surrounding ER membrane [58,59]. The ER maintains its continuity throughout this process, in contrast to other large organelles, such as the Golgi and mitochondria, which fragment [15,16,60,61]. There has been debate over the shape and arrangement of the ER in mitosis; however, recent work shows quite convincingly that the ER network becomes almost entirely cisternal through mitosis, with very few remaining tubules [62] (Figure 4). The intracellular localization of the ER also changes; it moves away from the mitotic spindle and chromosomes, towards the PM. It is not surprising that the ER should be moved to the cell periphery to avoid interfering with chromosome segregation, but how this change in mitotic ER shape and location is regulated is unknown. These shape changes could be due to cytoskeleton-directed movements and/or the inactivation of membrane shaping proteins.

Concluding remarks

Recent improvements in our ability to visualize the 3D structure of the ER in live cells, and at a higher resolution with EM, have revealed ER domains and functions that we did not know existed even a few years ago. Although we have come a long way in our understanding of factors that influence ER shape and function, it is clear that ER responsibilities extend well beyond traditionally-studied functions. The ER is extraordinarily dynamic, but we still have almost no information about the specific functions of these movements. Additionally, we now know that the ER forms stable contacts with the PM and nearly every other membrane-bound organelle in the cytoplasm. The tethering proteins that regulate contacts between the ER and PM, Golgi, vacuoles, endosomes, peroxisomes, and lipid droplets are unknown and only a few linkers have been identified for mitochondria. As a result, we have only begun to unravel the functions of ER–organelle contacts because they are currently difficult to perturb. Future work addressing these questions will greatly improve our understanding of ER functions in the cell.

Acknowledgements

This work was supported by NIH R01 GM083977 to G.K.V. and an NIH predoctoral training grant GM08759 to J.R.F. We thank M. West for providing electron microscope images and A. Rowland for helpful comments.

References

1 Baumann, O. and Walz, B. (2001) Endoplasmic reticulum of animal cells and its organization into structural and functional domains. *Int. Rev. Cytol.* 205, 149–214
2 Hetzer, M.W. *et al.* (2005) Pushing the envelope: structure, function, and dynamics of the nuclear periphery. *Annu. Rev. Cell Dev. Biol.* 21, 347–380
3 Bernales, S. *et al.* (2006) Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol.* 4, e423
4 West, M. *et al.* (2011) A 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. *J. Cell Biol.* 193, 333–346
5 Voeltz, G.K. *et al.* (2006) A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* 124, 573–586
6 Anderson, D.J. and Hetzer, M.W. (2008) Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation. *J. Cell Biol.* 182, 911–924
7 De Craene, J-O. *et al.* (2006) Rtn1p is involved in structuring the cortical endoplasmic reticulum. *Mol. Biol. Cell* 17, 3009–3020
8 Shibata, Y. *et al.* (2010) Mechanisms determining the morphology of the peripheral ER. *Cell* 143, 774–788
9 Sparkes, I. *et al.* (2010) Five Arabidopsis reticulon isoforms share endoplasmic reticulum location, topology, and membrane-shaping properties. *Plant Cell* 22, 1333–1343

