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Developmental Transitions and Dynamics of the Cortical ER of *Arabidopsis* Cells Seen with Green Fluorescent Protein

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Arabidopsis thaliana **plants were stably transformed with DNA encoding green fluorescent protein and with sequences ensuring retention in the endoplasmic reticulum (ER). Confocal laser scanning microscopy shows fluorescent ER in many cells of seedlings so allowing developmental changes to be documented. The arrangement of the cortical ER changes as cells mature in the hypocotyl and root epidermis. In the root, cells that have completed expansion have reticulate cortical ER resembling the ER described in many previous studies. Expanding cells, however, show extensive perforated sheets of cortical ER which transform quite abruptly into a loose reticulum at the basipetal end of the elongation zone. The reticulum compacts in trichoblasts beginning at sites where root hairs are about to emerge. The compacted form is maintained throughout the hair until growth ceases and the open reticulate form returns. All forms of cortical ER are dynamic and we use a color overlay method to distinguish stable and moving structures in a single composite image. Reticulate ER continuously rearranges its polygonal layout and perforations move and change their shape in the ER sheets of younger cells. ER deeper in the cell (i.e. not close to the plasma membrane) moves more actively so that almost no tubules remain stable even over short periods of less than one minute. The function of the perforated sheets of cortical ER present in growing cells is unknown.**

Key words: *Arabidopsis* — Confocal laser scanning microscopy — Cortical endoplasmic reticulum — Green fluorescent protein — Motility — Root hairs.

The endoplasmic reticulum (ER) forms a complex system of internal membranes that is virtually ubiquitous in eukaryotic cells. Many aspects of its detailed structure have been resolved only by electron microscopy (Staehelin 1997) but it can be difficult to appreciate the overall arrangement of the ER within cells by this technique unless serial sections are reconstructed or thick sections are used. Moreover, common fixation methods introduce many artefacts and all methods fail to show dynamic behaviour. This has strengthened efforts to image ER in vivo. Advanced optical methods make some analysis possible in favourable cell types without staining (Lichtscheidl and Url 1987, Lichtscheidl and Weiss 1988, Allen and Brown 1988) but analysis has been facilitated by the introduction of vital dyes such as dihexyloxacarbocyanine iodide (DiOC) (Quader and Schnepf 1986) which stains various endomembranes and is relatively non-toxic. This has dramatised the distinction between reticulate cortical ER, which does not undergo large scale movements, and the rapidly moving ER deeper in the cytoplasm (away from the plasma membrane).

Constructs delivering green fluorescent protein (GFP) to the ER (Boevink et al. 1996, 1998, Haseloff et al. 1997) provide further opportunities for in vivo study. GFP is a protein involved in jellyfish bioluminescence which has proved to be a versatile marker in many organisms (Chalfie and Kain 1998). The N-terminal signal peptide from *Arabidopsis* basic chitinase in the mGFP5 (Haseloff et al. 1997) construct delivers GFP to plant ER and a C-terminal HDEL sequence retains it. Readily visible levels of the protein accumulate in the ER of many *Arabidopsis* cell types without the toxicity which Haseloff et al. (1997) suggested prevents regeneration of transformants accumulating cytosolic GFP. *Arabidopsis* has two advantages for studying ER: the small seedlings allow ER to be observed in cells of minimally disturbed intact organs and the many *Arabidopsis* mutants relevant to ER structure and function can be analysed after crossing them with stable transformants expressing GFP in the wild type background.

As a basis for our studies of motility and secretion in *Arabidopsis* we made stable transformants carrying the mGFP5 construct in the wild type background and used confocal scanning laser microscopy to describe some major features of the ER. The reticulate cortical ER familiar from studies with vital dyes exists only in non-growing cells of the root and hypocotyl. Expanding cells have a novel arrangement of cortical ER that we call perforated sheets and this transforms to reticulate ER when cell expansion is coming to an end. The reticulate ER is again remodelled as root hairs emerge from epidermal cells just behind the ex-

Abbreviations: DIC, differential interference contrast; DiOC, dihexyloxacarbocyanine iodide; ER, endoplasmic reticulum; FITC, fluoroscein isothiocyanate; GFP, green fluorescent protein.

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pansion zone of the root. All forms of ER are dynamic and we introduce a color overlay method that shows stable and mobile ER components in a single composite image.

Material and Methods

Plant material—Seeds of *Arabidopsis thaliana* ecotype Columbia were surface sterilised and germinated at 20°C on agar in Petri dishes sealed with micropore Scotch 3M surgical tape. Media and conditions were described by Baskin et al. (1992). Plants for transformation and propagation were grown in pots containing a 1 : 1 : 1 mixture of vermiculite : sphagnum : sand using the same environmental conditions.

Plasmid and plant transformation—Purified DNA of an mGFP5 construct, pmGFP5-ER (Haseloff et al. 1997) (a generous gift from Jim Haseloff, MRC Laboratories, Cambridge, U.K.) was transformed into the *AGL1* strain of *Agrobacterium tumefaciens* by electroporation and *Arabidopsis* plants were transformed by vacuum infiltration (Bechtold et al. 1993). pmGFP5-ER is a plasmid consisting of a cauliflower mosaic virus promoter, modified GFP gene, and the HDEL amino acid sequence that codes for retention to the ER. Transformants were selected for kanamycin resistance in the T1 generation and positive plants screened for GFP using a long wavelength UV lamp. Single insert lines were selected in the T2 generation from their 3 : 1 segregation patterns for kanamycin resistance and stocks of homozygous T3 seed were obtained from six T2 plants that did not segregate kanamycin-sensitive plants in the T3.

