How and why does the endoplasmic reticulum move?

Becky Bola and Viki Allan

Faculty of Life Sciences, University of Manchester, The Michael Smith Building, Oxford Road, Manchester M13 9PT, U.K.

Abstract
The ER (endoplasmic reticulum) is a fascinating organelle that is highly dynamic, undergoing constant movement and reorganization. It has many key roles, including protein synthesis, folding and trafficking, calcium homoeostasis and lipid synthesis. It can expand in size when needed, and the balance between tubular and lamellar regions can be altered. The distribution and organization of the ER depends on both motile and static interactions with microtubules and the actin cytoskeleton. In the present paper, we review how the ER moves, and consider why this movement may be important for ER and cellular function.

Introduction
The ER (endoplasmic reticulum) is an extended network of lamellar membrane sheets and reticular tubules that spreads throughout the cell and is continuous with the nuclear envelope. In some cells, it is clearly divided into two types, rough ER and smooth ER, which are distinguished by the presence or absence of ribosomes on their surface (reviewed in [1]). Proteins destined for secretion, or that function within the organelles of the secretory and endocytic pathway, are synthesized at the rough ER. The lumen of the ER is much more oxidizing than the cytoplasm, which allows the formation of disulfide bonds necessary for proper protein folding. Newly synthesized proteins can then be transported from the ER to the Golgi apparatus, or be retained in the ER. The ER is thus a site for lipid and sterol synthesis, as well as being a major intracellular calcium store [1]. Last, but not least, the ER is a highly dynamic organelle that is continually moving and changing shape.

How is the ER moved?
In higher eukaryotes, the ER is moved to and maintained in its typical extended reticular network by the actions of microtubule motor proteins, and by direct linkage to microtubules [2–5] (Figure 1). Although myosin V may also play a part in short-range movement of ER in animal cells [1], actin-based motility is the primary means of moving the ER in plants [6]. In Saccharomyces cerevisiae, ER associates with the cell cortex via actin and myosin V, and also interacts statically with astral microtubules, which carry ER tubules into the growing bud [7].

Observation of fluorescently labelled ER in living cultured animal cells has revealed that most ER movement is driven by sliding of ER tubules along microtubules from the cell centre towards the periphery (e.g. [3,8]). It has long been believed that the microtubule motor protein kinesin-1 drives this movement, since it moves along microtubules towards their dynamic plus ends, transporting cargoes outwards [9] (Figure 1). Indeed, antisense oligonucleotides to kinesin-1 reduced extension of the ER into the cell periphery in astrocytes [10] and inhibited ER motility in dendrites [11].

Abbreviations used:
- actin, dynein, endoplasmic reticulum, kinesin, microtubule, migration.

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More recently, we have shown that outward ER tubule movement in Vero cells is inhibited by dominant-negative constructs that block kinesin-1 function [4]. Similar reagents also inhibit the extension of ER tubules in vitro [12], as does a function-blocking anti-kinesin-1 antibody [13]. Surprisingly, however, kinesin heavy chain knockout had no effect on ER position within mouse embryo fibroblasts [14]. Kinesin-1 association with the nuclear envelope is also needed for positioning the nucleus in polarized epithelial cells [15].

The fact that ER organization is normal in kinesin-knockout cells suggests that there must be another way to extend ER tubules into the cell periphery. Intriguingly, this occurs by the interaction between ER tubules and growing microtubule plus ends, via TACs (tip attachment complexes) (Figure 1) [3]. TACs are generated by the interaction of the microtubule plus end-binding protein EB1 and the ER-resident protein STIM1 (stromal interacting molecule 1) [2].

STIM1 plays an important role in cell calcium homoeostasis, as discussed below. Static interactions between ER tubules and microtubules, mediated by proteins such as CLIMP63 (63 kDa cytoskeleton-linking protein) [5,16,17] also play a part in stabilizing the extended ER network in vertebrate cells. TACs and kinesin-1 move the ER towards the cell periphery, but is there a balancing mechanism that carries ER tubules inwards? The ER slowly retracts back to the cell centre when microtubules are depolymerized in cultured amphibian cells [18], by association of ER tubules with inwardly moving actin filaments [3]. This mechanism is not prominent in mammalian cells, however [4]. Instead, in Vero cells, half the microtubule-dependent rapid ER tubule movements are directed towards the cell centre, driven by the minus
Figure 1 | Mechanisms of microtubule-based ER movement

Attachments can be static, as in the case of CLIMP63, or ER tubules can move along microtubules via the microtubule motors dynein and kinesin-1. Alternatively, ER tubules can be extended by attaching to growing microtubule tips (indicated by +), mediated by the interaction between the ER protein STIM1 and the plus end-binding protein EB1.

Does ER movement contribute to ER morphology and function?

