Dynamics and inheritance of the endoplasmic reticulum

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Summary

The endoplasmic reticulum (ER) consists of a polygonal array of interconnected tubules and sheets that spreads throughout the eukaryotic cell and is contiguous with the nuclear envelope. This elaborate structure is created and maintained by a constant remodeling process that involves the formation of new tubules, their cytoskeletal transport and homotypic fusion. Since the ER is a large, single-copy organelle, it must be actively segregated into daughter cells during cell division. Recent analysis in budding yeast indicates that ER inheritance involves the polarized transport of cytoplasmic ER tubules into newly formed buds along actin cables by a type V myosin. The tubules then become anchored to a site at the bud tip and this requires the Sec3p subunit of the exocyst complex. The ER is then propagated along the cortex of the bud to yield a cortical ER structure similar to that of the mother cell. In animal cells, the ER moves predominantly along microtubules, whereas actin fibers serve a complementary role. It is not yet clear to what extent the other components controlling ER distribution in yeast might be conserved in animal cells.

Key words: Endoplasmic reticulum, Microtubule, Motility, Inheritance, Segregation, Nuclear envelope, Actin

Introduction

The endoplasmic reticulum (ER) is arguably the most complex, multifunctional organelle of eukaryotic cells. Proteins are translocated across the ER membrane, and are folded and modified before they traverse the secretory pathway. The ER also plays a central role in other important processes, including Ca^{2+} sequestration, signaling and lipid synthesis. It is a complex structure that can have an extremely large surface area (Voeltz et al., 2002). It is composed of membrane sheets that enclose the nucleus (the nuclear envelope) and an elaborate interconnected network in the cytosol (the peripheral ER) (Fig. 1). During interphase, the peripheral ER in somatic cells consists of a polygonal network of interconnected tubules and cisternae. This network appears to emanate from the outer leaflet of the nuclear envelope and spread throughout the cytosol. The fine structure, spatial distribution and abundance of the peripheral ER vary among cell types, reflecting specialized functional requirements. In spite of its continuous appearance, the ER is organized into functionally and morphologically distinct domains. The rough ER, so named for the studded appearance of ribosomes bound to its surface, is involved in the synthesis of secretory and membrane proteins. The smooth ER is ribosome free, and the transitional ER is where carrier vesicles are formed (Baumann and Walz, 2001).

In many plants and some animal cells, the peripheral ER extends towards the inner surface of the plasma membrane and links to a reticular network underlying the plasma membrane (Staehelin, 1997). The majority of the peripheral ER in yeast cells is a cortical network whose arrangement is similar to that in higher eukaryotic cells (Pidoux and Armstrong, 1993; Prinz et al., 2000) (Fig. 2). Only a few individual linear ER tubules traverse the yeast cytosol to connect the cortical ER to the nuclear envelope.

The ER is an essential organelle that cannot be synthesized de novo and must therefore be partitioned into daughter cells prior to cell division. It is important to identify the molecular components that control ER dynamics and formation and to define the mechanisms that ensure its faithful inheritance in every cell cycle. Here, we discuss recent progress in these areas.

Molecular tracks and motors for ER movement

The peripheral ER undergoes constant rearrangement of its fine structure in virtually all cell types. This is believed to be crucial to maintain its characteristic structure. Linear extension of tubules that branch from pre-existing tubules and the sliding of one tubule along another are the principal mechanisms controlling the formation and elimination of polygonal ER structures in both live cells and cell-free systems (Dabora and Sheetz, 1988; Lee and Chen, 1988; Prinz et al., 2000). Single ER tubules extend into areas of cellular expansion, such as growth cones of neurons and leading edges of migrating adherent cells, prior to establishing a reticular ER network (Dailey and Bridgman, 1989; Waterman-Storer and Salmon, 1998). Retraction of ER tubules towards the cell center also occurs in animal cells (Dailey and Bridgman, 1989; Terasaki and Reese, 1994; Waterman-Storer and Salmon, 1998). The outward and inward movements are thought to generate a balance of opposing forces that control the distribution of the peripheral ER (Terasaki and Reese, 1994).