Microscopy and video—*Arabidopsis* seedlings grown on agar (Baskin et al. 1992) were mounted in liquid culture medium and observed with a BioRad MRC600 in the fluoroscein isothiocyanate (FITC) range, using a Zeiss Axioplan inverted microscope and 100× oil-immersion objective. The confocal pinhole was set to 3 scale divisions, giving a pinhole size of 1.5 mm. However, as the effective pinhole size is a function of the objective, the effective pinhole was 0.28 micron. Images were captured using BioRad software with up to 2.5 digital magnification. Rapid cytoplasmic movements precluded Kalman processing so all images are single-scan captured images. Kalman processing uses multiple scans of the same image to improve contrast and resolution, but with living material moving rapidly, such an improvement of the image was not possible. To provide a simple representation of movement in a single image, a series of three images collected 30 s apart were each given false colour (blue, green and red) and the images merged using the Confocal Assistant software package (software by Todd Clark Brelje, 1994; distributed by Bio-Rad Inc. at ftp://ftp.genetics.bio-rad.com/Public/confocal/cas/).

Movies of fluorescent images captured at 10 s intervals can be viewed at http://mac122.icu.ac.jp/gfp-er

Results

Transformants—Many primary transformants were readily obtained by vacuum infiltration and their lack of obvious morphological phenotype suggests that the GFP does not adversely affect growth or development. Some T3 seedlings did not express GFP or show localised loss of expression in certain cells but we readily found many seedlings strongly expressing GFP in most if not all cells.

Developmental changes in cortical ER in cells showing dispersed growth—The *Arabidopsis* root grows for many days sustained by expansion of both dividing and non-dividing cells which are arranged in a simple, predictable way along the root axis (Baskin et al. 1995). In contrast, the hypocotyl completes cell division during embryogenesis and the rate and distribution of cell expansion during germination is a complex function of time and light exposure (Gendreau et al. 1997). We therefore initially focussed on the seedling root to exploit the simple developmental sequence that can be reconstructed by scanning basipetally from the meristem through the expansion zone to where growth has ceased and root hairs are developing.

There is bright fluorescence near the cell and nuclear surfaces in meristematic cells although cortical ER is not clearly distinguishable (Fig. 1A, B). Cortical ER is seen in slightly larger cells in the expansion zone where it forms extended sheets penetrated by relatively small holes about 1 μm in diameter (Fig. 1C). Brightness can vary considerably across such perforated sheets. The sheets occupy a very shallow focal plane at the cell surface with more mobile ER visible deeper in the cell. Similar ER sheets are seen in the hypocotyl and will be discussed later in the context of dynamic changes. Cells at the basipetal end of the expansion zone sometimes show rather abrupt changes in the arrangement of the cortical ER (Fig. 1D). Perforations in the ER sheets seem to enlarge until membrane-free areas become separated by only a tubule and the areas of sheet-like membrane decrease. An unusual amount of punctate fluorescence often occurs in those transitional regions. Mature cells (Fig. 1E) have a reticulate pattern of cortical ER identical to that described in studies using cells that are unstained (Lichtscheidl and Url 1987, Lichtscheidl and Weiss 1988, Allen and Brown 1988), vitally stained (Drawert and Ruffer-Bock 1964, Quader and Schnepf 1986, Allen and Brown 1988, Lichtscheidl and Weiss 1988, Hepler et al. 1990, Knebel et al. 1990, McCauley and Hepler 1990) or have accumulated GFP (Boevink et al. 1996, 1998, Haseloff et al. 1997). Reticulate ER has tubules that delimit polygonal membrane-free areas, tubules which sometimes expand into small cisternae where they branch—the only remnants of the extensive membrane sheets that were so prominent in younger cells. The membrane-free areas are much larger than the perforations in the sheets of cortical ER in younger cells (compare Fig. 1C, E). Sheet, reticulate and transitional forms of cortical ER occur in cells of the hypocotyl and their dynamic behaviour will be discussed below.

Cortical ER in tip growing root hairs—Cells in the main body of the root and hypocotyl show dispersed growth, that is growth distributed evenly along their length. Root hairs emerge when specific epidermal cells (trichoblasts) that have only recently stopped elongating resume growth from a small region of their outer wall. The root hair that emerges shows tip growth (Heath 1990), ex-