10 Yang, Y.S. and Strittmatter, S.M. (2007) The reticulons: a family of proteins with diverse functions. *Genome Biol.* 8, 234
11 Shibata, Y. *et al.* (2006) Rough sheets and smooth tubules. *Cell* 126, 435–439
12 Puhka, M. *et al.* (2007) Endoplasmic reticulum remains continuous and undergoes sheet-to-tubule transformation during cell division in mammalian cells. *J. Cell Biol.* 179, 895–909
13 Schuck, S. *et al.* (2009) Membrane expansion alleviates endoplasmic reticulum stress independently of the unfolded protein response. *J. Cell Biol.* 187, 525–536
14 Hammond, A.T. and Glick, B.S. (2000) Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. *Mol. Biol. Cell* 11, 3013–3030
15 Ellenberg, J. and Lippincott-Schwartz, J. (1999) Dynamics and mobility of nuclear envelope proteins in interphase and mitotic cells revealed by green fluorescent protein chimeras. *Methods* 19, 362–372
16 Ellenberg, J. *et al.* (1997) Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. *J. Cell Biol.* 138, 1193–1206
17 Hu, J. *et al.* (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* 138, 549–561
18 Orso, G. *et al.* (2009) Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. *Nature* 460, 978–983
19 Park, S.H. *et al.* (2010) Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *J. Clin. Invest.* 120, 1097–1110
20 Bian, X. *et al.* (2011) Structures of the atlastin GTPase provide insight into homotypic fusion of endoplasmic reticulum membranes. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3976–3981
21 Moss, T.J. *et al.* (2011) Fusing a lasting relationship between ER tubules. *Trends Cell Biol.* 21, 416–423
22 Terasaki, M. *et al.* (1986) Microtubules and the endoplasmic reticulum are highly interdependent structures. *J. Cell Biol.* 103, 1557–1568
23 Grigoriev, I. *et al.* (2008) STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. *Curr. Biol.* 18, 177–182
24 Wozniak, M.J. *et al.* (2009) Role of kinesin-1 and cytoplasmic dynein in endoplasmic reticulum movement in VERO cells. *J. Cell Sci.* 122, 1979–1989
25 Friedman, J.R. *et al.* (2010) ER sliding dynamics and ER-mitochondrial contacts occur on acetylated microtubules. *J. Cell Biol.* 190, 363–375
26 Waterman-Storer, C.M. and Salmon, E.D. (1998) Endoplasmic reticulum membrane tubules are distributed by microtubules in living cells using three distinct mechanisms. *Curr. Biol.* 8, 798–806
27 Toyoshima, I. *et al.* (1992) Kinectin, a major kinesin-binding protein on ER. *J. Cell Biol.* 118, 1121–1131
28 English, A.R. *et al.* (2009) Peripheral ER structure and function. *Curr. Opin. Cell Biol.* 21, 596–602
29 Levine, T. and Loewen, C. (2006) Inter-organelle membrane contact sites: through a glass, darkly. *Curr. Opin. Cell Biol.* 18, 371–378
30 de Brito, O.M. and Scorrano, L. (2010) An intimate liaison: spatial organization of the endoplasmic reticulum-mitochondria relationship. *EMBO J.* 29, 2715–2723
31 Csordás, G. *et al.* (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* 174, 915–921
32 Csordás, G. *et al.* (2010) Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. *Mol. Cell* 39, 121–132
33 Boldogh, I.R. and Pon, L.A. (2007) Mitochondria on the move. *Trends Cell Biol.* 17, 502–510
34 Kornmann, B. *et al.* (2009) An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 325, 477–481
35 de Brito, O.M. and Scorrano, L. (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605–610
36 Hoppins, S. *et al.* (2007) The machines that divide and fuse mitochondria. *Annu. Rev. Biochem.* 76, 751–780
37 Giorgi, C. *et al.* (2009) Structural and functional link between the mitochondrial network and the endoplasmic reticulum. *Int. J. Biochem. Cell Biol.* 41, 1817–1827
38 Iwasawa, R. *et al.* (2011) Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction. *EMBO J.* 30, 556–568
39 Spang, A. (2009) On vesicle formation and tethering in the ER–Golgi shuttle. *Curr. Opin. Cell Biol.* 21, 531–536
40 Lev, S. (2010) Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat. Rev. Mol. Cell Biol.* 11, 739–750
41 Hanada, K. *et al.* (2009) CERT-mediated trafficking of ceramide. *Biochim. Biophys. Acta* 1791, 684–691
42 Peretti, D. *et al.* (2008) Coordinated lipid transfer between the endoplasmic reticulum and the Golgi complex requires the VAP proteins and is essential for Golgi-mediated transport. *Mol. Biol. Cell* 19, 3871–3884
