View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Oxford Brookes University: RADAR

RADAR

Research Archive and Digital Asset Repository

OXFORD BROOKES UNIVERSITY

Hawes, CR, Kiviniemi, P and Kriechbaumer, VC

The endoplasmic reticulum: a dynamic and well-connected organelle

Hawes, CR, Kiviniemi, P and Kriechbaumer, VC (2015) The endoplasmic reticulum: a dynamic and well-connected organelle. *Journal of Integrative Plant Biology*, 57 (1). pp. 50-62.

doi: 10.1111/jipb.12297

This version is available: https://radar.brookes.ac.uk/radar/items/3a7e5bcb-c558-4f89-b2db-7fa5b8ac5726/1/

Available on RADAR: February 2016

Copyright © and Moral Rights are retained by the author(s) and/ or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders.

This document is the postprint version of the journal article. Some differences between the published version and this version may remain and you are advised to consult the published version if you wish to cite from it.

WWW.BROOKES.AC.UK/GO/RADAR

Received Aug. 18, 2014
Accepted Oct. 9, 2014
Edited by: Tobias Baskin, University of Massachusetts, Amherst, USA
Article Type: Invited Reviews
Running Title: ER dynamics

The Endoplasmic reticulum: A dynamic and well connected organelle

Chris Hawes*, Petra Kiviniemi, Verena Kriechbaumer

Department of Biological &Medical Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

*Correspondence: chawes@brookes.ac.uk

Abstract

The endoplasmic reticulum forms the first compartment in a series of organelles which comprise the secretory pathway. It takes the form of an extremely dynamic and pleomorphic membrane bounded network of tubules and cisternae which have numerous different cellular functions. In this review we discuss the nature of endoplasmic reticulum structure and dynamics, its relationship with closely associated organelles, and its possible function as a highway for the distribution and delivery of a diverse range of structures from metabolic complexes to viral particles.

Keywords: Endomembranes, Golgi, myosin, actin, microtubules

INTRODUCTION

The plant early secretory pathway comprises the endoplasmic reticulum (ER) and the numerous cisternal stacks of the Golgi apparatus. The ER is a truly multifunctional organelle being not only the site of secretory protein production, folding, quality control (Brandizzi et al. 2003) and lipid biosynthesis (Wallis and Browse 2010), but it is also involved in many other functions including auxin regulation (Friml and Jones 2010), calcium homeostasis (Hong et al. 1999) and specialist functions such as oil and protein body formation (Huang 1996; Herman 2008).

More recently, although outside the scope of this review, in plants, as in mammalian cells, it is becoming apparent that the ER is intimately involved in the autophagic process. Induced ER stress has been reported to result in autophagic turnover of the ER membrane (Liu et al. 2012) and more recently, an autophagy related protein has been shown to define a domain connecting the ER to a specific compartment, the phagophore (Le Bars et al. 2014). Likewise, some evidence suggests that the ER may be involved in the formation of lytic vacuoles (Viotti et al. 2014).

Whilst historically the ER is split into two forms, rough or smooth depending on the presence of ribosomes attached to its surface, in the context of the majority of plant cells it is more appropriate to talk in terms of tubular or cisternal ER, both of which can support protein synthesis (Figure 1). There is little evidence in the literature indicating any relationship between the ratio of tubular to cisternal ER and productivity or biosynthetic capacity of the organelle, although a stereological electron microscopy analysis of ER form in the maize root cap has shown that in peripheral secretory cells the proportion of cisternal to tubular ER

increases significantly over that in the cap meristem and statocytes (Stephenson and Hawes 1986). It is often mentioned in the literature that the ER has a number of distinct sub-domains, although as it is become ever more clear that this is an extremely dynamic and fluid organelle (see below), the maintenance of any sub-domain, with the exception of protein storage bodies, as a physical entity separate from the rest of the organelle must be open to question.

The membrane of the outer nuclear envelope is continuous with the ER membrane, forming luminal continuity between the two organelles. It is likely that the ER also forms contacts with mitochondria, peroxisomes, the tonoplast and the plasma membrane as is well documented for mammalian cells. However in plants, it is the ER exit sites (ERES) mediating protein and membrane transfer between the ER and *cis*-Golgi cisternae that are the best characterised ER sub-domains (Hawes 2012; Langhans et al. 2012).

ENDOPLASMIC RETICULUM DYNAMICS

It has long been known that the ER is an extremely dynamic and pleomorphic organelle and has been previously investigated by video enhanced light microscopy (Allen and Brown 1988) and by fluorescence microscopy using vital dyes such as 3,3'-dihexyloxacarbocyanine ($DiOC_6$) (Quader and Schnepf 1986). Such studies also implicated the actin cytoskeleton as an organiser of the ER network (Quader et al. 1989; Quader 1990). However, it was the advent of fluorescent protein technology that permitted a true appreciation of the dynamic nature of the ER (Boevink et al. 1996).

The dynamics of the tubules of the ER can be seen in arabidopsis plants where fluorescent proteins have been targeted to the ER and highlight the well-known fusiform bodies that occur in many cortical and epidermal cell types in members of the Brassicaceae (Hawes et al. 2001). These large (several micron long) spindle shaped bodies have been shown to contain high levels of β-glucosidase and increase in number on exposure to stress such as wounding, and may have a role in defence against pests and pathogens (Matsushima et al. 2003; Yamada et al. 2008; Ogasawara et al. 2009). Based on electron microscopy electron microscopy, these bodies reside in the lumen of the ER, although they cause massive distortion of the tubules (Hawes et al. 2001).

The movement of these ER bodies is actin mediated and the implication is that, as there is no evidence of cytoskeletal elements within the lumen of the ER, their movement must reflect movement of the whole ER network. Such a hypothesis was vindicated when a construct comprising photo-activatable-GFP (paGFP) and the membrane spanning domain of the plant ER membrane chaperone, calnexin, was expressed in epidermal cells of tobacco leaves (Runions et al. 2006) and demonstrated movement of the ER membrane. On activation with 405 nm laser light the newly fluorescent ER membrane rapidly dispersed from the activation point in a vectorial manner, which was actin dependent, with a mobile fraction of the protein being as high as 95%. In the absence of actin, activated membrane diffused evenly through the ER from the activation spot. On prolonged activation of one small area of ER the whole network could become fluorescent in ten or so minutes, suggesting that the whole membrane surface, or at least all of the paGFP construct, could pass through the activation area in a relatively short period of time, implying that the whole surface of the ER is motile.

It is now clear that in leaf epidermal cells, there are at least three if not four distinct forms of movement of the ER: movement of the membrane surface, growth and shrinkage of tubules, conversion of tubules to cisternae and vice versa, and movement of the tubules forming the polygons of the cortical network which along with tubule growth permits polygon ring formation and closure (Sparkes et al. 2009a, 2009b; Griffing 2010); all these being distinct from the extremely rapid ER translocation caused by classic cytoplasmic streaming. Such movement which is myosin-based (see below, Sparkes et al. 2009b; Ueda et al. 2009) could be analysed from so called persistency maps of ER networks and these revealed that about 5% of the total ER imaged shows little movement and is very persistent. The significance of this static ER is not known, although the mapping also revealed sets of persistent puncta which could represent anchor points to the plasma membrane.

SHAPING THE ER

Apart from the obvious dynamics of the system the most remarkable feature of the cortical ER network is the polygonal arrangement of the tubules and their tendency to morph in to small cisternae, mainly at three-way junctions and for these to convert back to tubules. To date there is no real functional significance attributed to these membrane forms, although previously it has been suggested that this could be related to the secretory capacity of the cell (Stephenson and Hawes 1986). Recently it has been suggested that loss of the GTPase RHD3 (root hair defective 3, see below), which is involved in shaping the ER network,

disrupts the unfolded protein responseand this may indicate a direct relationship between ER form and function (Lai et al. 2014).

There are a number of proposed mechanisms by which proteins can induce membrane curvature in organelles or in vesicle formation including classic coats such as clathrin, external scaffolding such as BAR domain proteins and direct insertion into membranes (Shen et al. 2012). In yeast and mammalian cells, a number of proteins have been identified that are involved in ER membrane tubulation: the reticulons and DP1/Yop1p/REEP (De Craene et al. 2006; Voeltz et al. 2006; Yang and Strittmatter 2007; Shibata et al. 2009; Björk et al. 2013); in tubule fusion, the atlastins (Hu et al. 2009; Orso et al. 2009) and Rab 10 (English and Voeltz 2013); and in maintenance of cisternae, CLIMP-63, kinectin and p180 (Goyal and Blackstone 2013).