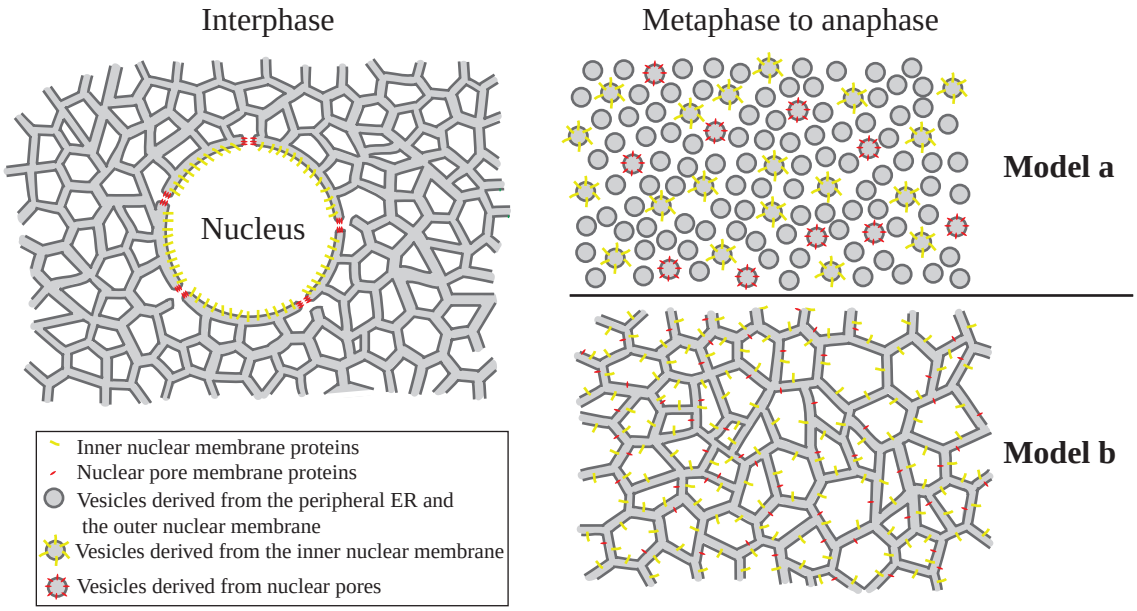

Fig. 1. Models of ER segregation in proliferating animal cells. In interphase (left panel), the peripheral ER forms an interconnected network that is contiguous with the outer membrane of the nuclear envelope. The outer nuclear membrane is biochemically similar to the peripheral ER membrane. By contrast, some integral membrane proteins are localized specifically to the inner nuclear membrane (yellow ovals) or to nuclear pores where the outer and inner nuclear membranes meet (red ovals). In one model of ER segregation (model a), the peripheral ER and the nuclear envelope undergo progressive vesiculation. By metaphase, the cell contains vesicles derived from the peripheral ER or outer nuclear membrane, and from the inner nuclear membrane or nuclear pore domains. Diffusion of these vesicles during metaphase and anaphase ensures the equal partition of ER elements. Another model (model b) predicts that the peripheral ER retains its integrity during mitosis. The nuclear envelope is absorbed into the peripheral ER. The inner nuclear membrane proteins and the nuclear pore integral membrane proteins are distributed throughout the peripheral ER network at early metaphase. Equal segregation of the ER network ensures the faithful partition of components of various ER domains.

Microtubules, kinesin and dynein in ER movement

Numerous studies implicate microtubules as tracks for ER extension and ER motility in interphase animal cells. There is generally a good correlation between the distribution of peripheral ER and microtubules in various animal cells (Terasaki et al., 1986). ER tubules and microtubules closely align over considerable distances and single-point attachment sites are evident at the light microscopic and ultrastructural levels (Baumann and Waltz, 2001). Video microscopy studies have revealed extension of ER tubules along microtubules during ER expansion in live cells and during reconstitution of the ER network from microsomes in cell-free systems (Terasaki et al., 1986; Dabora and Sheetz, 1988; Lee et al., 1989; Allan and Vale, 1991; Allan, 1995; Waterman-Storer et al., 1995; Waterman-Storer and Salmon, 1998). Interestingly, microtubule-dependent motility exclusively controls the rapid extension of ER tubules out to the cell periphery, whereas a slower movement of ER tubules towards the cell center is independent of microtubules (Terasaki and Reese, 1994; Waterman-Storer and Salmon, 1998). Indeed, prolonged disruption of microtubules in many animal cells causes a gradual collapse of the peripheral ER into large cytoplasmic patches or membrane aggregates that surround the nucleus (Louvard et al., 1982; Vogl et al., 1983; Terasaki et al., 1986). Microtubule-based local movements of ER tubules have also been shown to re-organize the fine structure of the ER network assembled in vitro (Dabora and Sheetz, 1988; Allan and Vale, 1991). In the fungus *Ustilago maydis*, microtubule-dependent motility is not required for ER distribution and maintenance but is required for reconstruction of the cortical ER network during recovery from disruption of the cell wall (Wedlich-Soldner et al., 2002).

