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ER – the key to the highway

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Abstract

The endoplasmic reticulum (ER) is the key organelle at the start of the secretory pathway and the list of its functions is continually growing. The ER organization as a tubular/cisternal network at the cortex of plant cells has recently been shown to be governed by the membrane tubulation proteins of the reticulon family working alongside plant atlastin homologues, members of the RHD3 group of proteins. Such a network has intimate connections with other organelles such as peroxisomes via peroxules, chloroplasts, Golgi bodies and at the cell cortex to the plasma membrane with cytoskeleton at so called “anchor/contact sites”. The ER network is by no means static displaying a range of different movements and acting as a sub-cellular highway supports the motility of organelles such as peroxisomes, mitochondria and Golgi bodies plus the transport of macromolecules such as viral movement proteins, nucleocapsid proteins and RNA. Here we highlight recent and exciting discoveries on the maintenance of the ER structure and its role on movement and biology of other organelles.

Keywords

Arabidopsis; endoplasmic reticulum; ER membrane; ER anchor/contact sites

Introduction

The ER is the gateway to the entire secretory pathway for the synthesis, modification, and quality control of most of the cell’s building blocks that traffic through the Golgi apparatus to reach their final destination [1,2]. It is also a calcium storage organelle and source of membranes necessary for the successful accomplishment of vital cellular processes, including direct biogenesis of other cell structures, such as peroxisomes, oil bodies, and autophagic bodies [3–5]. The ER is also a sensor for several cellular stresses and serves as a

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membrane scaffold for the binding of various signaling proteins, including essential membrane-tethered transcription factors, which evoke fast transcriptional responses upon stress and growth clues [6]. The plant ER assumes additional roles that are unique. It is a reservoir of receptors of plant-specific hormones [7,8] and it can serve as source of membrane to generate the lytic vacuole through a Golgi-independent pathway in meristem cells [9]. It also functions as a protein storage compartment in many tissues during growth and defense [10,11], and it entertains essential functional interactions for the synthesis of lipids with chloroplasts [12], which are found only in photosynthetic organisms. Finally, it is becoming ever more apparent that this dynamic network of tubules and cisternae acts as a major highway for the transport of organelles and macromolecules around the cells and to plasmodesmata [13,14].

The ER has a unique reticulated network structure characterized by the presence of interconnected tubules and cisternae, which develop from a conspicuous cisternal appearance in very young cells [15]. Although the ER is continuous with the nuclear envelope in all species, in mammalian cells, it extends throughout the cell; whereas in yeast and plant cells, the bulk of the ER is distributed primarily toward the plasma membrane and is commonly referred as “cortical ER” [16,17] (Fig. 1). In plant cells, the ER is most likely anchored through static points to the plasma membrane [18], and it extends in the body of the cell through the so-called “transvacuolar strands” [16,19,20]. A unique feature to plants is the intricate arrangement of ER in the phragmoplast, which gives rise to the cell plate during cell division and, at the same time, allows the formation of plasmodesmata [21]. These structures are unique to plants and allow for interconnections of the ER membrane between adjacent cells [22], in clear contrast to the individual ER of each cell in animals. Therefore, the plant ER represents a peculiar example of an organelle with intercellular extension throughout the plant. The ER distribution and functions in plant cells underscores that eukaryotes must have evolved specific mechanisms to suit the ER structure and function to the cell's specific features acquired during evolution. In this review we will discuss some of the most recent findings on plant ER structure and relationships with other organelles with which it entertains physical and functional contacts, and even transports them around the cytosol.