43 Eden, E.R. *et al.* (2010) Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat. Cell Biol.* 12, 267–272
44 Rocha, N. *et al.* (2009) Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. *J. Cell Biol.* 185, 1209–1225
45 Hoepfner, D. *et al.* (2005) Contribution of the endoplasmic reticulum to peroxisome formation. *Cell* 122, 85–95
46 Kim, P.K. *et al.* (2006) The origin and maintenance of mammalian peroxisomes involves a de novo PEX16-dependent pathway from the ER. *J. Cell Biol.* 173, 521–532
47 Raychaudhuri, S. and Prinz, W.A. (2008) Nonvesicular phospholipid transfer between peroxisomes and the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15785–15790
48 Jacquier, N. *et al.* (2011) Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. *J. Cell Sci.* 124, 2424–2437
49 Schulz, T.A. and Prinz, W.A. (2007) Sterol transport in yeast and the oxysterol binding protein homologue (OSH) family. *Biochim. Biophys. Acta* 1771, 769–780
50 Stefan, C.J. *et al.* (2011) Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. *Cell* 144, 389–401
51 Liou, J. *et al.* (2007) Live-cell imaging reveals sequential oligomerization and local plasma membrane targeting of stromal interaction molecule 1 after Ca^{2+} store depletion. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9301–9306
52 Orci, L. *et al.* (2009) STIM1-induced precortical and cortical subdomains of the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19358–19362
53 Park, C.Y. *et al.* (2009) STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1. *Cell* 136, 876–890
54 Desai, A. and Mitchison, T.J. (1997) Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 13, 83–117
55 Ramirez, O.A. and Couve, A. (2011) The endoplasmic reticulum and protein trafficking in dendrites and axons. *Trends Cell Biol.* 21, 219–227
56 Bola, B. and Allan, V. (2009) How and why does the endoplasmic reticulum move? *Biochem. Soc. Trans.* 37, 961–965
57 Zhang, X. *et al.* (2010) Kinectin-mediated endoplasmic reticulum dynamics supports focal adhesion growth in the cellular lamella. *J. Cell Sci.* 123, 3901–3912
58 Yang, L. *et al.* (1997) Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. *J. Cell Biol.* 137, 1199–1210
59 Hetzer, M.W. (2010) The nuclear envelope. *Cold Spring Harb. Perspect. Biol.* 2, a000539
60 Persico, A. *et al.* (2009) Mitotic inheritance of the Golgi complex. *FEBS Lett.* 583, 3857–3862
61 Taguchi, N. *et al.* (2007) Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J. Biol. Chem.* 282, 11521–11529
62 Lu, L. *et al.* (2009) Cisternal organization of the endoplasmic reticulum during mitosis. *Mol. Biol. Cell* 20, 3471–3480
63 Kiseleva, E. *et al.* (2007) Reticulon 4a/NogoA locates to regions of high membrane curvature and may have a role in nuclear envelope growth. *J. Struct. Biol.* 160, 224–235
64 Hu, J. *et al.* (2008) Membrane proteins of the endoplasmic reticulum induce high-curvature tubules. *Science* 319, 1247–1250
65 Tolley, N. *et al.* (2010) Transmembrane domain length is responsible for the ability of a plant reticulon to shape endoplasmic reticulum tubules in vivo. *Plant J.* 64, 411–418

66 Zurek, N. *et al.* (2011) Reticulon short hairpin transmembrane domains are used to shape ER tubules. *Traffic* 12, 28–41
67 Shibata, Y. *et al.* (2008) The reticulon and DP1/Yop1p proteins form immobile oligomers in the tubular endoplasmic reticulum. *J. Biol. Chem.* 283, 18892–18904
68 Prinz, W.A. *et al.* (2000) Mutants affecting the structure of the cortical endoplasmic reticulum in Saccharomyces cerevisiae. *J. Cell Biol.* 150, 461–474
69 Sparkes, I. *et al.* (2010) Movement and remodeling of the endoplasmic reticulum in nondividing cells of tobacco leaves. *Plant Cell* 21, 3937–3949
70 Ueda, H. *et al.* (2010) Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6894–6899
71 Du, Y. *et al.* (2004) Dynamics and inheritance of the endoplasmic reticulum. *J. Cell Sci.* 117, 2871–2878
72 Estrada, P. *et al.* (2003) Myo4p and She3p are required for cortical ER inheritance in Saccharomyces cerevisiae. *J. Cell Biol.* 163, 1255–1266
73 Cooney, J.R. *et al.* (2002) Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J. Neurosci.* 22, 2215–2224