

The Morphology and Dynamics of the ER

Hartmut Quader¹ (✉) · Michael Zachariadis²

¹Biocentre Klein Flottbek, University of Hamburg, Germany
quader@botanik.uni-hamburg.de

²Department of Botany, University of Athens, Greece

Abstract The endoplasmic reticulum (ER) is a non-uniform compartment in plants as regards its morphology and function. It extends as a highly anastomosing membranous network throughout the cytoplasm, is the major compartment of membrane biogenesis, and has been verified to function as the starting site for the secretory pathway. Early electron microscopy studies revealed three morphological ER sub-domains: the smooth ER, the rough ER, and the nuclear envelope. In the last two decades vital staining procedures, immunological methods, and green fluorescent protein technology in connection with confocal laser scanning microscopy have extended and augmented our knowledge regarding the morphology of the different ER domains, especially the three-dimensional transition between the cortical tubular network, long tubular strands, and lamellar sheets during interphase and mitosis. The cytoskeleton in connection with the respective motor proteins and cations like Ca^{2+} and H^{+} play a critical role in the regulation of ER organization in dividing, differentiating, and stressed cells. Although our understanding of ER morphology in plants has improved notably, our view still remains founded on a rather limited number of model cells.

1

Introduction

There is no standardized endoplasmic reticulum (ER) in plants. Descending from a single cell, in higher plants the zygote, plant cells may undergo rigorous morphological and metabolic changes before reaching their destination within an organism. Therefore, the course of development with periods of division and differentiation leads to various cell types, which will all have distinct ER organization according to their function. In the past few years our understanding of ER organization during the cell cycle, and in fully differentiated or differentiating cells during plant development, has improved considerably.

The ER extends as a highly anastomosing membranous network throughout the cytoplasm and represents in most plant cells the largest membrane system. It is the major compartment of membrane biogenesis and, ever since the membrane flow hypothesis was proposed (Mollenhauer and Morré 1980), also acts as the portal to the secretory pathway. It also contributes to several principal anabolic and catabolic cellular pathways, including the fine-tuning of the cytosolic Ca^{2+} concentration.

Morphologically the ER can be divided into three sub-domains: the smooth ER (sER), the rough ER (rER) (Fig. 1a), and the nuclear envelope (NE). In plants, desmotubule ER crossing plasmodesmata may be added as a fourth ER sub-domain linking the ER network of neighbouring cells. Plasmodesmata are structures which mediate intercellular communication (Staehelin 1996; see also Oparka and Wright, this volume). The amount of the NE remains almost constant during interphase, whereas the amount of the other ER sub-domains may vary according to metabolic demands.

Conventional ultrastructural studies showed longitudinally sectioned ER membrane fragments either without any associated ribosomes (the sER) or studded with ribosomes in a linear, spiral, or no clear arrangement (the rER). Ultrastructural studies using high-voltage electron microscopy have extended our view of ER organization in both animal (Walz 1982) and plant cells (Harris 1979). Such studies employed new post-fixation and post-staining techniques like osmium tetroxide/potassium ferricyanide (Hepler 1981) or zinc iodide/osmium tetroxide (Hawes et al. 1981), or high-pressure freezing (Craig and Staehelin 1988). These techniques not only provided evidence in favour of ER continuity, but also indicated that rER and sER occur in the form of flat, sheet-like sacs and tubular elements, referred to as cisternal ER (cER) and tubular ER (tER), respectively.

An even more complex view of ER morphology is obtained after visualizing the ER by means of light microscopy with ultraviolet microscopy, video-enhanced differential interference phase contrast microscopy (VeDIC) or fluorescence microscopy (Lichtscheidl and Hepler 1996). Actually, these procedures provided a reasonable insight into ER morphology some 40 years ago, but did not enjoy much recognition by the scientific community at the time (Drawert and Rüffer-Bock 1964; Url 1964). Light microscopy techniques, however, became the procedures of choice with the general availability of