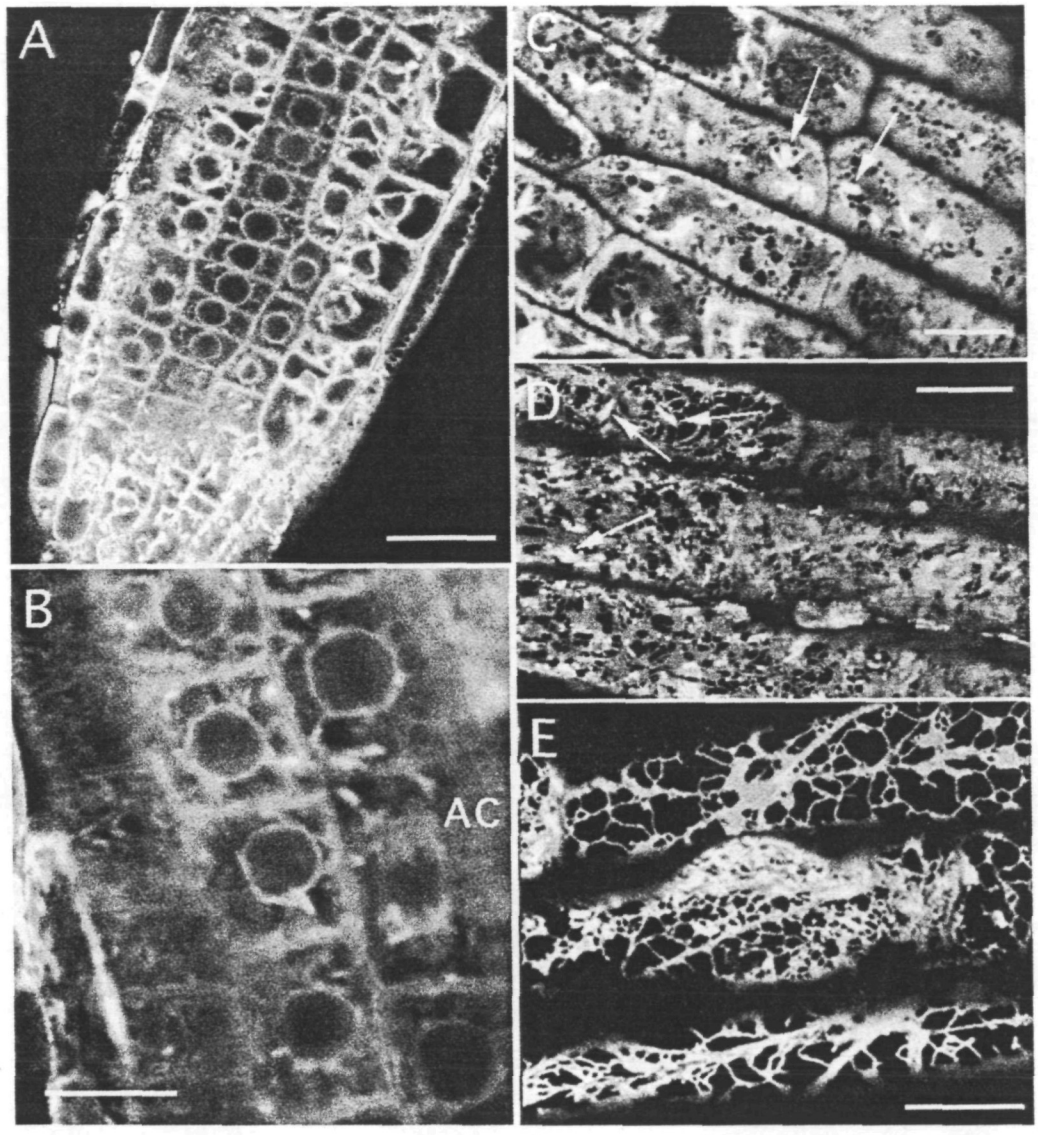

Fig. 1 Changes in cortical ER in cells of the root. (A) Survey view of the root apical meristem. The nuclear and cell surfaces are brightly fluorescent as are some parts of the intervening cytoplasm. Bar = 50 μm. (B) Cytoplasmic ER seen at higher magnification in various in meristematic cells. Cortical ER is not distinctly resolved in deeper tissue. The anaphase cell (AC) has no nuclear envelope and ER fluorescence is concentrated at the diffuse spindle poles. Bar = 20 μm. (C) Cortical ER in expanding cells. The extensive sheets show perforations about 1 μm in diameter and variations in fluorescent intensity between different parts of the sheet. Brightly fluorescent fusiform bodies are seen (arrows). Bar = 10 μm. (D) Cells at the base of the elongation zone showing what is thought to be an intermediate form of cortical ER which could arise as the holes in perforated sheets enlarge to form the mature reticulum. Cells showing this transitional form of cortical ER show an unusually large amount of punctate fluorescence. Larger, brightly fluorescent fusiform bodies are also seen (arrows). Bar = 10 μm. (E) Cortical reticulum in mature cells of the root. Irregular polygons are delimited by ER tubules that sometimes expand into cisternae where they intersect. The reticulum is more condensed at the site of root hair initiation. Bar = 10 μm.

tending in only a relatively short distal zone. Observations along a single root hair therefore include extending and non-extending regions of a single cell in a way that does not occur in cells showing dispersed growth. Given our findings linking ER arrangement to root cell extension, we were interested to see how the ER changes when growth resumes with root hair development.

Local condensation of the dispersed reticulum occurs at the site where root hair outgrowth is just commencing (Fig. 1E). Condensation subsequently spreads more widely through each trichoblast until their files stand out from the files of non-trichoblast epidermal cell files (Fig. 2A). The

elongating root hair has condensed reticulum that extends right into its tip (Fig. 2B) and ER organisation does not change markedly with increasing distance from the tip (Fig. 2C). Further changes in root hair ER are not seen until the hair reaches its maximum length and elongation growth ceases. The condensed reticulum then returns to an open form that is again present along the whole length of the hair (Fig. 2D).

ER deeper in the cytoplasm (not close to the plasma membrane)—The nuclear surface fluoresces prominently in interphase cells of the root meristem and elements of the ER extend short distances through the cytoplasm (Fig. 1A, B). The nuclear envelope is lost during mitosis but elements of the ER cluster around the diffuse spindle poles and remain in the cytoplasm (Fig. 1B). Cortical ER is readily distinguished from the deeper ER in both expanding and mature cells. Deeper ER in a hypocotyl cell that still has perforated sheets in its cortex extends through the system of cytoplasmic strands that span the vacuole (Fig. 3A). Large numbers of very bright, fusiform bodies are present. These are also seen in all cell types examined including expanding (Fig. 1C), transitional (Fig. 1D) and mature