In plants it has been shown that the reticulon family of proteins is most likely primarily responsible for tubulation of the ER (Tolley et al. 2008; Tolley et al. 2010, Chen et al. 2012). In arabidopsis at least 21 reticulons have been identified (Sparkes et al. 2010) and over-expression of these has been shown to induce a severe constriction phenotype in the tubular ER (Tolley et al. 2008) and can convert cisternal sheets into tubules (Tolley et al. 2010). The proteins are associated with curved ER membranes, tubules or edges of cisternae and are not regularly found on cisternal sheets (Sparkes et al. 2010). Reticulons are integral ER membrane proteins withan unusual topology in that they feature two major hydrophobic helical domains which are far too long to simply span the ER unit membrane. It is suggested that these form two "V" shaped wedges joined by a cytosolic loop, with C and N termini being oriented to the cytosol. The protein can then dimerise or oligomerise with itself creating differential tension in the ER membrane resulting in induction of curvature (Figure 2; Sparkes et al. 2010). Sparkes et al. (2010) demonstrated that at least five plant reticulons almost certainly exhibit the same topology and when the membrane spanning domains were shortened or totally removed, the proteins lost their ability to induce tubulation of the ER and could also reside on cisternae (Sparkes et al. 2010; Tolley et al. 2010).

Another family of proteins involved in the organisation of the ER network is the atlastins in mammals,withSey1p being the yeast functional ortholog (Hu et al. 2009). These are dynamin-related GTPases that are involved in the fusion of ER tubules (Liu et al. 2012). In plants it has long been known that the root hair deficient mutant (RHD3-1, Wang et al. 1997) is a GTPase that may function in the secretory pathway (Zheng et al. 2004). It has now been shown that *RHD3-1* in arabidopsis encodes the plant ortholog of Sey1p/atlastin and

expression of mutant forms results in an ER phenotype where the cortical geometrical array is disturbed, resulting in an ER network showing more long strands and less polygons (Chen et al. 2011). Again, it has been suggested that this may be due to a problem involving the fusion of ER tubules preventing polygon formation (Zhang et al. 2013). Interestingly Chen et al. (2011) reported that RHD3 and Sey1p cannot complement respective yeast and *Arabidopsis* mutants. However, it was reported by another group that RHD3 could complement yeast *sey1* mutants and was used in an assay to show that RHD3 can promote ER fusion in yeast (Zhang et al. 2013). A hint of another function for RHD3 comes from a study of ER membrane motility in expanding arabidopsis cotyledonary cells: in fully expanded epidermal cells in an *RHD3* mutant line, the mobility of the ER membrane marker protein CNX-GFP was reduced, suggesting some influence of RHD3 on the mobility of proteins in the lipid bilayer, thus potentially controlling bilayer properties (Stefano et al. 2014). A seed-specific isoform of RHD3 (RHD3-like 2 or RL-2) has also been shown to interact with reticulons and it is likely that these two families of protein act together to regulate the ER phenotype (Lee et al. 2013).

Anchor points

Although the majority of the ER network appears to be in a constantly dynamic stateas stated above a low percentage of tubulesappear to be static. Also, Sparkes et al. (2009b) demonstrated the existence of stable puncta distributed along the ER tubules, but not at three way junctions, which they suggested represent anchor sites on the plasma membrane. With the use of laser tweezers, it was previously shown that GFP-HDEL labelled endoplasmic reticulum could be remodelled in leaf epidermal cells by movement of laser-trapped fluorescent protein-tagged Golgi bodies and newly formed tubules could be captured at these putative anchor points and remodelled around them (Sparkes et al. 2009c; Figure 3). An analysis of cortical ER networks from time lapse series of leaf epidermal cells identified the presence of persistent puncta, which did not move with the ER network (Sparkes et al. 2009b). It has been suggested that junctions where the ER intersects with microtubules are stable and mark locations where actin mediated transport of organelles is interrupted (Hamada et al. 2012). This could be represented by the ER persistent puncta (Péña and Heinlein 2013). Such sites have been termed cortical microtubule-associated ER sites(C-MERS; Péña and Heinlein 2013).

In yeast and mammalian cells, it has been proposed that amongst a number of different proteins discovered at sites where the ER is anchored to the plasma membrane, members of the VAP family arebe particularly important. In yeast, the VAP homologue, Scs2, anchors

the ER to the plasma membrane, especially at the bud tipsand interacts with sterol transfer proteins (Loewen and Levine 2005; Loewen et al. 2007). Recently, it has been suggested that the static puncta found along the cortical ER tubules in leaf epidermal cells may represent the position of a multi-protein complex composed ofVAP27, the plant homologue of yeast Scs2, and NET3c, a member of a newly discoveredfamily of plant proteins called Networked (NET) that are involved in the attachment of membrane bounded organelles to the actin cytoskeleton (Deeks et al. 2012; Wang et al. 2014). As VAP27 was also shown to be a microtubule binding protein it was suggested that ER-plasma membranecontact sites in plants may also represent a nexus where the actin and microtubule cytoskeletons interact, thus creating sites at the cell cortex where the movement of motile organelles such as Golgi bodies may be interrupted (see below). It is worth noting that these contact sites are not found at three way junctions in the ER network which are formed by tubule fusion as suggested by Hamada et al. (2012). Various functions have been attributed to ER-plasma membrane contact sites in mammalian cells besides anchoring of the ER network. These include cell signalling, direct lipid transfer and calcium release (Manford et al. 2012). To date we have no evidence of any function atsuch sites in plants although lipid transfer and the transmission of pathogen-induced signals directly to the ER could be mediated at such contacts.

THE ER AS AN INTRACELLULAR HIGHWAY AND METABOLIC SURFACE

As the ER is highly dynamic with growing and retracting tubules and a mobile delimiting membrane, it is tailor made for the transport of material that is bound to its surface round the cell. Thus the membrane surface of the ER may function as a kind of highway transporting organelles and other structures. It has long been known that in many plant cell types Golgi bodies move with the surface of the ER (see below). Certainly, in tobacco leaf epidermal cells, it has been demonstrated that viral movement proteins and RNA can be located to the ER and even be transported along its surface to plasmodesmata (Heinlein et al. 1998; Schoelz et al. 2011). More recently it has been confirmed that the ER supports the movement of viruses with the triplegene block (TGB) organisationto plasmodesmata. For example, TGBp3 of Potexvirus targets curved ER tubules and helps bind TGBp2 plus viral RNA for transport to plasmodesmata for cell-to-cell transmission (Wu et al. 2011). Viral nucleocapsid proteins such as those of tomato spotted wilt tospovirus have also been shown to move on the surface of the ER in tobacco leaf epidermal cells as motile inclusions. Such movement is dependent on the actin-myosin system and is inhibited by the expression of a dominant negative myosin XI-K tail (Feng et al. 2013).

Functional protein complexes on the ER

A concept is also emerging of the ER offering an extensive surface for the location of blocks of metabolic enzymes, thus not only regulating the spatial organisation of metabolic pathways but enabling the mixing of metabolic products throughout the cytoplasm. Metabolic functions of all higher organisms require strict coordination at whole-organism as well as tissue, cellular, organellar, and molecular level. Organellar compartmentation provides the possibility for cells to bring enzymes and their substrates in closer proximity and increase local concentration for process optimisation. Down the structural hierarchy to the molecular level, such channelling and thereby optimization of substrate concentrations is achieved by the formation of functional multi-enzyme complexes. The concept of these so called metabolons was introduced in by Paul Srere in the context of glycolytic and Krebs cycle enzymes (Srere 1987). Sequential enzymes of a specialised biosynthetic pathway are transiently linked together by noncovalent binding typically stabilised by membrane or cytoskeletal anchoring (Hoppertand Mayer 1999).

Metabolons provide a variety of crucial advantages: these structures can improve catalytic efficiency in terms of turnover rate by bringing the active sites of enzymes converting adjacent steps in closer proximity. This also allows for substrate channelling, a direct passing-on of the product from an enzymatic reaction to act as substrate for the next biosynthetic step. Such a mechanism increases substrate concentration and turnover rates, prevents diffusion and metabolic interference, and is beneficial for labile or toxic intermediates (reviewed in Møller 2010). Such complex coordination has been shown for protein translocases found in endoplasmic reticulum, chloroplasts and mitochondria (Jarvis et al. 1998; Werhahnet al. 2001; Van den Berg et al. 2004) allowing coordinated and efficient transport potentially also preventing back-flux.