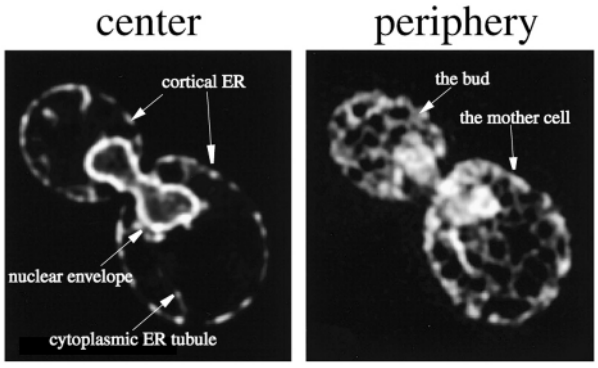

Fig. 2. The ER network in the budding yeast *S. cerevisiae*. Wild-type yeast cells expressing the ER membrane protein Sec63p fused to GFP were visualized by epifluorescence microscopy. The left panel reveals the appearance of cortical ER tubules and the nuclear envelope in the center of a large-budded cell. Cytoplasmic ER tubules that connect cortical ER and the nuclear envelope are readily seen at this focal plane. Focusing on the periphery of the cell (right panel) allows the visualization of the cortical network of interconnected tubules. Figure reproduced with permission from The Rockefeller University Press (Prinz et al., 2000). Both cortical ER and the nuclear envelope remain intact during mitosis.

Microtubule-dependent ER movement results from the activity of microtubule-associated motors as well as the polymerization of microtubules (Dabora and Sheetz, 1988; Vale and Hotani, 1988; Dailey and Bridgeman, 1989; Waterman-Storer et al., 1995; Waterman-Storer and Salmon, 1998; Lane and Allan, 1999). Motors can either drag membranes along underlying microtubules or drive the sliding of membrane-associated microtubules, whereas microtubule polymerization can promote the movement of an ER tubule bound to the dynamic microtubule tip (Waterman-Storer and Salmon, 1998). Both plus-end-directed motors of the kinesin family and the minus-end-directed dynein motor have been implicated in microtubule-based membrane movements (Lane and Allan, 1998).

The extension of ER tubules towards the fast-growing plus ends of microtubules in many differentiated animal cells implicates kinesin family proteins (Terasaki and Reese, 1994; Waterman-Storer and Salmon, 1998). Depletion of conventional kinesin by antisense oligonucleotides causes the retraction of the ER from the cell periphery in astrocytes (Feiguin et al., 1994). In some cells, conventional kinesin has been localized to the ER (Houliston and Elinson, 1991; Henson et al., 1992). Moreover, kinesin also associates with kinectin, an integral ER membrane protein (Toyoshima et al., 1992).

By contrast, ER tubules in *Xenopus* egg extracts move exclusively towards the minus ends of microtubules, driven by the activity of membrane-associated cytoplasmic dynein (Allan and Vale, 1991; Allan, 1995; Waterman-Storer et al., 1995; Niclas et al., 1996; Steffen et al., 1997). Cytoplasmic dynein, but not conventional kinesin, is required for cortical ER dynamics and reformation in the fungus *U. maydis* (Wedlich-Soldner et al., 2002). However, a combination of both dynein- and kinesin-dependent bidirectional movements of ER tubules along microtubules has been observed when membranes from *Xenopus* eggs are incubated with *Xenopus* tissue culture cell (XTC) cytosol extracts (Lane and Allan, 1999). The level of membrane-associated kinesin in egg microsomes is comparable with that in somatic cell microsomes. Pre-incubation of egg membranes with an antibody directed against kinesin heavy chain, thereby blocking motor activity, inhibits plus-end-directed ER tubule movements. Membrane-associated kinesin might thus become activated during development. How specialized cell types choose different motor complexes to drive motion of the peripheral ER is unknown.