An intriguing aspect of ER morphology is that tubular-reticular domains and lamellar regions often exist in the same cell, and are in direct continuity. The balance between the two states can be altered, since lamellar regions expand greatly when high levels of protein synthesis are required [1,25]. In keeping with this idea, removal of ribosomes by puromycin treatment increases the proportion of tubular ER, and a similar transformation is also seen during mitosis, when secretion is inhibited [26]. The formation of ER tubules is driven by the presence of reticulons and DP1/Yop1p [27], whereas tubule fusion to generate the reticular network is controlled by p97–p47 proteins [5]. Both of these processes are active in the absence of microtubules in vitro [28], suggesting that motility and/or anchoring is not important for determining ER morphology. This is not the case in vitro, however, since inhibiting either kinesin-1 or dynein activity leads to a pronounced increase in lamellar ER [4]. Whether this reflects a link between motility and reticulon function remains to be determined, but the idea that the tension generated by motors pulling on ER membranes facilitates the action of reticulons is an attractive one.

If loss of motility promotes lamellar ER accumulation, and lamellar ER is linked with high levels of protein synthesis, is there any link between ER motility and the protein synthetic and processing activity of the ER? This is not yet clear, but loss of microtubules or knockdown of CLIMP63 leads to an increase in the lateral mobility of translocon complexes [29]. Motor activity may also influence the efficiency of exit of newly synthesized material from the ER, since ER exit sites undergo kinesin-1- and dynein-dependent movement, and inhibition of kinesin-1 slows cargo exit [30].

Striking alterations in both ER morphology and motility can occur as cells progress through the cell cycle, although the details vary between cell types and organisms. Some of these changes are actin-dependent, such as the localization of a layer of ER under the plasma membrane in HeLa cells [31], and the formation of cortical ER clusters in mouse oocytes during meiosis I [21]. Similar aggregates form in mitotic Caenorhabditis elegans early embryos in a process regulated by Rab5 [32]. Furthermore, myosin V-driven ER movement is activated in metaphase Xenopus egg extracts compared with interphase extracts [33].

Cell cycle effects on microtubule-based ER movement are also very cell-type-dependent. For example, during meiosis in mouse oocytes, the ER clusters in the spindle during meiosis I via the activity of dynein [21]. Spindle pole accumulation of ER is also seen in dividing Drosophila and sea urchin early embryos [34,35], although a role for dynein has not yet been demonstrated. In cultured vertebrate cells, however, enrichment at spindle poles is not seen [26,31,36]. This fits with the observation that, whereas dynein-dependent ER tubule extension is active in interphase Xenopus egg extracts, it is inhibited in metaphase-arrested extracts owing to the release of the motor from the ER membrane following phosphorylation by Cdk1 [22,37]. However, ER-associated dynein is active during prophase, as it facilitates the rupture of the nuclear envelope [38]. Other ER-microtubule interactions are also under cell cycle control, since mitotic phosphorylation of CLIMP63 inhibits its binding to microtubules [17], whereas kinesin-1-driven ER movement in vitro is inhibited by metaphase cytosol (K. Brownhill and V. Allan, unpublished work).

One intriguing question is why the ER is spread throughout the cell during interphase when the next organelle on the secretory pathway is in the cell centre? Furthermore, why is it clustered at spindle poles in some early embryos? Perhaps
these distributions are important for the correct spatial regulation of calcium concentration within the cell, since the ER is a major calcium store. Some regions of the ER may be specialized for calcium control, such as vesicular ER domains in dendrites that are transported by kinesin-1 [11]. Direct associations between mitochondria and ER are involved in calcium homeostasis and lipid metabolism [1], and these interactions are dependent on a number of proteins, including mitofusin 2 [39], the inositol 1,4,5-trisphosphate receptor [40], and PACS-2 (phosphofurin acidic cluster sorting protein 2) [41]. Mitochondria–ER interactions certainly play an important part in generating ER morphology, because the ER fragments when the interactions are disrupted [39]. Strikingly, when calcium levels are raised, both the ER [42] and mitochondria [42,43] are less dynamic. Calcium is known to inhibit kinesin-1 function on mitochondria by promoting the binding of Miro to the motor domain [44], but it is unclear whether this mechanism regulates the movement of mitochondrial-associated ER, and whether this occurs in non-neuronal cells. It will also be important to determine whether altering calcium levels affects dynein- and kinesin-1-driven ER tubule extension.

Alterations in cytosolic calcium levels can also affect ER structure. When calcium levels increase, the ER can become fragmented (e.g. in Xenopus eggs following fertilization [45]). Following depletion of intracellular calcium, direct contacts form between the plasma membrane and the ER, mediated by STIM1 in the ER and the calcium-activated calcium channel component Orai1 in the plasma membrane, which then triggers flow of calcium from the external medium \(I_{\text{soc}}\) (store-operated calcium current) [46]. There are clear links between this ER function and microtubules, since depolymerization of microtubules or inhibition of kinesin or dynein reduces the \(I_{\text{soc}}\) [47,48]. Interestingly, although STIM1 links ER tubules to growing microtubules, this function (i.e. TAC formation) is not needed for generating \(I_{\text{soc}}\) [2]: this suggests that the two roles of STIM1 are unrelated.