In plants, metabolons have been described for several enzymatic pathways in secondary and primary metabolism (Winkel-Shirley 2002). Most metabolons involved in secondary metabolism comprise less stable interactions than reported for metabolons in primary metabolism, requiring refined microscopy methods and computational analysis to verify their existence (Bassard et al. 2012a). In secondary metabolism, the synthesis of phenylpropanoids (Stafford 1974) or flavonoids (Hrazdina and Wagner 1985; Winkel-Shirley 2001) was shown to be structured in metabolons and it was suggested that such multi-enzyme complexes allow for the production of specific metabolites from common intermediate products. The composition of enzymes in the flavonoid pathway complex can

vary resulting in varying end products (Hrazdina and Wagner 1985; Winkel-Shirley 2002;). Membrane-anchored cytochrome P450 enzymes are reported to serve as nucleation points and platforms for the metabolon formation. Most P450 monooxygenases are localised to the ER via an N-terminal tether, enhancing metabolon formation on this membrane system (Jørgensen et al. 2005; Ralston and Yu 2006). It is further suggested that cytochrome P450 localises even in specific ER subdomains and that this is facilitated by interactions between metabolon and the cytoskeletal elements (Bassardet al.2012b).

In sorghum, the production machinery of the cyanogenic glucoside, dhurrin, forms metabolons in specific in ER domains (Winkel 2004). Despite this biosynthetic pathway being rather simple, involving only two membrane-bound cytochrome P450 enzymes and a soluble glucosyltransferase, a metabolon formation between these three enzymes is surely beneficial as the reaction intermediate, p-hydroxym and elonitrile, is unstable.

In tapetal cells, four enzymes responsible for the biosynthesis of sporopollenin, the main building blocks of the outer layer of pollen walls, mainly localise to the ER. Co-localisation and protein-protein interaction of these enzymes indicate the existence of an ER-bound sporopollenin metabolon (Lallemand et al. 2013). The ER is a hotspot for active lipid synthesis and tapetal cells have been reported to show signs of a strong lipid metabolism, such as dilated ER and accumulation of lipid bodies (Murgia et al. 1991; Owen and Makaroff 1995; Hernandez-Pinzon et al. 1999). The high demand for sporopollenin would benefit from a quick and efficiently coordinated production of pollen cell wall componentsas afforded by the metabolon, allowing for rapid pollen development. Additionally, the sporopollenin synthetic enzymes also interact with cytochrome P450 membrane proteins, which can act as ER-membrane anchors for metabolon formation(Lallemand et al. 2013).

As many enzymes implicated in auxin biosynthesis have low turnover rates and substrate specificities, an indole acetic acid (IAA) synthase metabolon has been suggested (Müller and Weiler 2000) although purification and yeast-2-hybrid attempts so far failed to identify proteins involved (Müller and Weiler 2000; Kriechbaumer et al. 2006). This could potentially be due to such a complex being membrane-anchored, which would hinder detection by conventional approaches. This hypothesis is supported by the recent finding that a splice variant of YUCCA4 –a member of a family of 11 YUCCA genes encoding flavin-dependent monooxygenases (FMOs)- is localised to the ER via a C-terminal hydrophobic domain (Kriechbaumer et al. 2012). Additionally,a number of other YUCCA proteins are predicted, in silico*,* to have hydrophobic domains and signal peptides, indicating a wider significance for

enzyme localization in auxin biosynthesis (Kriechbaumer et al. 2012). Such a complex coordination could be valuable as the auxin precursor, indole-3-pyruvic acid, is very labile (Of course in aqueous solution, why would you be talking about its stability in acetone?). Interestingly, despite coding for an identical enzymatic FMO domain, (Figure 4A; Kriechbaumer et al. 2012) the ER membrane-bound splice variant YUCCA4.2 (Figure 4C) increases total IAA levels by about a third more than the cytosolic (Figure 4B) version when over-expressed in tobacco epidermal leaves (Figure 4D). As total IAA was normalised against leaf dry weight, this difference in productivity could indicate a certain level of protein stabilisation or enhanced accumulation on ER membranes.

Metabolon assembly can be connected with membrane areas of a specific lipid composition (Zajchowski and Robbins 2002) or membrane-structuring proteins, e.g. in plant secondary metabolism, putative metabolons containing P450 enzymes co-purified with reticulons (Bassard et al. 2012b), which as described above contribute to ER tubule shaping (Sparkes et al. 2010). Classically, the ER has been described as a homogenous structure with fluid movement and consisting of a lipid bilayer that allows for membrane-protein positioning (Singer and Nicholson 1972). Recent data indicates that lipids are capable of forming membrane-domains of specific compositions and that these “lipid rafts” are acting as platforms for metabolon formation (Zajchowski and Robbins 2002). The plant ER is a dynamic and constantly remodelling structure (see above) with rapid movement so that presumptive metabolons in rafts guided or driven by the actin/myosin cytoskeleton would likewise be rapidly moving (Bassard et al. 2012b); such plasticity allows for quicker exchanges between membrane-anchoring enzymes, cytosolic proteins and metabolites and therefore strongly enhancesmetabolic effectiveness. In a similar manner, rafts containing enzymatic production sites for defence molecules could benefit from such a transport, possibility in response to a pathogen attack or for localisation near pathogen entry sites to facilitate high local concentrations of defence compounds (Jørgensen et al. 2005).

Additionally, metabolons would not be limited to one organellar membrane system. For instance, partially fused membranes at interaction sites between the ER and plastids (Whatley et al. 1992, see below) would enable enzymes on both membranes to process nonpolar compounds in a transporter-independent manner (Mehrshahi et al. 2014). Such a mechanism would allow the development of membrane-spanning pathways and metabolons between organelles without the need for coevolution of transport facilities (Mehrshahi et al. 2014). A model of membrane fusion could be a potential platform for pathways producing non-polar compounds and spanning ER and plastid membranes. Examples for pathways

with these criteria are gibberellic acid biosynthesis (Zybailovet al. 2008) and desaturation processes in fatty acid and membrane lipid synthesis (Browse et al.1986; Miquel and Browse 1992).

ER/ORGANELLE CONTACTS

It has been shown across the kingdoms, that the ER can make close contacts with a number of organelles besides the plasma membrane (see above), such as mitochondria (Rowland and Voeltz 2012) and the Golgi apparatus, directly via structures such as ER exit sites and tubular vesicular carriers (Ladinsky et al. 1999; Mironov et al. 2003; Glick and Nakano 2009). Less is known about contacts between the ER and other plant organelles, although some potentially interesting stories are beginning to emerge (Mathur et al. 2012)

.

The Golgi apparatus

The Golgi apparatus lies at the heart of the secretory pathway and it modifies proteins and moves lipids around as well as being responsible for the biosynthesis of the major matrix polysaccharides of the cell wall. The interaction of the Golgi with the ER (Juniper et al. 1982) via ER exit sites is perhaps the most obvious of the interactions between the ER and other organelles (Hawes 2012; Langhans et al. 2012). It is now accepted that in vacuolated cells, Golgi bodies together with ER exit sites move over, or with, the surface of the ER (Brandizzi et al. 2002; DaSilva et al. 2004) and appear to be connected together, forming mobile secretory units. So much so that when Golgi bodies are trapped and manipulated by optical tweezers, the attached ER is remodelled behind the moving Golgi body (Figure 3; Sparkes et al. 2009c). Such tethering of the Golgi to the ER has been suggested to be mediated by members of the golgin family of coiled-coil peripheral Golgi proteins (Latijnhouwers et al. 2007; Osterrieder 2012). Such a close relationship between the Golgi and the ER has resulted in speculation that the ER exit sites, embedded in the curved ER surface, are not only responsible for the biogenesis of Golgi stacks, but that the Golgi bodies themselves could be considered to be a functional extension of an ER domain rather than an independent organelle (Hawes and Satiat-Jeunemaitre 2005; Hawes et al. 2010). The number of stacks in a cell would then reflect the secretory capacity of the cell, with ER exit sites multiplying (by fission?) and generating a new Golgi stack when required (Hawes et al. 2010).

A direct connection between the Golgi and ER does not necessarily imply permanent luminal continuity between the ER and *cis*-Golgi cisternae. It is now known that all the components of COPII carrier coats are present in plants and they are most likely required for the efficient transfer of cargo between the ER and Golgi (De Craene et al. 2014; Ito et al. 2014). However, whether COPII vesicle carriers are used in many cell types, other than a few specific tissues, is still an open and controversial question and tubular continuity for cargo transfer is a distinct possibility (Hawes 2012). However, the mobile secretory unit concept is gaining in acceptance (Langhans et al 2012; Lerich et al 2012) which suggests that all cargo transfer has to take place over a very narrow interface between the co-joined ER and Golgi (less than 300 nm), Thus, the requirement for directed vesicle vectors in such a confined space is questionable and we await more detailed high-resolution EM analysis such as that afforded by focussed ion beam SEM reconstruction, to answer this question.