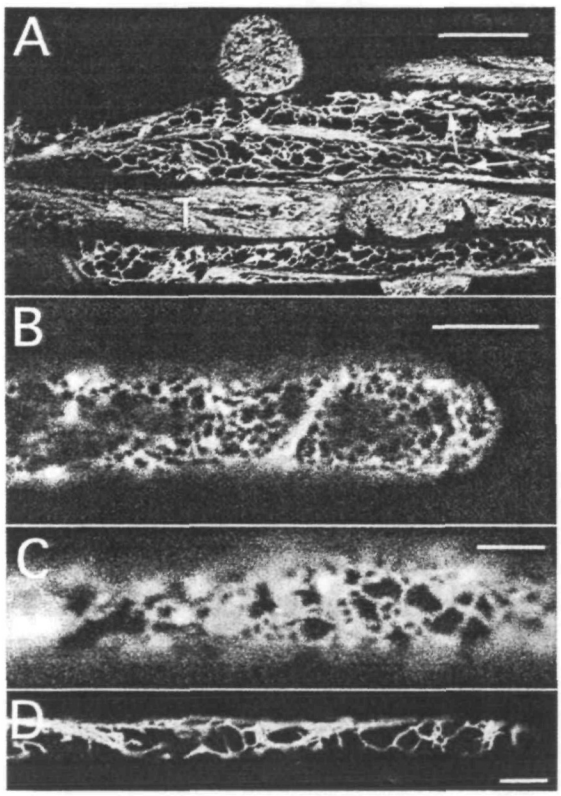

Fig. 2 Cortical ER in trichoblasts and root hairs. (A) Epidermal cells in the region of root hairs. The cell file containing trichoblasts (T) has a more condensed form of cortical ER than the neighbouring files from which root hairs do not arise. Brightly fluorescent fusiform bodies are seen (arrows). Bar = 10 μm. (B) Cortical ER near the tip of a growing root hair. The ER forms a condensed reticulum where many small membrane-free areas are separated by tubules. No large regions of sheet-like ER are seen. Bar = 10 μm. (C) Similar cortical ER as in (B) seen further back from the tip. The root hair is likely to be still extending but growth will not be occurring in this region which is well back from the tip. Bar = 3 μm. (D) An open reticulum of the type seen throughout a root hair that has stopped elongating (hair tip is at right). Bar = 10 μm.

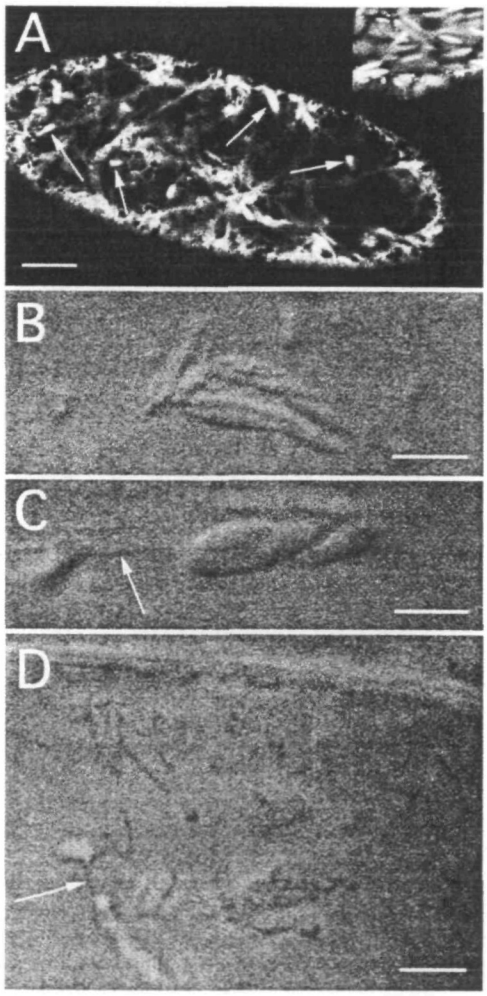

Fig. 3 ER deeper in the cell (not close to the plasma membrane) and fusiform bodies. (A) ER in a small hypocotyl cell seen in a focal plane below the cortical ER. The ER forms a network ramifying through the cytoplasmic strands. Numerous fusiform bodies fluoresce brightly (arrows). Inset shows cotyledon cells with strongly fluorescent fusiform bodies seen against chloroplasts visible by their red autofluorescence. Bar = 10 μm. (B) Differential interference contrast (DIC) image of presumed fusiform bodies in the cytoplasm of hypocotyl cells of the wild type. The bodies are apparently connected to thin, linear structures that might be ER tubules. Bar = 2 μm. (C) and (D) DIC images of presumed fusiform bodies in the cytoplasm of hypocotyl cells of the GFP-ER plant. The bodies are apparently connected to thin, linear structures (arrows) that might be ER tubules. Bars = 2 μm.

(Fig. 2A) root cells, root hairs (not shown) and cotyledon epidermal cells (Fig. 3A) where they are readily distinguished from the more ovoid chloroplasts with their red autofluorescence. Fusiform bodies sometimes appear in images of cortical ER (e.g. Fig. 1C, D) but do not appear to be part of the cortical ER since they move independently of