The proteins involved in shaping ER membranes have conserved and divergent features

ER tubules have a high-degree of membrane curvature. In mammalian and yeast cells, proteins called reticulons and DP1/REEP/Yop1 are required and sufficient to form an ER tubular network, most likely by stabilizing the high curvature of the tubules [23–25]. In *Arabidopsis*, there are 7 Yop1 homologs belonging to the HVA22 family and 21 reticulons, named RTNLB [26–28]. Overexpression of a number of RTNLBs in tobacco leaf epidermal cells is sufficient to constrict the ER lumen and convert ER sheets into tubules [29,30], suggesting a conserved role for these proteins. A role for HVA22 proteins in plant ER integrity is yet to be demonstrated. However the degree of identity with non-plant counterparts (i.e., 20–31% identity to DP1/Yop1p, which is similar to the identity between DP1 and Yop1p) [31] suggests functional conservation. Tubule origination from other

tubules, actin-myosin driven extension and fusion are at the basis of the dynamic rearrangements of the ER network. Atlastins (ATLs), a class of GTPases of the dynamin family in metazoans, have been proposed to mediate the process of homotypic fusion of ER tubules [32,33] helping establish and maintain the cortical network. Yeast and plants do not have sequence homologs of ATLs, but they have functional homologs proteins called Sey1p in *S. cerevisiae* and RHD3 in *A. thaliana* [34,35], with similar signature motifs of ATLs, including a large GTPase domain, a helical bundle domain, two closely spaced trans-membrane segments, and a short C-terminal tail. Similar to ATLs and Sey1p [36], RHD3 is a GTPase and mediates membrane fusion *in vitro* [37,38], supporting that the ER-associated dynamin-like GTPases share fusogenic activity. An Arabidopsis seed-specific version of RHD3 (RL2) has also been shown to co-immunoprecipitate with the seed specific reticulon RTNLB13. When expressed together they dramatically altered the ER network even in the absence of an active RHD3 GTPase domain [39] suggesting cooperation between the two proteins in modulating the ER network.

Loss of RHD3 or overexpression of RHD3 defective in the GTPase domain causes formation of aberrant ER morphology. The type of the ER defect is yet to be established but the structure resembles unbranched or largely bundled ER. Intriguingly, ER structure defects linked to simultaneous ablation of Sey1p and tubule-shaping proteins, Rtn1p or Yop1p, can be recovered by either Sey1p, human ATL, or RHD3 [32,37], but Sey1p, which can localize to the plant ER in heterologous expression experiments, seems to be unable to rescue the Arabidopsis growth phenotype associated with loss of RHD3 [40]. These results suggest, that differently from Sey1p in plant cells, RHD3 has functional/structural attributes that are sufficient to complement loss of Sey1p in yeast. A recent report shows that overexpression of the C-terminal region of RHD3 encompassing the two transmembrane domains and the C-terminal extension can disrupt ER integrity but that overexpression of the correspondent region Sey1p does not alter ER structure [41]. The observed functional differences between Sey1p and RHD3 may lay in the C-terminal extensions of Sey1p and RHD3, which have relatively little degree of conservation. Together these findings hint that the mechanisms underlying ER structure are not identical across the eukaryotic lineages. The consequences of loss of the ER structure integrity have been long ignored until recently. Loss of RHD3 does not visibly affect ER export of membrane and soluble bulk secretory reporters [38,40]. However, by analyzing the ability of the ER to invoke a cytoprotective signaling mechanism called unfolded protein response (UPR), Lai et al. [42] have demonstrated that, contrarily to other mutations that affect the ER structure, loss of RHD3 causes activation defects in one of the major UPR arms controlled by the conserved protein kinase and ribonuclease IRE1. These findings highlight that the loss of RHD3 causes specific consequences in ER functional homeostasis besides loss of organelle network integrity. Immediate open questions in the biology of RHD3 are related to the mechanisms that link loss of RHD3 to UPR management and the nature of the mechanisms that control RHD3 role in ER fusion *in vivo* at spatial and temporal levels.

ER contacts other organelles sites

In yeast and mammalian cells there are numerous reports on physiologically important connections between the ER and other organelles besides the Golgi apparatus, in particular

mitochondria [43] and the plasma membrane [44] (Figure 1). Yet in comparison the literature on plant ER anchor/contact sites is surprisingly sparse.