By definition ER exit sites are primarily involved in mediating transport of ER derived cargo to the *cis*-Golgi for further processing. However, an equivalent retrograde pathway most likely exists, as evidenced by work employing secretory pathway inhibiting drugs such as brefeldin A (BFA), and genetic inhibition of exit from the ER, both of which result in redistribution of Golgi membrane to the ER system (Osterrieder et al. 2009). Unfortunately, due to the lack of marker proteins little is known about these putative ER import sites. It has however been suggested that an ER SNARE (SYP72), which is distributed on puncta over the ER, marks putative ER import sites, because when Golgi bodies are stationary they co-locate with SYP72 puncta (Lerich et al. 2012). The authors of this study suggested a concept of mobile secretory units on the ER whereby Golgi bodies dock at SYP72-marked nodules to permit fusion of COPI retrograde vesicles at the ER import site which is geographically located with ER exit sites, thus explaining the stop-go movement of Golgi (Nebenführ et al. 1999). At the same time, Lerich et al. (2012) also suggested that COPII vesicles would bud from the ER exit sites when the Golgi bodies are motile. However, this work from tobacco BY2 and leaf cells did not present any direct evidence for COPI or COPII transport vesicles. Interestingly, Schoberer et al. (2010) demonstrated that upon deconstruction of the Golgi with GTP-locked Sar1p or brefeldin, membrane retrieval to the ER and release of Golgi matrix proteins commenced at the *trans*-face of the Golgi stacks, sequentially working back to the *cis*-face, indicating the existence of a retrograde transport pathway from the *trans*-Golgi to the ER.

Interestingly, from numerous observations it is apparent that Golgi bodies and their associated ER exit sites are only attached to curved ER membranes – the surface of tubules

or the curved edges of cisternae (Hawes unpublished). Such a situation has been reported in yeast where high-curvature domains of the ER, containing reticulon 1, are associated with ER exit sites and in mutants with more cisternal ER, ER exit sites are still restricted to high curvature ER and the rims of cisternae (Okamoto et al. 2012). It has yet to be shown if there is any involvement of the tubule-inducing reticulons in the organisation of the exit sites and attached Golgi.

Chloroplasts

As mentioned above, the existence of functional continuity between ER and plastid envelopes has been extrapolated from the ability of enzymes in the tocopherol and carotenoid synthetic pathways that are normally resident in the plastid to complement their loss of function mutant when targeted to the lumen of the ER (Mehrshahi et al. 2014). However, direct evidence for membrane continuities between the ER and plastids haspreviously been shown by freeze-fracture electron microscopy (Whatley et al. 1991). Contacts between ER and chloroplasts have also been demonstrated in cytoplasm from burst protoplasts by using optical tweezers and the authors suggested this indicated a functional relationship between the structures *in vivo* (Andersson et al. 2007). It has also been shown using fluorescent protein expression and live cell imaging that that the tubular extensions from plastids, known as stomules, can align along the ER but the functional significance of this alignment has yet to be elucidated (Schattat et al. 2011). Such contacts between the ER and chloroplasts most likely mediate lipid transfer between the organelles (Hurlock et al. 2014).

Peroxisomes

Peroxules, tubular extensions from peroxisomes are particularly apparent in cells under conditions of oxidative stress. Sinclair et al. (2009) have shown, again using live cell imaging, that peroxules can extend and retract along ER tubules. In an arabidopsis mutant (*apm1/drp3a),*peroxisomes were extremely elongated and phenotypically looked similar to the ER network, and also co-aligned with the ER. Such data suggest direct connection or interaction between the two organelles. Although in yeast and mammalian cells it has been shown that the ER is directly involved in peroxisome biogenesis (Smith and Aitchison 2013), there is little evidence that this is the case in plants (Sparkes et al. 2005), although some plant peroxisomal prtoeins have been shown to traffic from the ER (Baker and Paudyal 2014).

MYOSINS AND ENDOMEMBRANE MOTILITY

As described earlier, the ER displays a diverse range of movements which are driven by the cell's actomyosin system with members of the myosin XI family being the key motor proteins driving the movements (Sparkes et al. 2009b, Griffing etal.2014). Whilst a knock-out approach to dissect myosin function is desirable (Peremyslov et al. 2008, 2010), due to the predicted functional redundancy of such a large group (13 myosin XIs in arabidopsis), a dominant negative mutant approach is often taken to assess the role of individual myosin isoforms (Sparkes et al. 2008; Avisar et al. 2009). Using such an approach, Griffing et al. (2014) reported differential effects of the expression of various myosin tail domain fusions on ER modelling in the cortical epidermal cells of tobacco leaves. It is predicted that these proteins will out compete or dimerise with their equivalent native myosins or accessory factors, thus effectively knocking out or knocking down their function (Sparkes 2010). Persistency mapping of the ER in the presence of plants expressing a number of myosin constructs, showed that four different myosin tail domains (XI-C, XI-E, XI-K, XI-1) affected ER tubule persistency and the persistence of small cisternae, another (XI-I) increased the level of cisternae formation in the network, and a fifth (XI-2) reduced overall ER remodelling (Griffing et al. 2014) had the effect of an overall reduction in the level of ER remodelling. This approach whilst not showing the sub-cellular location of myosin isoforms is extremely effective in inhibiting myosin function and thus motility of specific organelles. Ueda et al. (2010) employed a mutant approach (single, double, and triple) to assess the role of myosin on ER streaming in arabidopsis cotyledon petioles rather than the fine details of the cortical network. They concluded the myosin XI-K was primarily but not wholly responsible for driving ER streaming, to a certain extent supporting the role of XI-K in the organisation of the cortical ER network (Sparkes et al. 2009b).

Taken together all of these data suggest different myosin isoforms have evolved unique roles in regulating the structure of the ER network.

MYOSINS AND GOLGI MOTILITY

It is now generally accepted that in many cell types, especially highly vacuolated cells, Golgi bodies can be extremely motile in the cytoplasm (Boevink et al.1998, Nebenführ et al. 1999, Sparkes et al. 2008). Such motility is actin-based, as it ceases on disruption of the actin network with cytochalasin or latrunculin (Boevink et al. 1998; Sparkes et al. 2008) and is somehow myosin driven, insofar as expression of a number of myosin IX tail domain constructs or RNAi inhibits or slows Golgi motility (Avisar et al. 2008, 2009; Sparkes et al. 2008). With 13 myosin IX genes identified in arabidopsis combined with the fact that it took

the construction of double,triple, and even quadruple myosin mutants toaffect Golgi motility (Prokhnevsky et al. 2008; Peremyslov et al. 2010), it must be assumed that there is a considerable degree of functional redundancy within the family.

Because of the difficulty of knocking down myosin function exactly how force is exerted on individual Golgi stacks is a matter for conjecture. One hypothesis has been that the Golgi bodies move with the surface of the ER which itself is motile. As discussed above, by photo-activating a GFP-tagged calnexin transmembrane-domain construct, expressed in tobacco leaf epidermal cells, a directional and actin-dependant movement of the fluorescent protein in the ER membrane was reported (Runions et al. 2006). Co-expression with a Golgi marker showed the directionality of movement to be the same as that of ER-associated Golgi bodies and it is known that in this tissue Golgi bodies are tightly associated with the ER. Subsequently it was shown that this movement of the photo-activated GFP was not induced by movement of the attached Golgi bodies but could be observed after Golgi disruption with brefeldin A (Sparkes et al. 2009b) indicating that Golgi body movement is not responsible for movement of the ER surface.

A DIL domain, homologous to the yeast secretory vesicle binding domain from Myo2p, identified in the tails of 12 arabidopsis myosin XI proteins has been show to locate to Golgi, ER, peroxisomes, plasma membrane, nuclei and un-identified vesicles (Sattarzadeh et al. 2011). Only one construct, which containedto theDIL domain from myosin XI-G, labelled the Golgi and ER only. Recently the same group (Sattarzadh et al. 2013) expressed fluorescent protein fusions of another sub-domain from 11 members of the myosin XI family in tobacco leaf epidermal cells and reported organelle labelling. The subdomain is homologous to ones found in yeast class V (myo2p) myosin tails that confer organelle specificity. Of the subdomain fusions, termed PAL-domains, seven labelled Golgi bodies and five labelled mitochondria. Interestingly, and unlike expression of complete myosin tail domains (Sparkes et al. 2008, Avisar et al. 2009), specific organelle movements were not compromised until several constructswere co-expressed. This could be due to the level of expression of the PAL domain constructs being too low to out-compete native myosin binding sites or due to non-specific labelling of the organelles which results in no competition with any putative membrane bound myosin receptors on the organelles. Interestingly none of these domain constructs were reported to label the ER, which as discussed above has been shown to exhibit myosin-dependent movement. Therefore, at this juncture we have confusing evidence from organelle labelling and movement inhibition about which myosin isoforms are involved in Golgi motility. In fact,and no solid evidence to confirm that complete myosin

dimers directly decorate the surface of organelles they are supposed to move inthe early secretory pathway (Buchnik et al. 2015 this issue).