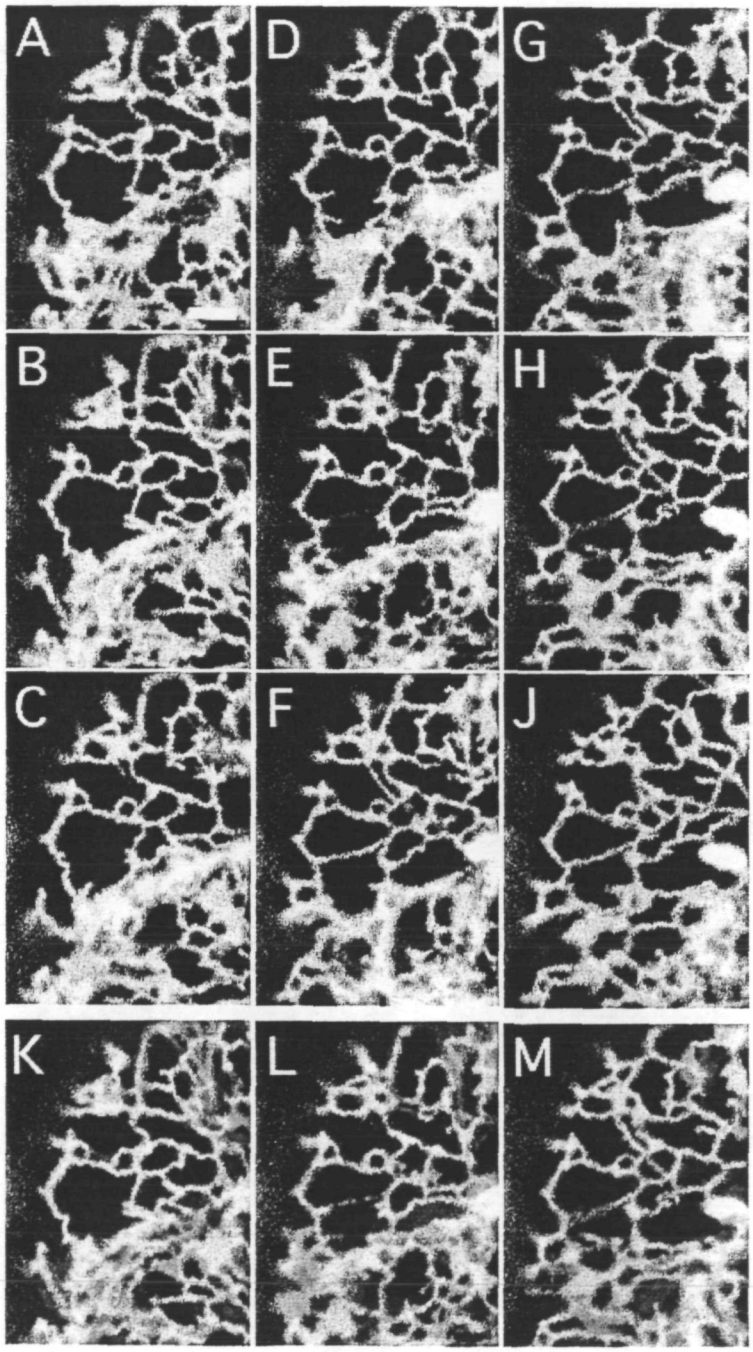

Fig. 4 Images of the same area of cortical ER in mature hypocotyl cells acquired at 30 s intervals and showing the dynamic nature of the reticulum. (A) to (J) High magnification view of a limited area of cortical reticulum in which widespread remodelling can be seen to occur over the period covered by the images. (K) to (M) Color overlay images formed by overlaying red, blue and green pseudocolor images of three sequential images seen in (A) to (J). Tubules that were stable over the period covered by the three overlaid images appear white. Structures that move show as multiple images either in the original colors or in new colors arising from combining two of the colors. (K) Color overlay of images (A), (B) and (C). (L) Color overlay of images (D), (E) and (F). (M) Color overlay of images (G), (H) and (J). Bar = 1 μm. Images (A) to (J) and others of the same region can be seen as a movie at http://mac122.icu.ac.jp/gfp-er.

it. We assume they are components of the deeper cytoplasm coming into the same focal plane as some of the cortical ER. Fusiform bodies are seen as structures with higher refractive index in Differential Interference Contrast (DIC) images of both wild type and GFP-transformants (Fig. 3B, C, D) showing that they are not artefacts induced by high level expression of a foreign protein. They often appear to be continuous with narrower structures that likewise have higher refractive index than the cytosol (arrows in Fig. 3B, C, D).

ER dynamics—The dynamics of various forms of ER are illustrated in movies of ≤ 40 images captured at 10 s intervals that can be viewed at http://mac122.icu.ac.jp/gfp-er

As reported by others, the reticulate cortical ER does not show large scale translocations but is continuously remodelled as tubules extend, merge and move their branch points. A series of nine images spanning about four minutes illustrate the dynamics of cortical reticulum tubules in a small part of a mature hypocotyl cell (Fig. 4A to J). Many rearrangements of the reticulum can be seen even in a small region such as this. A color overlay method which highlights moving and non-moving regions can facilitate analysis of such images. Red, green and blue versions of the images in Figure 4A to C are overlaid in Figure 4K, Fig. 4D to F are overlaid in Figure 4L, and Figure 4G to J are overlaid in Figure 4M. The three images of a stable tubule lie in register and show white whereas the three images of a mobile tubule may appear separately in their originally assigned pseudocolors or may partly overlap generating one or more new colors. Structures appearing stable (white) in the overlay from one set of three images are often seen to move in the overlay from another set. The color overlay directs attention to sites of stability and change that can then be verified in the component images.

Movement is less dramatic in the perforated sheets of growing cells but movies (at http://mac122.icu.ac.jp/gfp-er) still show a highly dynamic system. Changes in the cortical ER in part of a hypocotyl cell over a period of about one minute are shown in Figure 5A. Most pores have a simple ovoid shape but some, such as that arrowed in Figure 5A are larger and have a more complex appearance that changes with time: the same structure can appear horseshoe shaped, as a circle with central disc and in others seem to resolve into two pores. The area circled in Figure 5A shows another complex pore and three simple pores. The complex pore remains in a relatively constant position but changes its appearance whereas the simple pores show positional changes and size changes. The area contained within the rectangle shows three pores whose relative positions rapidly change over the six images. The ER deeper in the cell and other organelles are in continuous large scale motion in a plane that in some cases (arrowhead in Fig. 5A) can be seen in the same plane as the cortical ER. In the time lapse movie, accentuated movements of pores in the cortical ER are seen immediately adjacent to the streaming