Earlier work showed the existence of ER contact sites with other organelles [45]. More recently, live cell imaging experiments coupled with optical laser tweezers have shown that the ER is attached to Golgi stacks as well as chloroplasts, whereby pulling Golgi stacks or chloroplasts away from the ER results in ER extensions following the other organelles [46–48]. The existence of connections between the ER and the Golgi or chloroplasts apparatus are consistent with a functional relationship of the ER with these organelles. A tight association between chloroplasts and the ER may facilitate ER-localized synthesis of membrane and storage lipids that is dependent on plastid-synthesized precursors [12,47], but also for other metabolites. Indeed, complementation experiments have demonstrated that mutants with compromised plastidic metabolic pathways (i.e. tocopherol and carotenoid pathways) may be complemented by retargeting key plastid enzymes to the ER lumen [49]. These findings support the existence of functional membrane contacts between the ER and chloroplasts that enable access of the ER-retargeted plastid enzymes to nonpolar metabolites in the plastid envelope. While the nature of these sites is yet to be established [50], it is plausible that specialized protein networks may be in place to stabilize the ER-chloroplast interface [51]. It will be interesting to test in the future whether ER-chloroplast contacts occur on specialized domains of the chloroplasts, including the unique plastid membrane extrusions, known as stromules, which have been shown to be in close and extensive proximity with the ER [52–54].

Similar to chloroplast stromules, extensions from peroxisomes (peroxules) formed under conditions of oxidative stress were found to extend and retract along ER tubules [55]. In a similar fashion tubular peroxisomes in the *Arabidopsis apm1/drp3a* mutant have been shown to take on the form similar to a portion of an ER network. Two-color fluorescent protein expression in these peroxisomes and the ER showed that the elongated peroxisomes very closely aligned to and followed the dynamics of adjacent ER tubules. This suggests that the two organelles are indeed connected but no evidence was found of any luminal connectivity [56].

Probably the best characterized ER-organelle interaction is that supporting protein export from the ER to the Golgi, which occurs at very small interfaces, known as ER exit sites (ERES). These are marked by elements of the COPII machinery that is responsible for ER protein export [57] (Figure 2). With the exception of Sec16, which is believed to provide a regulatory role for COPII assembly [58–60], the sequences of COPII proteins are largely conserved among eukaryotes [61]. A functional fluorescent protein fusion to the *Arabidopsis* SEC16A was shown to accumulate in the cytoplasm as well as in ring or cup-shaped subdomains of the ER, similar to COPII component and SEC16-interactor Sec13 [60,62]. Although it is possible that partially uncoated COPII carriers are associated with the Golgi membrane prior to membrane fusion [63], it has been shown in photobleaching experiments that SEC16A has a slower membrane-binding turnover rate and a smaller mobile fraction compared to the COPII coat components SEC24A and SEC13 at membranes, supporting that SEC16A is not largely associated with partially uncoated carriers at the Golgi [60]. These findings support a model whereby plant Golgi stacks and ERES are associated as a