Although it is apparent that myosins play a major role in Golgi motility, it has been shown that the plasma membrane-anchored arabidopsis actin-binding and -nucleating protein, formin 1,apparently influences Golgi motility, presumably through interfering with and inducing rearrangements of the actin cytoskeleton (Martinière et al. 2011). This over-expression of formin 1 results in a major rearrangement of the cortical actin cytoskeleton and abrogation of Golgi movement.Interestingly the extracellular domain of the formin 1 was proposed to interact with the cell wall and deletions in this domain reduce its capacity to induce remodelling of the actin cytoskeleton and to decrease Golgi body motility. These results uncoverda direct link between the cell wall and endomembrane system dynamics.

MICROTUBULES AND MEMBRANE MOTILITY

The role of microtubules in the organelles of the endomembrane system is starting to attract attention (Brandizzi and Wasteneys 2013). For instance, in the giant intermodal cells of the characean algae it is well documented that the dramatic cytoplasmic streaming is actin-myosin-mediated. However, it appears that the cortical ER which is far less dynamic lies over the microtubule cytoskeleton and it is microtubules that are responsible for organising the ER during elongation of these cells (Foinssner et al. 2009). This is a situation more akin to mammalian cell ER organisation as opposed to the actin-based system in higher plants. However, a direct microtubule ER interaction has been reported in protodermal leaf cells of various angiosperms (Giannoutsou et al. 2012). Here the cortical ER co-located with the preprophase bands of microtubules forming a ring-like structure. This was not found in root tip preprophase bands where it was suggested that the microtubules are less acetylated and less stable.

In many cell types, the movement of Golgi bodies is by no means smooth or regular and they often exhibit stop and go motility. Originally it was suggested that they paused at exit sites to pick up cargo (Nebenführ et al. 1999). Although it was originally suggested that microtubules have no effect on Golgi motility (Brandizzi et al. 2002), more recently is has been suggested that Golgi bodies pause on microtubules (Hamada et al. 2012), possibly at sites where they release vesicles containing CesA complexes for cellulose biosynthesis to specific sites on the plasma membrane for secretion (Crowell et al. 2009, Vick et al. 2012). However, it appears that the actin cytoskeleton regulates CesA delivery to the plasma membrane and small CesA compartments can be actin-associated even if localised insertion

of the CesA complex is microtubule-mediated (Sampathkumar et al. 2012; Bashline et al. 2014). It was concluded that in arabidopsis hypocotyl cells, only about 30% of CesA insertion events at the plasma membrane are associated with Golgi pausing. Thus, it is unclear whether Golgi bodies genuinely pause to permit exocytotic events or pause due to their movement being physically obstructed by barriers on their ER tracks such as the puncta of the NET3C/VAP27 anchor points (Wang et al. 2014). This could explain thee supposed association of Golgi bodies with microtubules.

OUTLOOK

From the above it can be seen that the ER as an extensive network of tubules and cisternae is a truly multifunctional and dynamic organelle. Recent years have witnessed major advances in our understanding of the dynamics of the ER network and of its potential roles for example in intracellular transport and as a metabolic surface, whilst we are discovering some of the structural proteins that regulate its form. However, there are many more questions to be addressed and answered. For instance: How is the transition between tubular and cisternal ER regulated and mediated? What is the role of the ER in protein folding and subcellular compartmentation of metabolic processes? How do myosins interact with the organelle? What is the true role of microtubules in the organisation of the system and the function of contacts points at the plasma membrane? Mediating such processes we can predict the requirement for a myriad of structural and regulatory proteins such as myosin adaptors, reticulon and RHD3 interactors, plus regulatory Rab-GTPases as well as potential novel and unprecedented regulatory mechanisms. This is indeed still an exciting period for plant endomembrane research.

ACKNOWLEDGMENTS

We would like to acknowledge the support and contributions of our collaborators to some of the work discussed here, namely, Imogen Sparkes (Exeter, UK), Lorenzo Frigerio (Warwick, UK), Karl Oparka (Edinburgh, UK), Pengwei Wang (Durham, UK) and Ben Abell (Sheffield Hallam, UK). Some of the work reported here was financed by BBSRC (332/C19286, BB/J005959/1) and Leverhulme Trust Grants (F/00 382/G) to CH.

REFERENCES

Allen NS, Brown DT (1988) Dynamics of the endoplasmic reticulum in living onion epidermal cells in relation to microtubules, microfilaments and intracellular particle movement. **Cell Motil, Cytoskel** 24, 153–163

Andersson MX, Goksor M, Sandelius AS (2007) Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. **J Biol Chem** 282, 1170–1174

Avisar D, Abu-Abied M, Belausov E, Sadot E, Hawes C, Sparkes IA (2009) A comparative study of the involvement of 17 *Arabidopsis* myosin family members on the motility of Golgi and other organelles**. Plant Physiol** 150, 700–709

Avisar D, Prokhnevsky AI, Makarova KS, Koonin EV, Dolja VV (2008) Myosin XI-K is required for rapid trafficking of Golgi stacks, peroxisomes and mitochondria in leaf cells of *Nicotiana benthamiana*. **Plant Physiol** 146, 1098–1108

Baker A, Paudyal R (2014) The birth and death of peroxisomes. **Curr Ops Plant Biol** In press.

Bashline L, Shundai L, Gu Y (2014) The trafficking of the cellulose synthase complex in higher plants. **Ann Bot** doi:10.1093/aob/mcu040

Bassard J-E, Mutterer J, Duval F, Werck-Reichhart D (2012a) A novel method for monitoring the localization of cytochromes P450 and other endoplasmic reticulum membrane associated proteins: a tool for investigating the formation of metabolons. **FEBS J** 279, 1576–1583

Bassard J-E, Richert L, Geerinck J, Renault H, Duval F, Ullmann P, Schmitt M, Meyer E, Mutterer J, Boerjan W, De Jaeger G, Mely Y, Goossens A, Werck-Reichhart D (2012b) Protein–protein and protein–membrane associations in the lignin pathway. **Plant Cell** 24, 4465–4482

Björk SM, Hurt CM, Ho VK, Angelotti T (2013) REEPs are membrane shaping adaptor proteins that module specific G-protein-coupled receptor trafficking by affecting ER cargo capacity. **PLoS One** 8, e76366

Boevink P, Santa Cruz S, Hawes C, Harris N, Oparka K (1996) Virus-mediated deliver of the green fluorescent protein to the endoplasmic reticulum of plant cells. **Plant J** 10, 935–941

Boevink P, Oparka K, Santa Cruz S, Martin B, Betteridge A, Hawes C (1998) Stacks on tracks: The plant Golgi apparatus traffics on an actin/ER network**. Plant J** 15, 441–447

Brandizzi F, Snapp EL, Oberts A, Lippincott-Schwartz J, Hawes C (2002) Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: Evidence from selective photobleaching. **Plant Cell** 14, 1293–1309

Brandizzi F, Hanton S, Pinto DaSilva LL, Boevink P, Evans D, Oparka K, Denecke J, Hawes C (2003) ER quality control can lead to retrograde transport from the ER lumen to the cytoplasm and the nucleoplasm in plants. **Plant J** 34, 269–281

Brandizzi F, Wasteneys GO (2013) Cytoskeleton-dependent endomembrane organization in plant cells: An emerging role for microtubules. **Plant J** 75, 339–349

Browse J, Warwick N, Somerville CR, Slack CR (1986) Fluxes through the prokaryotic and eukaryotic pathways of lipid synthesis in the '16:3' plant *Arabidopsis thaliana*. **Biochem J** 235, 25–31

Buchnik L, Abu-Abied M, Sadot E (2015) The role of plant myosins in motile organelles: Is a direct interaction required? **J Integr Plant Biol** 56, this issue

Chen J, Stefano G, Brandizzi F, Zheng H (2011) Arabidopsis RHD3 mediates the generation of the tubular ER network and is required of Golgi distribution and motility in plant cells**. J Cell Sci** 124, 2241–2252

Chen J, Doyle C, Qi A, Zheng H. (2012) The endoplasmic reticulum: a social network in plant cells. **J. Integrative Plant Biol** 54, 840-850

Chuong SDX, Good, AG, Taylor GJ, Freeman MC, Moorhead GBG Muench DG (2004) Large-scale identification of tubulin-binding proteins provides insight on subcellular trafficking, metabolic channelling and signalling in plant cells. **Mol Cell Proteomics** 3, 970–983

Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof YD, Schumacher K, Gonneau M, Höfte H, Vernhettes S (2009) Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. **Plant Cell** 21, 1141–1154