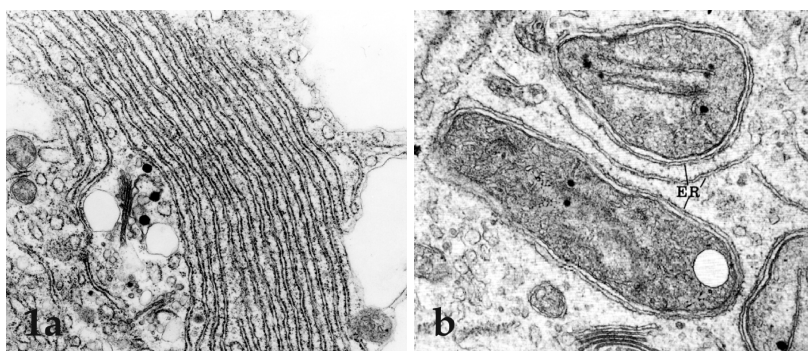


Fig. 1 Electron microscopic images of conventionally fixed ER. **a** Stack of rough ER in a pea root cell with a dictyosome at a close distance (courtesy of D.G. Robinson, Heidelberg). **b** ER in close association with chloroplasts (courtesy of P. Apostolakis, Athens)

VeDIC and confocal laser scanning microscopy (CLSM). ER visualization by CLSM profited enormously from the introduction of selective fluorescent stains about 20 years ago (Terasaki et al. 1984; Quader and Schnepf 1986; Hepler and Gunning 1998), and from indirect immunofluorescence techniques using antibodies that recognize the ER retention signal of ER luminal proteins (Napier et al. 1992) or ER resident proteins like the Ca^{2+} -binding protein calreticulin (Denecke et al. 1995). About a decade ago, green fluorescent protein (GFP) technology (Boevink et al. 1996; Haseloff et al. 1997; Hawes et al. 2001) provided an even greater input into ER morphology studies. A significant benefit of CLSM is the possibility of carrying out three-dimensional studies of ER organization in living cells (Hepler and Gunning 1998; Ridge et al. 1999; Cutler and Erhard 2002).

In general, ER morphology and its dynamics depend on the particular functions the cells of different tissues will have to perform for the sake of the organ, and thus of the whole organism. In the past, particular aspects of the organization and function of plant cell ER have been reviewed by several authors (Hepler et al. 1990; Lichtscheidl and Hepler 1996; Staehelin 1997; Hawes et al. 2001). In this chapter, we discuss the changes of ER morphology in meristem and differentiated cells, as related to cell cycle stages or physiological conditions.

2

ER Morphology and the Cytoskeleton

Many metabolic pathways in plant cells are under strict spatial control, as is apparent from distinct organelle distributions which are guaranteed by the two major cytoskeletal elements: microtubules (MTs) and actin filaments (AFs). MTs and AFs function in organelle patterning and motility as relatively stiff tracks along which the organelles are transported via specific motor proteins like dynein or kinesin (for MTs), and myosin (for AFs).

The controlled arrangement of the ER membrane meshwork throughout the cytoplasm is not conceivable without a guiding skeleton. In animal cells, MTs form tracks along which the ER network is constituted and altered in connection with the appropriate motor protein (Lee and Chen 1988; Terasaki 2000). In plant cells, ER tubules and small ER sheets have also been observed by electron microscopy in close nearness to MTs in the cell cortex of developing guard cells (Hodge and Palevitz 1984) and in pollen tubes (Lancelle et al. 1987). Most distinct are the MT-ER associations found during the mitotic stages (Hepler 1980) and during cytokinesis (Segui-Simarro et al. 2004). However, all the examples of spatial nearness between MTs and the ER during interphase resemble static situations, and bear the blemish that MTs and the ER have not been detected to co-align in plant cells over a longer distance. It thus seems very doubtful that these scarce contact sites are sufficient for the

observed controlled dynamic changes of the ER pattern. In vivo observations of ER organization and motility in onion epidermal cells show no perturbation in the presence of MT disassembling drugs, such as colchicine (Knebel et al. 1990), oryzalin, or trifluralin (Quader et al. 1989), when applied at concentrations specific for plant cells and for reasonable experimental periods. These results strongly support the view that MTs are not involved in ER organization in interphase and highly differentiated cells, although it has been claimed that MTs might be involved in the polar distribution of membranes including the ER (Mathur et al. 2003). A different situation may exist during mitosis and cytokinesis (see below).