secretory unit [62,64–66], possibly to sustain efficient membrane exchange at the interface of highly motile stacks with the ER membrane. Therefore, while it is also possible that ERES may transiently exist as independent structures from Golgi stacks to initiate the biogenesis of additional stacks or to function as additional export foci to deliver cargo to Golgi that come in close association, ERES are in continuous association with motile Golgi stacks, at least in highly vacuolated cells. This organization is unique to plants. An immediate question arising from these findings relates to the nature of the connections that facilitate the maintenance of the ER/ERES/Golgi system in plants. The organization of the ERES is presumably defined by proteins that impose curvature on defined areas of the ER, as it occurs for ER tubulation (Golgi stacks are not found on the surface of cisternal sheets), as well as proteins that modify lipid bilayer composition in these areas. For example, in mammalian cells local lipid remodeling by phosphorylation has been invoked as necessary for Sar1-mediated ER export and COPII nucleation [67]. Similar mechanisms may be in place also in plants. Concentration of COPII carriers at the plant ERES may be maintained by analogous proteins to the mammalian and *C. elegans* ERES-localized TFG-proteins that can interact with Sec16 and regulate secretion [68]. It has been proposed that TFG-proteins may generate a matrix at ERES, which could enable local accumulation of COPII components for efficient vesicle formation and egress. While plants do not have a close sequence TFG-homologue, plant proteins with analogous function to TFG-proteins may exist. Alternatively, it is also possible that plants may not require TFG-proteins. In plants Golgi and ER are in close proximity and COPII vesicles have rarely been observed [63]. It is possible that COPII vesicles are rapidly consumed given the close proximity of the two membrane surfaces and this would make the existence of a COPII carrier concentrating matrix at the ER/Golgi interface superfluous. On the other hand, fast production and consumption of COPII carriers could facilitate dynamic attachment of the ER with the Golgi. In mammalian cells the TRAPP complex at the Golgi interacts with the COPII component Sec23 and tethers COPII vesicles at the Golgi [69]. In plants, similar mechanisms may be in place to sustain not only fidelity of Golgi targeting by COPII carriers but also attachment of the COPII carriers in formation with the Golgi membrane that could be short-lived but sufficient to anchor dynamically the two organelles. Under a similar light, it cannot be excluded that tubular connections, which have been seen in electron microscopy studies at the ER/Golgi interface [70], may be the result of a transient ER/Golgi membrane continuity due to fusion of forming ER export carriers with the Golgi membrane (Figure 2).

Due to the fact that a great proportion of the ER in vacuolated cells is organised as a polygonal network of tubules and small cisternae at the cell cortex and that this organization, although slightly disrupted, remains in place even after depolymerization of the associated actin network, it has been proposed that there may be specific anchor/contact sites anchoring tubules to the plasma membrane [18,71]. Indeed a mapping analysis of cortical ER networks identified the presence of persistent puncta associated with the cortical ER [18]. More recently VAP27, a homologue of the yeast protein Scs2 (a homologue of a mammalian VAP protein), which is proposed to play a role in anchoring the ER to the plasma membrane, has been described [72]. This protein located to both to the ER and to stationary puncta, which were proposed to be putative anchor sites. Interestingly VAP27 was also shown to co-locate and interact with NET3c at the cortical puncta associated with the ER where it would form

complexes with actin and microtubules. Net3c is a member of a newly reported family of proteins involved in the attachment of membrane bounded organelles to the actin cytoskeleton [73] and fluorescent protein fusions plus immunolabelling demonstrated that the protein associated with immobile puncta on the ER [72]. Thus it is possible that at the cortex of cells there exists a nexus where a complex of VAP27 and Net3c cross links the actin and microtubule intersections with the cortical ER, and presumably through to the plasma membrane.

ER as a highway for other organelles

It has long been known that the ER is a highly dynamic organelle showing considerable movement in cells [74]. However, it is important to stress that besides getting swept up in classic cytoplasmic streaming, the cortical ER network displays a range of far more subtle movements such as growth and retraction of tubules across the polygons of the cortical network, flow of membrane over the surface of the ER and the conversion of tubules to cisternae and vice versa [18,19]. Such movements are driven by the acto-myosin system with members of the myosin XI family being the key motors controlling ER dynamics [75–77]. Interestingly, inhibition of myosin activity by the expression of various myosin tail domain fluorescent protein fusions indicated a differential effect of myosins in ER modelling when analysed by persistency mapping of the network [75]. Expression of 4 different mutant myosins affected tubule persistency and the persistent nature of small cisternae, one myosin increased the level of cisternalization whilst another reduced overall ER remodelling [75]. Such data indicates that distinct myosins may have evolved specific roles in controlling the organization of the ER network.