Actin and myosin V in ER movement

In contrast to animal cells, plant and budding yeast cells use actin as tracks for cortical ER. Ultrastructural studies of plant cells have revealed the close association of ER tubules and actin bundles within the cortical cytoplasm (Goosen-de Roo et al., 1983; Lichtscheidl et al., 1990; White et al., 1994), and video microscopy of cytoplasmic streaming has shown sliding of the ER network along stationary actin cables in lower-plant characean algae cells (Kachar and Reese, 1988). Even though most cortical ER tubules do not co-align with cortical actin filaments in yeast, the disruption of actin assembly results in a rapid and dramatic decrease in cortical ER dynamics (Prinz et al., 2000).

Although microtubules are the major molecular tracks for the ER in animal cells, peripheral ER tubules do move along actin filaments in regions devoid of microtubules. Smooth ER moves on actin cables from the main cell body to microvilli in locust photoreceptor cells (Stürmer et al., 1995). The absence of smooth ER in the dendritic spine of Purkinje cells from dilute-lethal mice lacking the actin-based motor myosin Va indicates that actin is required for transporting ER from the dendritic shaft to the dendritic spine (Takagishi et al., 1996; Bridgman, 1999). The photoreceptor microvilli region and the Purkinje dendritic spines contain abundant actin filaments but lack microtubules (Landis and Reese, 1983; Hirokawa, 1989; Stürmer et al., 1995). Even in animal cells in which microtubules are known to control outward ER extension, the transport of ER tubules towards the cell center is perturbed when actin assembly or myosin motor function is disrupted (Terasaki and Reese, 1994; Waterman-Storer and Salmon, 1998). However, no dramatic effect on the overall morphology of the peripheral ER is evident in these cells. Formation of the ER network in metaphase *Xenopus* egg extracts requires actin polymerization and myosin V activity when microtubule-dependent ER movement is blocked (Wöllert et al., 2002).

Components of the ER fusion machinery

The homotypic fusion of ER membranes is key for the formation and maintenance of the ER. The fusion of intersecting tubules contributes to the formation of new polygons, whereas the fusion of two junctional tubules results in the net loss of a polygon within the peripheral ER network. The nuclear envelope of most higher eukaryotic cells breaks down during prometaphase, and homotypic membrane fusion is required to reassemble daughter nuclei at the end of mitosis. Analysis of these fusion reactions suggests that the mechanism is similar to that of other membrane fusion reactions; hence, we only briefly summarize key findings here.

Fusion of both peripheral-ER-derived and nuclear-envelope-derived microsomes is sensitive to N-ethylmaleimide (NEM) and nonhydrolyzable ATP analogs (Dabora and Sheetz, 1988; Allan and Vale, 1991; Newport and Dunphy, 1992; Lavoie et al., 1996; Drier and Rapoport, 2000). As in the case of other membrane fusion reactions, this sensitivity reflects the requirement for a type II AAA-family ATPase. Whereas most other fusion events require NEM-sensitive factor (NSF), fusion of ER membranes requires the related ATPase p97 or its yeast homolog Cdc48p (Latterich et al., 1995; Roy et al., 2000; Hetzer et al., 2001). The soluble NSF attachment receptors (SNAREs) Ufe1p and syntaxin 5 have been implicated in Cdc48p/p97-regulated ER membrane fusion in vitro (Patel et al., 1998; Roy et al., 2000). Ufe1p also functions in Golgi-to-ER trafficking, and syntaxin 5 is required for ER-to-Golgi transport (Lewis and Pelham, 1996; Rowe et al., 1998). It remains unclear whether or not Cdc48p/p97, like NSF, functions as a molecular chaperone to disassemble SNARE complexes after membrane fusion.