DaSilva LL, Snapp EL, Denecke J, Lippincott-Schwartz J, Hawes C, Brandizzi F (2004) Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. **Plant Cell** 16, 1753–1771

De Craene J-O, Courte,F, Rinaldi B, Fitterer C, Herranz MC, Schmitt-Keichinger C, Ritzenthaler C, Friant S (2014) Study of the plant COPII vesicle coat subunits by functional complementation of yeast *Saccharomyces cerevisiae* mutants. **PLOS One** 9, e90072

De Craene J-O, Coleman J, Estrada de Martin P, Pypaert M, Anderson S, Yates JR III, Ferro-Novick S, Novick, P (2006) Rtn1p is involved in structuring the cortical endoplasmic reticulum**. Mol Biol Cell** 17, 3009–3020

Deeks MJ, Calcutt JR, Ingle EK, Hawkins TJ, Chapman S, Richardson AC, Mentlak DA, Dixon MR, Cartwright F, Smertenko AP, Oparka K, Hussey PJ (2012) A superfamily of actin-binding proteins at the actin-membrane nexus of higher plants**. Curr Biol** 22, 1595–1600

English AR, Voeltz GK (2013) Rab10 GTPase regulates ER dynamics and morphology. **Nat Cell Biol** 15, 169–178

Feng Z, Chen X, Bao Y, Dong J, Zhang Z, Tao X (2013) Nucleopcapsid of tomato spotted wilt tospovirus form mobile particles the traffic on an actin/endoplasmic reticulum network driven by myosin XI-K. **New Phyt** 200, 1212–1224

Foinssner I, Menzel D, Wasteney GO (2009) Microtubule-dependent motility and orientation of the cortical endoplasmic reticulum in elongating characean intermodal cells. **Cell Motil Cytoskel** 66,142–155

Friml J, Jones AR (2010) Endoplasmic reticulum: The rising compartment in auxin biology. **Plant Phys** 154, 458–462

Giannoutsou E, Galatis B, Zachariadis M, Apostolakos P (2012). Formation of an endoplasmic reticulum ring associated with acetylated microtubules in the angiosperm preprophase band. **Cytoskeleton** 69, 252–265

Glick B, Nakano A (2009) Membrane traffic within the Golgi apparatus. **Ann Rev Cell Dev Biol** 25, 113–132

Goyal U, Blackstone C (2013) Untangling the web: Mechanisms underlying ER network formation**. Biochim Biophys Acta**1833, 2492–2498

Griffing LR, Gao H, Sparkes I (2014) ER network dynamics are differentially controlled by myosins XI-K, XI-C, XI-E, XI-I, and XI-2. **Front Plant Sci** DOI 10.3389/fpls.2014.00218

Griffing LR (2010) Networking in the endoplasmic reticulum. **Bioch Soc Trans**. 38, 747–753

Hamada T, Tominaga M, Fukaya T, Nakamura M, Nakano A, Watanabe Y, Hashimoto T, Baskin TI (2012) RNA processing bodies, mitochondria and endoplasmic reticulum tubule junctions frequently pause at cortical microtubules. **Plant Cell Physiol**. 53, 699–708

Hawes C, Saint-Jore C, Martin B, Zheng, H-Q (2001) ER conformed as the location of mystery organelles in *Arabidopsis* plants expressing GFP. **Trends Plant Sci**.6, 245–246

Hawes C, Satiat-Jeunemaitre B (2005) The plant Golgi apparatus – Going with the flow. **Biochim Biophys Acta**1744, 93–107

Hawes C, Schoberer J, Hummel E, Osterrieder A. (2010). Biogenesis of the plant Golgi apparatus. **Biochem Soc Trans** 38, 761–767

Hawes C (2012) The ER/Golgi interface - Is there anything in-between? **Front Plant Sci** 18, 73

Heinlein M, Padgett HS, Gens JS, Pickard BG, Casper SJ, Epel BL, Beachy RN (1998) Changing patterns of localization of the tobacco mosaic virus movment protein and replicase to the endoplasmic reticulum and microtubules during infection. **Plant Cell** 10, 1107–1120

Herman EM (2008) Endoplasmic reticulum bodies: Solving the insoluble. **Curr Opin Plant Biol** 11, 672–679

Hernández-Pinzón I, Ross JH, Barnes KA, Damant AP, Murphy DJ (1999) Composition and role of tapetal lipid bodies in the biogenesis of the pollen coat of *Brassica napus*. **Planta** 208, 588–598

Hong B, Ichida A, Wang Y, Gens JS, Pickard BG, Harper JF (1999) Identification of a calmodulin-regulated Ca^{++}-ATPase in the endoplasmic reticulum. **Plant Physiol.**119, 1165–1175.

Hoppert M, Mayer F (1999) Principles of macromolecular organization and cell function in bacteria and archaea, **Cell Biochem Biophys** 31, 247–284

Hrazdina G, Wagner GJ (1985) Metabolic pathways as enzyme complexes: Evidence for the synthesis of phenylpropanoids and flavonoids on membrane associated enzyme complexes. **Arch Biochem Biophys** 237, 88–100

Hurlock AK, Roston RL, Wang K, Benning C (2014) Lipid trafficking in plant cells. **Traffic** doi. 10.1111/tra.12187

Hu J, Shibata Y, Zhu P-P, Voss C, Rismanchi N, Prinz WA, Rapoport TA, Blackstone C (2009) A class of dynamin-like GTPases involved in the generation of the tubular ER network. **Cell** 138, 549–561

Huang AHC (1996) Oleosins and oil bodies in seeds and other organs. **Plant Physiol** 110, 1055–1061

Ito Y, Uemura T, Nakano A (2014) Formation and maintenance of the Golgi apparatus in plant cell**. Int Rev Cell Mol Biol** 310, 221–287

Jarvis P, Chen LJ, Li H, Peto CA, Fankhauser C, Chory J (1998) An *Arabidopsis* mutant defective in the plastid general protein import apparatus. **Science** 28, 100–103

Jørgensen K, Rasmussen AV, Morant M, Nielsen AH, Bjarnholt N, Zagrobelny M, Bak S, Møller BL (2005) Metabolon formation and metabolic channelling in the biosynthesis of plant natural products. **Curr Opin Plant Biol** 8, 280–291.

Juniper BE, Hawes CR, Horne JC (1982) The relationships between the dictyosomes and the forms of endoplasmic reticulum in plant cells with different export programs**. Bot Gaz** 143, 135–145

Kriechbaumer V, Wang P, Hawes C, Abell BM (2012) Alternative splicing of the auxin biosynthesis gene *YUCCA4* determines its subcellular compartmentation. **Plant J** 70, 292–302

Lallemand B, Erhardt M, Heitz T, Legrand M (2013) Sporopollenin biosynthetic enzymes interact and constitute a metabolon localized to the endoplasmic reticulum at tapetum cells. **Plant Physiol** 162, 616–625

Langhans M, Meckel T, Kress A, Lerich A, Robinson DG (2012). ERES (ER exit sites) and the “secretory unit concept”. **J Microsc** 247, 48–59

Ladinsky MS, Mastronade DN, McIntosh JR, Howell KE, Staehelin LA (1999) Golgi structure in three dimensions: Functional insights from normal rat kidney cell**. J. Cell Biol** 144, 1135–1149.

Lai YS, Stefano G, Brandizzi F (2014) ER stress signalling requires RHD3, a functionally conserved ER-shaping GTPase. **J Cell Sci** In press.

Latijnhouwers M, Gillespie T, Boevink P, Kriechbaumer V, Hawes C, Carvalho CM. (2007). Localization and domain characterization of *Arabidopsis* golgin candidates.**J Exp Bot** 48, 4373–4386

Lee H, Sparkes I, Gattolin S, Snowden CJ, Roberts LM, Hawes C, Frigerio L (2013) An *Arabidopsis* reticulon and the atlastin homologue RHD3-like2 act synergistically in shaping the tubular endoplasmic reticulum. **New Phytol** 197, 481–489

Lerich A, Hillmer S, Langhans M, Scheuring D, van Bentum P, Robinson DG (2012) ER import sites and their relationship to ER exit sites: A new model for bidirectional ER-Golgi transport in higher plant. **Front Plant Sci** 3, 143 doi: 10.3389/fpls.2012.00143

Liu TJ, Bian X, Sun S, Hu X, Klemm RW, Prinz WA, Rapoport TA (2012) Lipid interaction of the C-terminus and association of the transmembrane segments facilitate atlastin-mediated homotypic endoplasmic reticulum fusion. **Proc Natl Acad Sci USA** 109, 2146–2154

Loewen CJR, Levine TP (2005) A highly conserved binding site in vesicle-associated membrane protein-associated protein (VAP) for the FFAT motif of lipid binding proteins. **J Biol Chem** 280, 14097–14104

Loewen CJR, Young BP, Tavassoli S, Levine TP (2007) Inheritance of cortical ER in yeast is required for normal septin organization**. J Cell Biol** 5, 467–483

Manford AG, Stefan CJ, Yuan HL, MacGurn JA, Emr SD (2012) ER-to-plasma membrane tethering proteins regulate cell signalling and ER morphology. **Dev Cell** 23, 1129–1140

Martinière A, Gayral P, Hawes C, Runions J (2011) Building bridges: Formin 1 of *Arabidopsis* forms a connection between the cell wall and actin cytoskeleton. **Plant J** 66, 354–365

Mathur J, Mammone A, Barton KA (2012) Organelle extensions in plant cells. J. Integrative Plant Biol. 54, 851-867.