Electron microscopy has revealed a spatial proximity between AF bundles and ER elements in fusiform cambium cells (Goosen de Roo et al. 1983), in developing guard cells (Palevitz and Hodge 1984), in differentiated root statocytes (Hensel 1987), in onion epidermis cells (Quader et al. 1987), in parenchyma cells of *Drosera* (Lichtscheidl et al. 1990), and in internodal cells of the giant alga *Chara* (Kachar and Reese 1988). Sliding of ER tubules along AFs has been convincingly demonstrated by in vitro video microscopy studies after gently extracting the cytoplasm of internodal cells of giant algae such as *Nitella* (Higashi-Fujime 1988) and *Chara* (Kachar and Reese 1988). We approached this question in onion bulb scale epidermal cells by following the redistribution of the ER after previously dislocating it by centrifugation (Quader et al. 1987). The ER starts to relocate in the form of bundles of long ER tubules and attains its former pattern after several hours depending on the centrifugal force applied. However, no recovery is observed in the presence of the AF disassembling drug cytochalasin D. This causes the transformation of parts of the tER into flat lamellar ER sheets (Quader et al. 1989). Lamellae form, in particular, at sites in the polygonal network where organelles accumulate (Quader et al. 1996). Depending on the physiological activity of the cells studied, the lamellar ER sheets may occupy large areas of the peripheral ER network. In mesophyll cells from *Vallisneria*, cytochalasin D also caused a change of the polygonal cortical ER tubules into lamellar sheets (Liebe and Menzel 1995).

AFs can only function as a framework for ER organization in correlation with a linking motor protein creating the force for ER motility. Using perfused internodal cells of *C. corallina*, Williamson (1979) showed that myosin-like filaments are associated in a stationary way with the ER at one end, while the other end is apparently in loose contact with the sub-cortical AF bundles. These filaments were later proved to be myosin (Grolig et al. 1988). The involvement of myosin in the dynamic distribution of cell organelles including the ER was confirmed in onion epidermal cells by localizing myosin through immunofluorescence, and by blocking its activity with the sulfhydryl reagent *N*-ethylmaleimide (NEM, Liebe and Quader 1994). Displacement of the ER by centrifugation leads to the dislocation of the ER and of myosin to the region of the centrifugal pole. After centrifugation, the previous ER and myosin pat-

tern was gradually restored. NEM, which leads to the complete inhibition of actomyosin-dependent organelle movement (Kohno and Shimmen 1988), not only arrested ER translocation, but also caused in onion epidermal cells the partial conversion of tER elements into lamellar sheets, whereas this agent apparently only froze the polygonal network in the alga *Vallisneria* (Liebe and Menzel 1995). Blocking the action of the myosin ATPase by 2,3-butanedione 2-monoxime caused the bulging and dilation of cortical ER tubules in the vicinity of plasmodesmata (Samaj et al. 2000).

In conclusion, while it is generally accepted for many algae and differentiated higher plant cells that ER organization depends on an intact actomyosin system, little is known regarding the situation in mosses and fern protonema cells.

3

ER Morphology and the Changing Demands of the Cell Cycle

While ultrastructural methods are suitable for studying the details of ER structure in all cell types, the use of fluorochromes such as the vital stain 3,3'-dihexyloxycarbocyanine iodide, DiOC₆(3) (Terasaki et al. 1984) and GFP technology (Haseloff et al. 1997) are superior in visualizing dynamic changes in ER organization in three dimensions. Although DiOC₆(3) and other fluorochromes appear to stain the great majority of different ER domains in plant cells, there is still no proof that all domains are equally labelled by the dyes or by GFP-tagged ER-targeted molecules.

3.1

ER Organization in Interphase Cells

Although the ER was first described by light microscopists, a more precise portrayal became possible only when Ledbetter and Porter (1963) introduced glutaraldehyde as a fixative for the electron microscopy of ultrathin sections. This technique, however, has a major disadvantage because it yields a rather limited three-dimensional view of cellular structures due to the minute thickness of the sections. Nevertheless, improvement in staining techniques gave a first glimpse into the 3-D pattern of the ER, and showed that it is an interconnected membrane system of tubules and flat sheets in remotely related differentiated interphase cells such as storage parenchyma cells of legume cotyledons, maize root cells, stamen hair cells, epidermal cells including guard cells, and moss caulonemata (Hepler et al. 1990). A distinct part of the ER was found in the cell cortex, largely present as a tubular network in close proximity to the plasma membrane (PM). In the vicinity of the nucleus it often exists as lamellar sheets covered with ribosomes. Lamellar sheets are not restricted to the nuclear region but may also occur embedded in the cortical



<http://www.springer.com/978-3-540-32531-4>

The Plant Endoplasmic Reticulum

Robinson, D.G. (Ed.)

2006, X, 338 p., Hardcover

ISBN: 978-3-540-32531-4