It is this complex organization of ER movement that suggests that the membrane surface of the organelle may act as a highway supporting the translocation of other organelles and structures (Figure 3). This is further supported by the evidence that a verified increase in ER and organelles streaming during cell expansion was negatively affected by a loss of ER integrity due to RHD3 mutation, independently from the actin cytoskeleton [78]. The evidence that loss of RHD3 causes changes in the ER membrane fluidity has led to the suggestion that the ER may influence the movement of other organelles either through membrane-or protein-mediated connections between heterologous membranes [78]. In addition to organelles the ER also moves other structures. For example, it has been suggested that the ER is the transport vector for the movement to plasmodesmata of viruses with the triple gene block (TGB) organization. For instance TGBp3 of Potexvirus is thought to target curved ER tubules and help sequester TGBp2 and viral RNA for transport to plasmodesmata for cell-cell transmission [13]. The nucleocapsid protein of tomato spotted wilt tospovirus has also been shown to move around tobacco leaf epidermal cells as motile inclusions on the surface of the ER. Again this movement is as expected dependent on the actin-myosin system and is inhibited by the expression of a dominant negative myosin XI-K tail [79]. Together all these exciting findings point towards a new model that the ER has a larger key role in homeostasis and dynamics of other organelles than previously believed. The critical path forward is now to identify the factors that enable the ER to communicate with other membrane and define their role in growth and defence.

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Highlights

- Conserved and divergent features of the plant ER proteins involved in shaping ER membranes.
- ER entertains not only functional but also physical interactions with other organelles.
- New role of the ER as a propulsive force for the movement of other organelles independently from the cytoskeleton.

Figure 1. Schematic representation of the organization of a plant cell

Organelles such as ER, Golgi stacks (GA), plasma membrane (PM), nucleus (N), chloroplasts (CP), plasmodesmata (PD) and cell wall (CW) are represented. The diagram (not in scale) illustrates points of contact between the ER and other organelles for which evidence is emerging in the literature. Points of contact include those between the ER, chloroplasts, Golgi and plasma membrane. Unlike for other organelles, a physiological relevance of putative points of contacts between peroxisomes and mitochondria as well as the nature of the components of the points of contact with the ER have yet to be uncovered.

Figure 2. Representation of the ER organization with respect to chloroplasts and Golgi

The chloroplast outer envelope faces the ER membrane; **model A** and **model B** represent two possible working hypotheses for transorganellar complementation [50]. The first model is based on the presence of transporter proteins while the second model assumes the existence of a hemifusion mechanism.

The cis-face of the Golgi apparatus faces the ER membrane. At the interface between the two organelles, the COPII machinery facilitates membrane export from the ER to the Golgi apparatus at the ERES. Functional fluorescent protein fusions of an earlier component of the COPII machinery such as SEC16 localizes at the ERES where they accumulate in cup-like structures [60]. Unlike the Sar1-GEF SEC12 that localizes throughout the ER network, other components of the COPII coat have been found to associate at the ER/Golgi interface. The demonstrated attachment of the ER and Golgi in plant cells [46] in combination with the evidence that Golgi movement depends not only on actin [80] but also on ER streaming [78]

possibly explains how ER export to the Golgi can occur while Golgi stacks are in movement [64], in that ERES would export membrane to Golgi stacks that are relatively static with respect to the ER [57,64,65].

Figure 3. The ER is in close association with Golgi, mitochondria, peroxisomes and chloroplasts Confocal images from time-lapse sequences acquired in Arabidopsis leaf epidermal cells that co-express a soluble ER marker and specific markers of the organelles, with the exception of chloroplasts, which are visualized through chlorophyll autofluorescence. Note that overtime, the organelles appear to travel over the ER network. Time is indicated at the top right corner of the images in seconds. Additional material can be found on Gunning's DVD [14] Scale bar = 5 micron.