Matsushima R, Kondo M, Nishimura M, Hara-Nishimura I (2003) A novel ER-derived compartment, the ER body, selectively accumulates a beta-glucosidase with an ER-retention signal in *Arabidopsis*. **Plant J** 33, 493–502

Mehrshahi P, Johnny C, DellaPenna D (2014) Redefining the metabolic continuity of chloroplasts and ER**. Trends Plant Sci**, doi: 10.1016/j.tplants.2014.02.013.

Miquel M, Browse J (1992) *Arabidopsis* mutants deficient in polyunsaturated fatty acid synthesis. Biochemical and genetic characterization of a plant oleoyl-phosphatidylcholine desaturase. **J Biol Chem** 267, 1502–1509

Mironov AA, Mironov AA Jr., Beznoussenko GV, Trucco A, Lupetti P, Smith JD, Geerts WJC, Koster AJ, Burger KNJ, Martone ME, Deerinck TJ, Ellisman MH, Luini A (2003) ER-Golgi-carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains. **Dev Cell** 5, 1–20

Møller BL (2010) Dynamic metabolons. **Science** 330, 1328–1329

Müller A, Weiler EW (2000) IAA-synthase, an enzyme complex from *Arabidopsis thaliana* catalyzing the formation of indole-3-acetic acid from (S)-tryptophan**. Biol Chem** 381, 679–686

Murgia M, Charzynska M, Rougier M, Cresti M (1991) Secretory tapetum of *Brassica oleracea* L.: Polarity and ultrastructural features. **Sex Plant Reprod** 4, 28–35

Nebenführ A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, Meehl JB, Staehelin LA (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system**. Plant Phys** 121, 1127–1142

Ogasawara K, Yamada K, Christeller JT, Kondo M, Hatsugai N, Hara-Nishimura I, Nishimura M (2009) Constitutive and inducible ER bodies of *Arabidopsis thaliana* accumulate distinct beta-glucosidases. **Plant Cell Physiol** 50, 480–488

Okamoto M, Kurokawa K, Matsuura-Tokita K, Saito C, Hirata R, Nakano A. (2012) High-curvature domains of the ER are important for the organization of ER exit sites in *Saccharomyces cerevisiae*. **J Cell Sci** 125, 3412–3420

Osterrieder A, Hummel E, Carvalho CM, Hawes (2010). Golgi membrane dynamics after induction of a dominant-negative mutant Sar1 GTPase in tobacco. **J Exp Bot** 61, 405–422

Osterrieder A (2012) Tales of tethers and tentacles: Golgins in plants. **J Microsc** 247, 68–77

Orso G, Pendin D, Liu S, Tosetto J, Moss TJ, Faust JE, Micaroni M, Egorova A, Martinuzzi A, McNew JA, Daga A (2009) Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. **Nature** 460, 978–983

Owen HA, Makaroff CA (1995) Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L.) Heynh. ecotype Wassilewskija (Brassicaceae). **Protoplasma** 185, 7–21

Pẽña EJ, Heinlein M (2013) Cortical microtubule-associated ER sites: Organisation centres of cell polarity and communication. **Curr Opin Plant Biol** 16, 1–10

Peremyslov VV, Prokhnevsky AI, Avisar D, Dolja VV (2008). Two class XI myosins function in organelle trafficking and root hair development in *Arabidopsis thaliana*. **Plant Physiol** 146, 1109–1116

Prokhnevsky AL, Peremyslov VV, Dolja VV (2008). Overlapping functions of the four class XI myosins in *Arabidopsis* growth, root hair elongation, and organelle motility**. Proc Natl Acad Sci USA** 105, 19744–19749

Peremyslov VV, Prokhnevsky AI, Dolja VV (2010) Class XI myosins are required for development, cell expansion, and F-actin organization in *Arabidopsis*. **Plant Cell** 22, 1883–1897.

Quader H, Schnepf E (1986) Endoplasmic reticulum and cytoplasmic streaming: Fluorescence microscopical obsevations in adaxial epidermis cells of onion bulb scales. **Protoplasma**131, 250–252

Quader H, Hofmann A, Schnepf E (1989) Reorganization of the endoplasmic reticulum in epidermal cells of onion bulb scales after cold stress: Involvement of cytoskeletal elements. **Planta**177, 273–280

Quader H (1990) Formation and disintegration of cisternae of the endoplasmic reticulum visualized in live cells by conventional fluorescence and confocal laser scanning microscopy: Evidence for the involvement of calcium and the cytoskeleton**. Protoplasma** 155, 166–175

Ralston L, Yu O (2006) Metabolons involving plant cytochrome P450. **Phytochem Rev** 5, 459–472

Rowland AA, Voeltz GK (2012) Endoplasmic reticulum-mitochondria contacts: Function of the junction. **Nat Rev Mol Cell Biol** 13, 607–625.

Runions J, Brach T, Kuhner S, Hawes C (2006) Photoactivation of GFP reveals protein dynamics within the endoplasmic reticulum membrane. **J Exp Bot** 57, 43–50

Sampathkumar A, Gutierrez R, McFarlane HE, Bringmann M, Lindeboom J, Emons AM, Samuels L, Ketelaar T, Ehrhardt DW, Persson S (2013) Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in *Arabidopsis* interphase cells. **Plant Physiol** 162, 675–688

Sattarzadeh A, Schmeltzer E, Hanson MR (2011) Analysis of organelle targeting by DIL domains of the *Arabidopsis* myosin XI family**. Front. Plant Sci** 2, 72, doi: 10.3389/fpls.2011.00072.

Sattarzadeh A, Schmeltzer E, Hanson MR (2013) *Arabidopsis* myosin XI sub-domains homologous to the yeast myo2p organelle inheritance sub-domain target sub-cellular structures in plant cells. **Front Plant Sci** 4, 407, doi: 10.3389/fpls.2013.00407

Schoberer J, Runions J, Steinkellner H, Strasser R, Hawes C, Osterrieder A (2010) Sequential depletion and acquisition of proteins during Golgi stack disassembly and reformation. **Traffic** 11, 1429–1444

Schoelz JE, Harries PA, Nelson RS (2011) Intracellular transport of plant viruses: Finding the door out of the cell**. Mol Plant** 4, 813–831

Schattat M, Barton K, Baudisch B, Klosgen RB, Mathur J (2011) Plastid stromule branching coincides with contiguous endoplasmic reticulum dynamics**. Plant Physiol** 155, 1667–1677

Shen H, Pirruccello M, De Camilli P (2012) Snapshot: Membrane curvature sensors and generators**. Cell** 150, 1300

Shibata Y, Hu J, Kozlov MM, Rapoport TA (2009) Mechanisms shaping the membrane of cellular organelles. **Ann Rev Cell Dev Biol** 25, 329–354

Sinclair AM, Trobacher CP, Mathur N, Greenwood JS, Mathur J (2009). Peroxule extension over ER-defined paths constitutes a rapid subcellular response to hydroxyl stress**. Plant J** 59, 231–242

Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes.**Science**175, 720–731

Smith JJ, Aitchison JD (2013) Peroxisomes take shape. **Nat Rev Mol Cell Biol** 14, 803–817

Sparkes IA (2010) Motoring around the plant cell: Insights from plant myosins. **Biochem Soc Trans** 38, 833–838

Sparkes IA, Frigerio L, Tolley N, Hawes C (2009a) The plant endoplasmic reticulum: A cell-wide web. **Biochem J** 423, 145–155

Sparkes I, Runions J, Hawes C, Griffing L (2009b) Movement and remodeling of the endoplasmic reticulum in non-dividing cells of tobacco leaves. **Plant Cell** 21, 3937–3949

Sparkes IA, Ketelaar T, de Ruijter NC, Hawes C. (2009c) Grab a Golgi: Laser trapping of Golgi bodies reveals in vivo interactions with the endoplasmic reticulum**. Traffic** 10, 567–571