Fusion of ER-enriched microsomes, like most other fusion reactions, is also sensitive to nonhydrolyzable GTP analogs (Allan and Vale, 1991; Boman et al., 1992; Newport and Dunphy, 1992; Lavoie et al., 1996; Macaulay and Forbes, 1996; Dreier and Rapport, 2000). Investigation of the molecular mechanism underlying the requirement for GTP hydrolysis has shown that Ran, a GTPase of the Ras superfamily, plays a central role in membrane fusion during nuclear envelope

assembly (Demeter et al., 1995; Hetzer et al., 2000; Zhang and Clarke, 2000; Zhang and Clarke, 2001; Askjaer et al., 2002). The identity of the GTPase needed for the fusion of peripheral ER membranes has not yet been established.

Partitioning of the ER during the cell cycle

ER segregation during mitosis in animal cells

A hallmark of mitosis in most animal cells is the breakdown of the nuclear envelope during mitotic prometaphase. Electron microscopy indicates that the nuclear envelope undergoes progressive and complete fragmentation. In some cell types (rat thyroid epithelial cells, HeLa cells), most of the peripheral ER similarly disassembles to form small vesicles that scatter throughout the cytoplasm (Robbins and Gonatas, 1964; Zeligs and Wollman, 1979). Mitotic *Xenopus* egg extracts can induce the disassembly of purified nuclear envelopes from interphase animal cells (Lohka and Maller, 1985; Newport and Spann, 1987). Moreover, egg or embryo extracts from *Xenopus*, sea urchin and *Drosophila* contain nuclear-membrane-derived and ER-derived vesicles that form functional nuclear envelopes in the presence of purified chromatin or an elaborate ER network of interconnected tubules in the absence of chromatin (Drier and Rapoport, 2000; Collas and Courvalin, 2000; Paiemant and Bergeron, 2001). These observations led to a vesiculation model for ER inheritance in which the nuclear envelope and the peripheral ER of animal cells both fragment to form numerous discrete vesicles during mitosis to facilitate partitioning of the ER between daughter cells (Fig. 1). In this model, the peripheral ER network and the nuclear envelope reassemble largely by fusion reactions at the end of mitosis.

Recent studies have provided evidence for an alternative model of ER inheritance. First, the peripheral ER network in most animal somatic cells appears to retain its integrity during mitosis and shows no significant fragmentation (Zeligs and Wollman, 1979; Waterman-Storer et al., 1993; Ioshii et al., 1995; Ellenberg et al., 1997; Terasaki, 2000; Axelsson and Warren, 2004; Pecot and Malhotra, 2004). Second, integral membrane proteins that are concentrated in the inner nuclear membrane or in the nuclear pore region become uniformly distributed throughout the peripheral ER when the envelope breaks down. These proteins re-concentrate around chromosomes when the envelope reassembles (Ellenberg et al., 1997; Yang et al., 1997). Furthermore, photobleaching experiments have revealed that a green fluorescent protein (GFP)-tagged integral membrane protein, which is essentially immobile in the interphase nuclear membrane, becomes highly mobile throughout the peripheral ER network during mitosis (Ellenberg et al., 1997). These findings prompted the proposal that the entire nuclear envelope is absorbed into the peripheral ER network during mitosis and its components are partitioned together with peripheral ER membranes (Fig. 1). The accumulation of peripheral ER at the mitotic poles in embryonic systems indicates that microtubules and microtubule motors are likely to be involved (Henson et al., 1989; Terasaki, 2000; Bobinnec et al., 2003). However, the molecular details of the process remain largely unknown.

ER segregation in yeast

The nuclear envelope in yeast cells remains intact during mitosis (Fig. 2). In addition, the cortical ER in both budding and fission yeast maintains its continuous and reticular appearance throughout the cell cycle (Preuss et al., 1991; Pidoux and Armstrong, 1993; Prinz et al., 2000; Du et al., 2001). The nuclear envelope in budding yeast is partitioned by an ordered, multi-stage process involving migration into the mother-bud neck and extension towards the ends of the mother and daughter cells (Shaw et al., 1998). Microtubule-dependent motor transport is required for migration and extension of the nuclear envelope (Huffaker et al., 1988; Jacobs et al., 1988; Shaw et al., 1997).