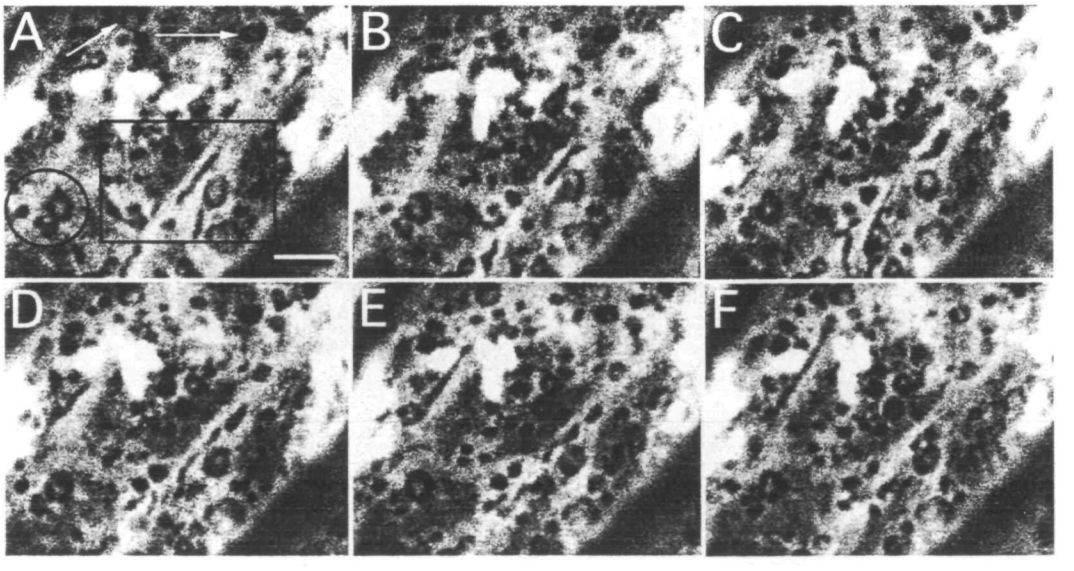

Fig. 5 Sequential images (A to F) covering about 60 s and showing part of a hypocotyl cell containing cortical ER in the form of a perforated sheet. Two sorts of perforations are seen. First, large complex pores (examples arrowed in A) whose detailed appearance changes from image to image but whose position remains relatively stable and second, smaller pores of simpler appearance whose size changes and position can change in time, as shown in this succession of images. The circle marked in A contains a complex pore and three simple pores that illustrate this behaviour; as seen by following successive images. The rectangle in A encloses an area of pores that undergo particularly active displacements (seen in successive images) as a result of the movement of nearby organelles. Bar = 3 μm. These images and others of the same region can be seen as a movie at http://mac122.icu.ac.jp/gfp-er.

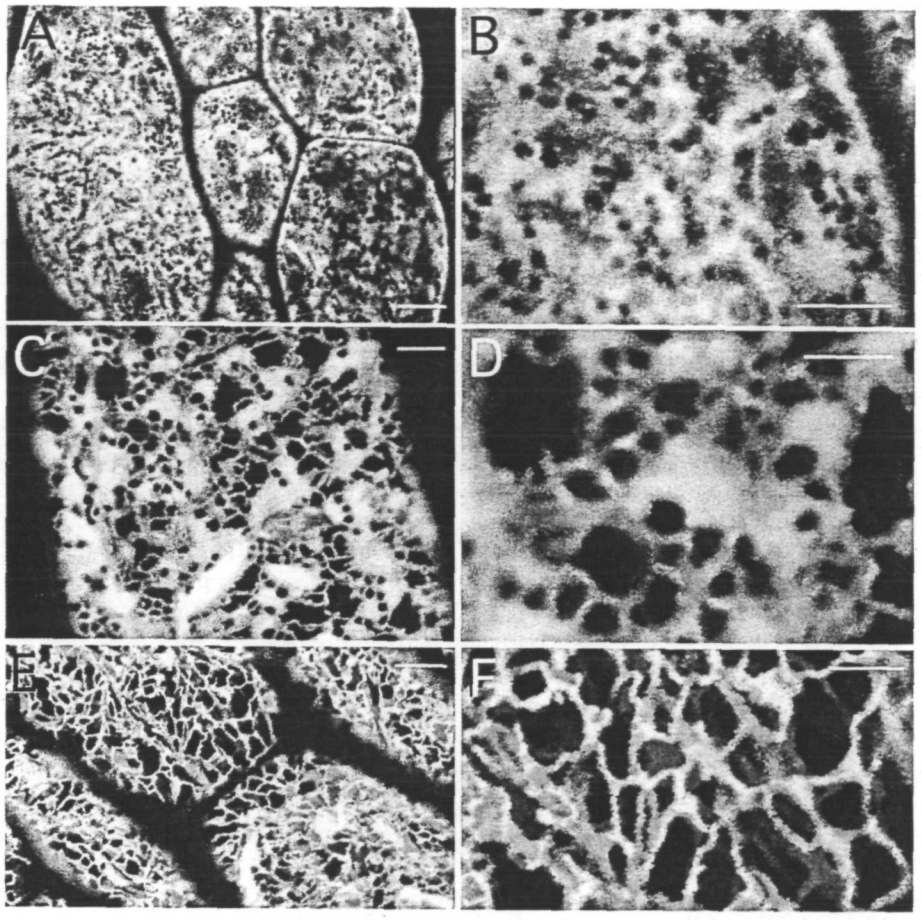

Fig. 6 Cortical ER in hypocotyl cells. Color overlay images formed by merging red, blue and green pseudocolored images taken of the same field at approximately 30 s intervals. Stable structures show white by superposition while moving structures show either as multiple images in the original colours or as a new color from combining two of the original colors. (A) Color overlay image of perforated sheets of cortical ER in several neighbouring cells. Bar = 10 μm. (B) Detail of (A). Bar = 5 μm. (C) Color overlay image of ER representing the presumed transitional stage between the perforated sheet and the mature reticulum. Bar = 8 μm. (D) Detail of (C) showing the colored fringes around many of the pores in which the changes in shape of the pores are recorded. Bar = 2 μm. (E) Color overlay image of mature reticulum in several neighbouring cells showing a minority of non-mobile tubules appearing white. Bar = 8 μm. (F) Detail of (E). Bar = 2 μm.

cytoplasm. These are probably connected to displacement effects caused by the passage of bulky organelles in the streaming cytoplasm or perhaps to intermittent force generation related to nearby organelle movements. Color overlay imaging illustrates the widespread movements of pores in a group of small hypocotyl cells (Fig. 6A, B). Movements and shape changes also occur in the larger pore sizes present in ER undergoing the sheet to reticulum transition in the hypocotyl (Fig. 6C, D). Stable tubules can be picked out even in survey views covering the reticulate ER of several cells (Fig. 6E, F) even when many of the mobile tubules are so active that their detailed behavior is hard to resolve in overlay.