**ER motility in cell migration**

Regulated cell migration is pivotal to survival. In order for cells to migrate in a fibroblast-like fashion, they must be able to bind to, and be released from, the ECM (extracellular matrix). Interactions between the cell and ECM are primarily mediated by integrins. These transmembrane receptors bind the ECM via their extracellular domains, while their cytoplasmic domains contact the actin cytoskeleton [49–51]. Cellular adhesion to the ECM proceeds via a series of spatially and temporally defined molecular steps. The primary attachment may be mediated by cell-associated proteoglycans. Subsequently, focal complexes form, and these mature first into focal adhesions and finally into fibrillar adhesions [51]. These adhesive complexes interact with a wide variety of both structural and signalling molecules within the cell.

Multiple lines of evidence suggest that movement of the ER is involved in cell migration. Several ER-resident proteins such as kinectin, calreticulin and PTP1B (protein tyrosine phosphatase 1B) have been shown to interact with or influence focal adhesion components [52–57]. Moreover, blocking kinesin-1 function inhibits focal adhesion disassembly [58,59]. As the ER is transported by kinesin-1, then this mechanism could effectively deliver several important factors at once.

A number of studies surrounding the ER-localized PTP1B support this theory. Not only does PTP1B co-localize with focal adhesions, but also, when the ER targeting sequence of PTP1B is deleted, then focal adhesions mature more slowly or not at all, and are less stable [53]. Moreover, expressing a catalytically inactive form of PTP1B impairs cell spreading and adhesion to fibronectin, as well as the formation of actin stress fibres and focal adhesions [52]. PTP1B co-immunoprecipitates with \(\beta1\) integrin and co-localizes with the focal adhesion component vinculin at the ends of actin stress fibres, suggesting that it forms a part of an integrin-containing complex [52]. PTP1B plays a key role in the mediation of signalling downstream of focal adhesions by relieving the inhibition of Src kinase caused by phosphorylation of Tyr527 [54], and by influencing the activity of FAK (focal adhesion kinase) [52]. Following integrin engagement, FAK and Src then phosphorylate multiple substrates, leading to the subsequent activation of signalling pathways such as the extracellular signal-regulated kinase 1 and 2 cascades [54,60,61]. These data would suggest that ER-localized PTP1B is a key regulator of integrin-based signalling pathways and plays an important role in focal adhesion formation.

A further link between kinesin-1, the ER and cell–ECM adhesion is that kinectin may interact with kinesin-1, integrins and RhoGTPases. Kinectin is a heterodimeric integral transmembrane protein that is largely localized to the ER, and which acts as a membrane anchor for both kinesin-1 and translation elongation factor 18 on the ER [62,63]. Kinectin is thought to activate kinesin-1 by triggering its unfolding into an active conformational state, enhancing its microtubule-stimulated ATPase activity [63]. Kinectin also appears to influence the actin cytoskeleton via RhoG, which is a member of the Rho family of GTPases whose inhibition hinders cell spreading and migration [64,65]. Kinectin binds RhoGTP, and this interaction may trigger kinesin-1-mediated membrane transport [66]. However, kinectin also binds the GTP-bound forms of Rac1, Cdc42 and RhoA [67,68], and activated RhoG may itself activate Rac1- and Cdc42-controlled pathways to trigger the formation of lamellipodia and filopodia [64,66]. The morphogenic action of RhoG on the cytoskeleton seems to be distinct from that of Rac1 and Cdc42, however, in that it is dependent on kinectin and kinesin-1 [66], and requires an intact microtubule network [64].

Kinectin may be linked directly to adhesion, as when beads coated with fibronectin were added to cells to induce the formation of integrin-based adhesion complexes, 57% of cellular kinectin was recruited to the bead-bound complexes [56]. Also observed, but to a lesser extent, was a co-accumulation of other ER-resident proteins such as calreticulin, possibly indicating that the ER comes into contact with adhesion complexes. This led to the hypothesis that the function of kinectin clustering might be to help direct...
newly synthesized proteins to sites of integrin clustering [56]. However, this is unlikely, given that membrane and secreted proteins would first need to traffic through the Golgi, which is located in the cell centre. However, this link between a putative kinesin-1 receptor localized to ER membranes and focal adhesion components is intriguing, and together with the effects of PTP1B on adhesion formation and dynamics, support the hypothesis that direct contact between ER tubules and focal adhesions plays a role in cell migration.

Concluding remarks

Although we know a fair amount about how movement of the ER is generated at the molecular level, there is much still to be learned about why this motility is important. Moreover, is it needed for all ER functions, or only a subset? The links between movement and ER morphology are also poorly understood. Another key topic for the future is how the interaction between the ER and other organelles affects ER dynamics. We need answers to these questions if we are truly to understand the function of the ER.

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