The cortical ER in budding yeast is also inherited in an ordered fashion. The bud acquires ER tubules along its cortex during early S phase, after secretory vesicles appear in the bud, yet well before migration of the nucleus into the neck (Preuss et al., 1991; Du et al., 2001). Prolonged disruption of microtubules does not have any detectable effects on the inheritance or dynamics of the cortical ER (Prinz et al., 2000; Du et al., 2001). Thus, in spite of the tubular connections between the cortical ER and the nuclear envelope (Preuss et al., 1991; Koning et al., 1996; Prinz et al., 2000), partitioning of these two structures differs – at least with regards to their dependence on microtubules. Strikingly, both immunoelectron microscopy and GFP fluorescence microscopy have revealed that cytoplasmic tubules are the first ER elements to be present in newly developed buds (Preuss et al., 1991; Du et al., 2001). These ER tubules are typically oriented along the mother-bud axis. Tubules are also readily observed in larger buds that already contain cortical ER. Time-lapse video microscopy has revealed bud-directed extensions of these tubules and their subsequent propagation to the cortex of growing buds (Wiederkehr et al., 2003; Estrada et al., 2003). This indicates that cortical ER components are partitioned into the bud in the form of linear tubules. Interestingly, the apical tip of the bud appears to act as a docking site for these tubules. ER tubules generally appear to be directed to the bud tip before they spread along the periphery of the bud (Wiederkehr et al., 2003). For a tubule that has reached the bud tip, the range of undulations at a given point is inversely proportional to its distance from the bud tip, suggesting a stable association at the bud tip (Fehrenbacher et al., 2002). Such attachment might facilitate retention of ER membranes within the bud and/or enable entire tubules to be pulled into the bud as it grows. These observations suggest that cortical ER inheritance is a multi-stage process involving the extension of ER tubules along the axis, tubule anchorage at the bud tip, uniform distribution along the bud cortex, and formation of a polygonal cortical network (Fig. 3).

The ongoing formation of a cortical, reticular network in the bud derived from long cytoplasmic ER tubules might explain why the cortical ER network in the bud appears to be more dynamic than in the mother cell (Prinz et al., 2000). Notably, the peripheral ER in animal cells recovering from microtubule disruption is reconstructed by a similar process that involves extension of linear tubules over a significant distance and requiring subsequent reticulation (Lee et al., 1989). In addition, linear tubule extension appears to be the major mechanism driving peripheral ER expansion into animal cell axoplasm (Dailey and Bridgman, 1989; Waterman-Storer and Salmon, 1998).

The linear nature of bud-directed extension of ER tubules suggests that cytoskeletal tracks are involved. Since

microtubule function is not required for ER segregation, actin is the most likely candidate. Actin cables are required for the bud-directed delivery of several yeast membrane compartments, including secretory vesicles, mitochondrial tubules, vacuole segregation structures and the late Golgi (Govindan et al., 1995; Hill et al., 1996; Simon et al., 1997; Rossanese et al., 2001). Disruption of actin polymerization significantly reduces cortical ER dynamics in daughter cells (Prinz et al., 2000). Furthermore, cytoplasmic ER tubules oriented along the major axis become immobilized upon actin depolymerization, which indicates that actin is required for the transport of ER tubules into the bud (Estrada et al., 2003). Nevertheless, actin is dispensable for the maintenance of cortical ER structures, which suggests that, once formed, the ER can be maintained by an actin-independent mechanism (Prinz et al., 2000). This is consistent with the observation that most cortical ER tubules do not colocalize with cortical actin cables. It remains to be determined whether the ER structures actively segregating into the bud are among the small fraction of ER tubules that do colocalize with actin cables.

Genetic analysis of cortical ER inheritance

Several genetic screens have identified potential components of the ER inheritance machinery (Prinz et al., 2000; Du et al., 2001; Estrada et al., 2003). The first screen found that the cortical ER structure is disrupted in several temperature-sensitive mutants exhibiting defective membrane traffic between the ER and Golgi (*sec27-95*, *sec21-1*, *ret1-3*, *sec23-1* and *ufe1-1*) (Prinz et al., 2000). Following a shift to the restrictive temperature, the ER is transformed from interconnected tubules into sheets at the cell periphery in these mutants. The same screen also found that several mutants exhibiting defective targeting of ribosome-nascent polypeptide chains to the ER membrane (*srp101-47*, *srp102-510* and *sec65-1*) accumulate sheets of ER in the cytosol. Interestingly, both groups of mutants display pronounced defects in ER inheritance at the restrictive temperature. The delay in ER inheritance might be caused by defects in the formation and/or stabilization of segregating ER tubules. Alternatively, the large ER sheets that form in these mutants may not be efficiently transported.