Discussion

Our observations confirm GFP's value as a marker for ER in plant cells, demonstrate previously unreported transitions in the form of the cortical ER and show the value of a simple video method to identify stable and mobile ER components.

Cortical ER in cells expanding by dispersed growth—Cortical ER was recognisable in early electron micrographs of glutaraldehyde fixed cells (Porter and Machado 1960) and by special contrasting techniques (Harris 1979, Hepler 1981). ER dynamics seen in living cells emphasised the distinctiveness of cortical ER which, although constantly restructured, does not move with the ER tubules and other organelles of the deeper, streaming cytoplasm (Drawert

and Ruffer-Bock 1964, Quader and Schnepf 1986, Lichtscheidl and Weiss 1988, Allen and Brown 1988, Haseloff et al. 1997, Boevink et al. 1996, 1998). The cortical ER seen in all these studies is predominantly reticulate resembling what we see in mature cells of the *Arabidopsis* root and hypocotyl as well as in root hairs that have ceased elongating. Root and hypocotyl cells that are still expanding, however, have extensive perforated sheets of cortical ER, structures that cannot be directly equated with cortical ER whether seen by electron microscopy (Staehelin 1997) or in living cells. The fenestrated lamellae seen by electron microscopists (Hepler 1981, Hepler et al. 1990) are smaller than our perforated sheets and their holes ($<0.1\,\mu m$ in diameter) are much smaller than those we see. Previous studies with DiOC or GFP did not show extensive perforated sheets perhaps because only non-growing or slowly growing cells occur in onion bulb epidermis (Drawert and Ruffer-Bock 1964, Quader and Schnepf 1986, Lichtscheidl and Url 1987, Lichtscheidl and Weiss 1988, Allen and Brown 1988, Knebel et al. 1990, Quader 1990, Quader and Fast 1990, Quader and Liebe 1995) or tobacco leaves (Boevink et al. 1996, 1998).

Converting sheets to tubules—Cortical sheets keep pace with the rapid growth in root cells but break down in cells that have ceased or almost ceased growing. The areas remaining as sheets shrink and thin to tubules separating the enlarging perforations. Such changes probably involve loss of cortical membranes that could be transferred to the deeper ER or be broken down. Many sites of punctate fluorescence are seen only where this large scale remodelling of cortical ER is likely to be occurring. These could be Golgi bodies if, for some reason, retrieval of GFP from the Golgi is transiently less effective. Transformations of cisternae to tubules have been reported after various cellular perturbations (Quader 1990, Quader and Fast 1990, Quader and Liebe 1995) and when light induces chloroplast movements in *Vallisneria* (Liebe and Menzel 1995).

ER in tip growing root hairs—Root hairs emerge from cells that have recently undergone a sheet to dispersed reticulum transition as their expansion by dispersed growth came to an end. The transition is not reversed when root hair growth resumes but the reticulum does condense, first at the site where outgrowth begins and later in the rest of the trichoblast. The condensed form of reticulum is then retained in the root hair until its growth ends when it reverts to a dispersed reticulum. The root hair therefore shows that there is not an invariable association between growth and a sheet form of cortical ER. Moreover, during tip growth of the root hair, there is no difference between the arrangement of ER in growing regions near the tip and non-growing regions more basally. The condensed reticulum of root hairs seems more extreme than the "somewhat tighter ER network" seen with DiOC in tip growing branch cells of *Funaria hygrometrica* (McCauley and Hepler 1990) but this moss shows the same trend of an increased density of cortical membranes in growing cells. It also lacks an obvious distinction between the ER in the growing tip and that in the non-growing regions below.

Fusiform bodies—Highly fluorescent fusiform bodies are abundant in all the cell types of *Arabidopsis* we observed in transformants and comparable structures are visible with DIC optics in both transformed and wild type cells. They were previously proposed to be plastids labelled by imprecise targetting of GFP to the ER (Haseloff et al. 1997) but using our unpublished data Gunning (1998) argued that they are dilated ER cisternae with dense contents. Such swollen cisternae have been found in the *Arabidopsis* root cap (Kordyum et al. 1983) in addition to the many other members of the Brassicaceae that Gunning (1998) listed. The DIC images we present (Fig. 3) show apparent continuity with narrower structures that could be ER tubules, consistent with the swollen ER found in all tissue examined. However, in both our fluorescent images and those of Haseloff et al. (1997), only rare images suggest continuity with ER tubules.

ER mobility and color overlay imaging—The GFP-containing cortical reticulum in mature *Arabidopsis* cells shows mobility resembling that previously described after DiOC staining (Quader and Schnepf 1986, Knebel et al. 1990) or in unstained cells (Lichtscheidl and Url 1987, Lichtscheidl and Weiss 1988, Allen and Brown 1988). The color overlay method conveniently records where movement has or has not occurred for all structures within the image. Cross checking the three overlaid images confirms that stable structures are correctly identified. To reconstruct such information without color overlay requires comparing each structure individually through the image sequence and becomes almost impossible in large area survey views. Color overlays can help make visible any large scale patterns in the sites of activity or inactivity by summarising them in one image. Immobile structures stand out sharply in white even when details of the mobile tubules are lost in survey views.

The color overlay method depends on the movement of optical boundaries whose number will vary according to the particular structure being imaged. The dispersed cortical reticulum is rich in boundaries whereas boundaries in perforated sheets are mainly confined to the small and scattered perforations. Comparisons between such very different structures based on the vividness of colors could therefore be misleading but color overlay can quickly show that movements of perforations are very widespread in survey views of several cells.