Sparkes I, Teanby N, Hawes C (2008) Truncated myosin XI tail fusions inhibit peroxisome, Golgi and mitochondrial movement in tobacco leaf epidermal cells: A genetic tool for the next generation. **J Exp Bot** 59, 2499–2512

Sparkes IA, Hawes C, Baker A (2005) AtPex2 and AtPEX10 are targeted to peroxisomes independently of known endoplasmic reticulum trafficking routes. **Plant Physiol** 139, 690–700

Sparkes I, Tolley N, Aller I, Svozil , Osterrieder A, Botchway, S, Mueller C, Frigerio L Hawes C (2010). Five plant reticulon isoforms share ER localisation, topology, ER membrane shaping properties. **Plant Cell** 22, 1333–1343

Srere PA (1987) Complexes of sequential metabolic enzymes. Annu Rev Biochem 56, 89–124

Stafford HA (1974) Possible multienzyme complexes regulating the formation of C6-C3 phenolic compounds and lignins in higher plants. **Recent Adv Phytochem** 8, 53–79

Stefano G, Renna L, Brandizzi F (2014) The endoplasmic reticulum exerts control over organelle streaming during cell expansion. **J Cell Sci** 127, 947–953

Stefano G, Hawes C, Brandizzi F (2014) ER – the key to the highway. **Curr. Op. Plant Biol**. DOI: 10.1016/j.pbi.2014.09.001

Stephenson JLM, and Hawes CR (1986) Stereology and stereometry of endoplasmic reticulum during differentiation in the maize root cap. **Protoplasma** 131, 32–46

Tolley N, Sparkes IA, Hunter PA, Craddock CP, Nuttall J, Roberts LM, Hawes C, Pedrazzini E, Frigerio, L (2008) Over expression of a plant reticulon remodels the lumen of the cortical endoplasmic reticulum but does not perturb protein transport. **Traffic** 9, 94–102

Tolley N, Sparkes I, Craddock CP, Eastmond PJ, Runions J, Hawes C, Frigerio L (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

Ueda H, Yokota E, Kutsuna N, Shimada T, Tamura K, Shimmen T, Hasezawa S, Dolja VV, Hara-Nishimura I (2010) Myosin-dependent endoplasmic reticulum motility and F-actin organization in plant cells**. Proc Natl Acad Sci USA** 107, 6894–6899

Van den Berg B, Clemons WM, Collinson I, Modis Y, Hartmann E, Harrison SC, Rapoport TA (2004) X-ray structure of a protein conducting channel. **Nature** 427, 36–44

Viotti C, KrügerK, Krebs M, Neubert C, Finka F, Lupanga U, Scheuring D, Boutté Y, Frescatada-Rosa M, Wolfenstetter S, Sauer N, Hillmer S, Grebe M Schumacher K (2013) The endoplasmic reticulum is the main membrane source for biogenesis of the lytic vacuole in *Arabidopsis*. **Plant Cell** 25, 3434–3449

Vick JK, Nebenführ A (2012) Putting on the breaks: regulating organelle movements in plant cells. **J. Integrative Plant Biol.** 54, 868-874

Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA (2006) A class of membrane proteins shaping the tubular endoplasmic reticulum. **Cell** 124, 573–586

Wang H, Lockwood SK, Hoeltzel MF, Schiefelbein JW (1997) The *ROOT HAIR DEFECTIVE3* gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in *Arabidopsis*. **Genes Dev** 11, 799–811

Wang P, Hawkings T, Richardson C, Cummins I, Deeks M, Sparkes I, Hawes C, Hussey P (2014) The plant cytoskeleton, NET3C and VAP27 mediates the link between the plasma membrane and endoplasmic reticulum. **Curr Biol** 24, 1397–1405

Wallis JG, Browse J (2010) Lipid biochemists salute the genome. **Plant J** 61, 1092–1106

Werhahn W, Niemeyer A, Jansch L, Kruft V, Schmitz UK, Braun H (2001) Purification and characterization of the preproteintranslocase of the outer mitochondrial membrane from *Arabidopsis*. Identification of multiple forms of TOM20. **Plant Physiol** 125, 943–954

Whatley JM, McLean B, Juniper BE (1991) Continuity of chloroplast and endoplasmic-reticulum membranes in *Phaseolus vulgaris.* **New Phytol** 117, 209–217

Winkel BS (2004) Metabolicchanneling in plants. **Annu Rev Plant Biol** 55, 85–107

Winkel-Shirley B (2002) Evidence for enzyme complexes in the phenylpropanoid and flavonoid pathways**. Physiol Plant**.107, 142–149

Winkel-Shirley B (2001) Flavonoid biosynthesis: A colorful model for genetics, biochemistry, cell biology and biotechnology. **Plant Physiol** 126, 485–493

Wu C-H, Lee S-C, Wang C-W (2011) Viral protein targeting to the cortical endoplasmic reticulum is required for cell-cell spreading in plants. **J Cell Biol** 193, 52–535

Yamada K, Nagano AJ, Nishina M, Hara-Nishimura I, Nishimura M (2008) NAI2 is an endoplasmic reticulum body component that enables ER body formation in *Arabidopsis thaliana*. **Plant Cell** 20, 2529–2540

Yang YS, Strittmatter SM (2007) The reticulons: A family of proteins with diverse functions. **Genome Biol** 8, 234, doi: 10.1186/gb-2007-8-12-234

Zajchowski LD, Robbins SM (2002) Lipid rafts and little caves**. Eur J Biochem** 269, 737–752

Zhang M, Wu F, Shi J, Zhu Y, Zhu Z, Gong Q, Hu J (2013) ROOT HAIR DEFECTIVE family of dynamin-like GTPases mediates homotypic endoplasmic reticulum fusion and is essential for arabidopsis development**. Plant Physiol** 163, 713–720

Zheng H, Kunst L, Hawes C, Moore I (2004) A GFP-based assay reveals a role for RHD3 in transport between the endoplasmic reticulum and Golgi apparatus**. Plant J** 37, 398–414

Zybailov B, Rutschow H, Friso G, Rudella A, Emanuelsson O, Sun Q, van Wijk KJ (2008) Sorting signals, N-terminal modifications and abundance of the chloroplast proteome**. PLoS ONE**3, e1994

Figure Legends:

Figure 1. Reconstruction of the cortical ER from two adjacent pea root meristem cells

The endomembrane system was selectively osmium impregnated and serial block face imaging carried out with an FEI-Quanta field emission scanning EM fitted with a 3-View sectioning system (courtesy of Dr. Toby Starbourg, University of Manchester). Reconstruction of the ER from the serial sections was carried out with Imaris (Bitplane) software. The complex ER network either side of a lateral cell wall (not contrasted) was pseudo coloured red and blue.

Figure 2. Reticulons in plants

(A) Diagram showing the proposed insertion of reticulons into the ER membrane. **(B)** Cross section of an ER tubule showing how reticulons interact with each other to form a series of V-shaped wedges to induce membrane curvature (note–only on half of the lipid bilayer is depicted). **(C)** Expression of reticulon 3-YFP in a transformed arabidopsis leaf, clearly showing the cortical tubular ER network. Bar = 5 μm.

Figure 3. Frames taken from an time-series of an optical tweezer experiment

An arabidopsis leaf in which Golgi bodies are marked with a red fluorescent protein and ER with GFP. Two Golgi bodies were trapped (white arrows) and an attached ER tubule follows them as they are pulled through the cortical cytoplasm. At 9 seconds the Golgi bodies pick up another ER tubule which fuses with the trailing tubule and forms a new three way junction. At 31 seconds the trailing ER tubule attaches to a putative plasma membrane contact site (yellow arrow) and remains anchored to it. Figure adapted from Sparkes et al. (2009C).

Figure 4. YUCCA4 alternative localisations and auxin synthesis

(A) Intron-exon structures for two splice variants with alternative 3´end processing of Yucca4. One of them, YUCCA4.2 has a hydrophobic C-terminal transmembrane domainderived from intron 3 sequence that is spliced out in YUCCA4.1. **(B)** YFP-YUCCA4.1 cytosolic localisation; insert shows labelling of nucleus but not the nuclear envelope which is typical for cytosolic fluorescent proteins. **(C)** YFP-YUCCA4.2 localises at the ER. Panels A, B and C are adapted from Kriechbaumer et al. (2012) **(D)** The two alternatively localised enzymes were over-expressed in tobacco epidermal leaves followed by total IAA extraction and analysis by thin layer chromatography (TLC). Over-expression of the membrane-anchored YUCCA4.2 in results in increased level of total IAA compared to over-expression of the cytosolic YUCCA4.1. Percentages are normalised to YUCCA4.2. Empty vector control (-) and an IAA standard are shown (unpublished data, Kriechbaumer et al.).