A second screen revealed that defects in Aux1p/Swa2p cause a dramatic delay in cortical ER inheritance (Du et al., 2001). Aux1p/Swa2p was previously implicated as a J-domain-containing co-chaperone that stimulates the activity of the chaperone Hsc70 in the uncoating of clathrin-coated vesicles (Gall et al., 2000; Pishvaee et al., 2000). However, several lines of evidence suggest that the function of Aux1p/Swa2p in ER inheritance is uncoupled from its role in clathrin-mediated membrane transport (Du et al., 2001). First, deletion of the J-domain causes defects in clathrin uncoating but not in cortical ER inheritance. Second, loss of function of either Chc1p (the clathrin heavy chain) or Arf1p, two Aux1p/Swa2p-interacting proteins that are required for the formation of clathrin-coated vesicles, does not perturb cortical ER inheritance. Third, the localization of Aux1p/Swa2p to the ER in subcellular fractionation studies supports a direct role of this protein on the ER. Future studies on the execution point of Aux1p/Swa2p in ER inheritance and on the identification of its binding partners on the ER might provide clues to the function of this protein in ER inheritance.

A direct role for actin in cortical ER inheritance was revealed in a third genetic screen by the finding that Myo4p is required for the delivery of cortical ER into the daughter cells of budding yeast (Estrada et al., 2003). Myo4p is a type V myosin motor that is responsible for localization of ASH1 and IST2 mRNAs to the bud tip (Long et al., 1997; Takizawa et al., 2000). RNA cargo is linked to Myo4p by the RNA-binding protein She2p and the Myo4p-interacting protein She3p (Böhl et al., 2000; Long et al., 2000; Takizawa and Vale, 2000). Interestingly, She3p, but not She2p, is required for cortical ER inheritance (Estrada et al., 2003). Cells carrying a point mutation in the ATP-binding domain of Myo4p fail to segregate cortical ER into daughter cells. The motor activity of Myo4p must therefore be required for this process. Measurements of the rate of ER tip tubule movement have led to the conclusion that the Myo4p-She3p complex plays a role in forming and extending ER tubules into daughter cells along actin cables via an unidentified ER-associated adaptor. Myo4p and actin might also be required for the stabilization and orientation of linear ER tubules, as treatment of cells with the actin polymerization inhibitor latrunculin A or the loss of Myo4p results in a substantial reduction in the number of cytoplasmic ER tubules present in newly formed buds (Estrada et al., 2003).

The Sec3p protein is also required for cortical ER inheritance (Wiederkehr et al., 2003). Sec3p is a component of the exocyst, a conserved octameric complex required for tethering secretory vesicles to exocytic sites on the plasma membrane. Components of the exocyst are concentrated at sites of