Detailed behaviour of pores in the perforated sheets is best seen by time lapse movies or by comparing sequential still images. A sub-population of perforations appear larger and show a more variable appearance from image to image. In some images they appear horseshoe shaped, a

shape previously described on the plasma membrane of high pressure frozen cells (Staehelin and Chapman 1987, Craig and Staehelin 1988) and suggested to be sites of recent secretory vesicle fusion. The size discrepancies (1 to 2 μm in our study, 0.1 to 0.2 μm for the electron microscope structures) suggest that the two structures are unrelated. The limited translational movements we observe for the large complex pores might reflect some sort of anchorage. The majority of pores, however, appear in our images as simple holes, they show more movement and may be affected by movements of nearby organelles.

In summary, we have shown that the cortical ER of expanding cells forms perforated sheets, quite distinct from the familiar open reticulum with small cisternae found in older cells, and that growing root hairs show a condensed form of the cortical reticulum rather than perforated sheets. The functional significance of the perforated sheets is uncertain but we suggest they may influence access of Golgi vesicles to the plasma membrane and the radial diffusion of ions particularly the Ca^{2+} involved in Golgi secretion.

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References

Allen, N.S. and Brown, D.T. (1988) Dynamics of the endoplasmic reticulum in living onion epidermal cells in relation to microtubules, microfilaments and intracellular particle movement. *Cell Motil. Cytoskel.* 10: 153–163.

Baskin, T.I., Betzner, A.S., Hoggart, R., Cork, A. and Williamson, R.E. (1992) Root morphology mutants in *Arabidopsis thaliana*. *Aust. J. Plant Physiol.* 19: 427–437.

Baskin, T.I., Cork, A., Williamson, R.E. and Gorst, J.R. (1995) STUNTED PLANT, a gene required for expansion in rapidly elongating but not in dividing cell and mediating root growth responses to applied cytokinin. *Plant Physiol.* 107: 233–243.

Bechtold, N., Ellis, J. and Pelletier, G. (1993) In planta *Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *Comptes Rend. Acad. Sci. Paris* 316: 1194–1199.

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

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

Chalfie, N. and Kain, S. (1998) *Green Fluorescent Protein: Strategies, Applications and Protocols.* Wiley, New York.

Craig, S. and Staehelin, L.A. (1988) High pressure freezing of intact plant tissues. Evaluation and characterization of novel features of the endoplasmic reticulum and associated membrane systems. *Eur. J. Cell Biol.* 46: 80–93.

Drawert, H. and Ruffer-Bock, U. (1964) Fluorochromierung von Endoplasmatischen Reticulum, Dictyosomen und Chondriosomen mit Tetracyclin. *Ber. Deutsch. Bot. Ges.* 77: 440–449.

Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M. and Höfte, H. (1997) Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. *Plant Physiol.* 114: 295–305.

Gunning, B.E.S. (1998) The identity of mystery organelles in *Arabidopsis* plants expressing GFP. *Trends Plant Sci.* 3: 417.

Harris, N. (1979) Endoplasmic reticulum in developing seeds of *Vicia faba*. A high voltage electron microscopic study. *Planta* 146: 63–69.

Haseloff, J., Siemering, K.R., Prasher, D.C. and Hodge, S. (1997) Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants. *Proc. Nat. Acad. Sci. USA* 94: 2122–2127.

Heath, I.B. (1990) *Tip Growth in Plant and Fungal Cells.* Academic Press, San Diego.

Hepler, P.K. (1981) The structure of the endoplasmic reticulum revealed by osmium tetroxide-potassium ferricyanide staining. *Eur. J. Cell Biol.* 26: 102–110.

Hepler, P.K., Palevitz, B.A., Lancelle, S.A., McCauley, M.M. and Lichtscheidl, I. (1990) Cortical endoplasmic reticulum in plants. *J. Cell Sci.* 96: 355–373.

Knebel, W., Quader, H. and Schnepf, E. (1990) Mobile and immobile endoplasmic reticulum in onion bulb epidermal cells: short- and long-term observations with a confocal laser scanning electron microscope. *Eur. J. Cell Biol.* 52: 328–340.

Kordyum, V.A., Tarasenko, V.A. and Chernyaeva, I.I. (1983) Root cap ultrastructure of *Arabidopsis thaliana* under control of microgravity. *Arabidopsis Information Service* 20: http://genome-www.stanford.edu/Arabidopsis/ais/1983/kordy-1983-aacvr.html

Lichtscheidl, I. and Url, W.G. (1987) Investigation of the protoplasm of *Allium cepa* inner epidermal cells using ultraviolet microscopy. *Eur. J. Cell Biol.* 43: 93–97.

Lichtscheidl, I.K. and Weiss, D.G. (1988) Visualization of submicroscopic structures in the cytoplasm of *Allium cepa* inner epidermal cells by video-enhanced contrast light microscopy. *Eur. J. Cell Biol.* 46: 376–382.

Liebe, S. and Menzel, D. (1995) Actomyosin-based motility of endoplasmic reticulum and chloroplasts in *Vallisneria* mesophyll cells. *Biol. Cell.* 85: 207–222.

McCauley, M.M. and Hepler, P.K. (1990) Visualization of the endoplasmic reticulum in living buds and branches of the moss *Funaria hygrometrica* by confocal laser scanning microscopy. *Development* 109: 753–764.

Porter, K.R. and Machado, R.D. (1960) Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. *J. Biophys. Biochem. Cytol.* 7: 167–180.

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.

Quader, H. and Fast, H. (1990) Influence of cytosolic pH changes on the organisation of the endoplasmic reticulum in epidermal cells of onion bulb scales: acidification by loading with weak organic acids. *Protoplasma* 157: 216–224.

Quader, H. and Liebe, S. (1995) Actin filament-independent formation of tubular endoplasmic reticulum in onion epidermis cells. *J. Plant Physiol.* 145: 71–77.

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

Staehelin, L.A. (1997) The plant ER: a dynamic organelle composed of a large number of discrete functional domains. *Plant J.* 11: 1151–1165.

Staehelin, L.A. and Chapman, R.L. (1987) Secretion and membrane recycling in plant cells: novel intermediary structures in ultrarapidly frozen sycamore and carrot suspension-culture cells. *Planta* 171: 43–57.

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