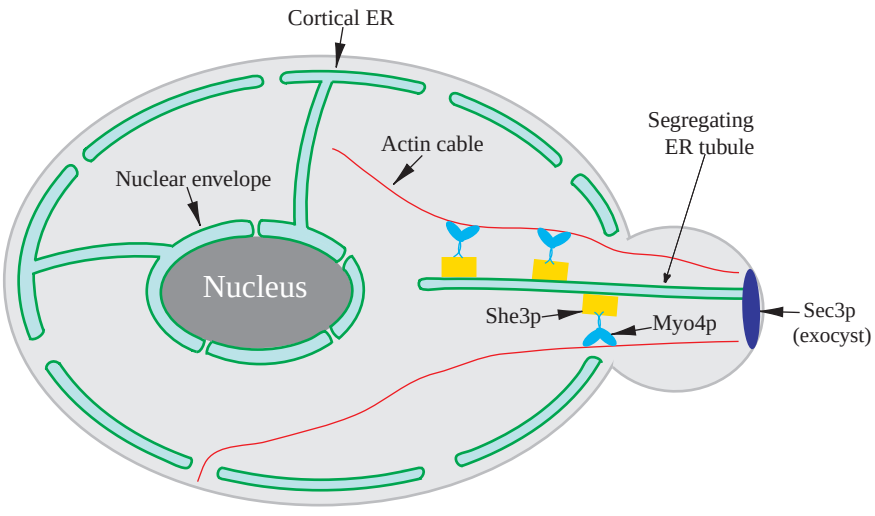

Fig. 3. A model for cortical ER inheritance in *S. cerevisiae*. Myo4p transports ER tubules into the daughter cell along actin cables. She3p functions as an adaptor protein that links Myo4p to the segregating ER tubule. Sec3p stabilizes the cortical association of ER tubules delivered into the bud. The ER tubules are propagated along the bud periphery and form an interconnected tubular network.

active membrane expansion in both yeast and mammalian cells (Guo et al., 2000). In budding yeast, these sites include the bud tip during early apical growth and the mother-bud neck during cytokinesis (Finger and Novick, 1998).

Sec3p is unique among the eight components of the exocyst in several respects. First, the polarized localization of Sec3p is independent of ongoing secretion, the actin cytoskeleton, and the function of the other subunits of the exocyst. This suggests that it acts as a spatial landmark for sites of secretion (Finger et al., 1998). Second, under certain growth conditions, *SEC3* is not essential for growth and secretion (Wiederkehr et al., 2003). Cells lacking Sec3p exhibit a rounder morphology and a broader distribution of exocytic markers, which indicates that they have defects in polarized secretion. Moreover, *sec3* mutants display normal ER-to-Golgi transport, yet have an aberrant distribution of ER (Finger and Novick, 1997). By using GFP-tagged ER-resident proteins to follow the distribution of the ER in live yeast cells, one can show that loss of Sec3p significantly delays cortical ER inheritance (Wiederkehr et al., 2003). Interestingly, even late in the cell cycle, a significant number of *sec3Δ* cells contain cytoplasmic tubules along the mother-bud axis as the only inherited ER elements. Time-lapse microscopy has further revealed that tubules continue to move across the neck but fail to be stably retained in *sec3Δ* mutant buds. These findings suggest Sec3p functions as an anchor for ER tubules that move to the daughter cell, which would be analogous to its role in tethering exocytic vesicles to sites of secretion.

A possible clue to the nature of the association between Sec3p and the ER came from independent studies demonstrating genetic and physical interactions between exocyst subunits and components of the Sec61 complex. The Sec61p complex is the core component of the protein translocation channel in ER membranes (Johnson and van Waes, 1999). Overexpression of different subunits of the Sec61 complex partially suppresses the temperature sensitivity of various exocyst mutants (Toikkanen et al., 1996; Toikkanen et al., 2003). Sec3p overexpression causes GFP-tagged Sec61p to accumulate at the bud tip without effecting localization of another ER marker or ER membranes at this site (Wiederkehr et al., 2003). In addition, exocyst components and the β-subunit of the Sec61 complex co-precipitate both in yeast cell lysates and mammalian cell extracts (Toikkanen et al., 2003; Lipschutz et al., 2003). Whether the apparent interaction between the exocyst and translocon contributes to ER tubule retention in the bud cortex has yet to be addressed.

Concluding remarks

At a superficial level, the structure and distribution of the ER appears to vary considerably among different organisms and cell types. However, in all cells, the ER maintains its reticular nature by a constant interplay of tubule formation and homotypic fusion. Furthermore, all cells have mechanisms to disperse the ER throughout the entire cell volume. The cytoskeletal tracks, motors and cortical anchors used to achieve this distribution might vary among organisms. For instance, yeast and plants use an actin-dependent and type-V-myosin-dependent mechanism, whereas animal cells use a microtubule-dependent mechanism. However, even in animal cells, actin-based movement plays a supporting role, and is used in yeast microtubules for inheritance of the nuclear ER. As further progress is made in identifying the machinery responsible for establishing and maintaining the structure and distribution of the ER, common themes will no doubt emerge. It will be interesting to see whether animal cells use a cortical anchor for the ER that is analogous to that of Sec3p at the tip of the yeast bud. Through such studies, we hope to learn not only how the ER achieves and maintains its unique morphology but also why this morphology is important for specific ER functions.

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