Photoactivation of GFP reveals protein dynamics within the endoplasmic reticulum membrane

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Abstract

Components of the plant cell secretory pathway, including the endoplasmic reticulum and Golgi apparatus, are in constant motion. The photoactivation of GFP has been used to determine that proteins within the membrane of the ER flow as the ER is remodelled. Measurement of the rate at which activated GFP moves away from the activation spot shows that this motion is much faster than would be expected if membrane components moved simply by diffusion. Treatment with latrunculin to depolymerize the actin cytoskeleton stops ER remodelling and reduces the rate of GFP movement to that expected from diffusion alone. This suggests that myosin binds directly or indirectly to ER membrane proteins and actively moves them around over the actin scaffold. Tracking of Golgi body movement was used to demonstrate that they move at the same rate and in the same direction as do photoactivated ER surface proteins. Golgi bodies, therefore, move with, and not over, the surface of the ER. These observations support the current theory of continuity between Golgi bodies and discrete ER exit sites in the ER membrane.

Key words: Actin cytoskeleton, endoplasmic reticulum, GFP, Golgi, photoactivatable GFP.

Introduction

The endoplasmic reticulum (ER) and Golgi apparatus are in constant, actin-dependent motion within the cortical cytoplasm of leaf epidermal cells of Nicotiana tabacum (Quader et al., 1987; Boevink et al., 1998). Newly folded proteins move along the anterograde pathway from the ER to Golgi bodies for modification and secretion to their cellular destination. Theoretical mechanisms of ER/Golgi body association have been revised and updated frequently (Staehelin and Moore, 1995; Batoko et al., 2000; Nebenführ and Staehelin, 2001; Robinson, 2003; Hawes, 2005). Obvious physical continuity is seldom observed between the ER and Golgi stacks at the light or electron microscope level, but there are reports of tubular connections in some cell types (Brandizzi et al., 2002). A major question to be resolved is whether the entire ER surface is competent to export proteins which are then picked up by motile Golgi stacks (Boevink et al., 1998) or whether the Golgi stacks stop at discrete ER exit sites (ERES) as proposed by Nebenführ et al. (1999)? Recent evidence suggests that, in fact, ERES are in connection with the individual Golgi stacks either permanently (daSilva et al., 2004), or at least transiently (Yang et al., 2005) and that the ERES/Golgi stack complex moves over or within the surface of the ER.

Fluorescent protein fusions to structural proteins of the secretory pathway are an ideal way to study the dynamic nature of the system (Brandizzi et al., 2004). When green fluorescent protein (GFP) is targeted to the ER and retained due to an HDEL signal (Boevink et al., 1996; Haseloff et al., 1997; Batoko et al., 2000), a polygonal, reticulate network becomes visible in the cortical cytoplasm of living cells. This network remodels relatively slowly compared with the fast moving strands of ER that radiate through it in a co-planar fashion or that traverse the cell as trans-vacuolar strands. GFP modified thus, remains within the lumen of the ER and is, therefore, not a useful tool for observation of the ER membrane as a separate entity. What is required is an ER membrane-bound fluorescent protein that will enable monitoring of ER surface movement contemporaneously with tracking of Golgi stacks. Such a system would allow investigation as to whether Golgi bodies move with, or over the ER surface and, ultimately, would help define the mechanism at the ER/Golgi interface more precisely.

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To this end, photoactivatable GFP (PAGFP; Patterson and Lippincott-Schwartz, 2002) fused to the trans-membrane domains of Arabidopsis thaliana calnexin (Huang et al., 1993; Irons et al., 2003) have been utilized. Irons et al. (2003) observed that calnexin overexpression in the ER membrane results in flat sheets of ER that are not normally observed in GFP-HDEL expressing plants. These sheets remain connected in a network by normal-appearing ER tubules and plants appear unaffected by the change in ER morphology. When activated, the calnexin-PAGFP (CX-PAGFP) construct fluorescently marks the ER membrane and, if a small enough region of membrane has been activated, its movement can subsequently be tracked by time-lapse imaging. The Golgi apparatus was marked at the same time using a construct consisting of the trans-membrane domain of a rat sialyl transferase (Boevink et al., 1998) fused to red fluorescent protein (mRFP). Two different software approaches were required to correlate movement of the ER membrane (a diffuse structure), and Golgi bodies (discrete structures). Ultimately, it was possible to quantify the speed and direction of movement of each.

Materials and methods

Marking the ER membrane with photoactivatable GFP

PAGFP was fused to the 3’ end of the calnexin trans-membrane domain to produce CX-PAGFP. In this orientation, PAGFP is anchored to the ER membrane but is located within the cell cytoplasm which makes it more readily photoactivatable. The calnexin fragment was PCR amplified from the vector pVKH18En6 spGFP5CX (Irons et al., 2003) using the primers JR023 (forward GCTTCTAGAGGTATGATCGGAACCTGATTGAGAAGCCGAG) to add an XhoI restriction site and an ATG start codon, and JR008 (reverse TGAGGATCCATTTACACGTCAGTTGGCCTTTTGCC) to remove the TAG stop codon and add a BamHI restriction site (266 bp). PAGFP (provided by George Patterson and Jennifer Lippincott-Schwartz) was PCR amplified with the primers JR016 (forward GCTTCTAGAGGATCGATTTACACGTCAGTTGGCCTTTTGCC) to add a XhoI restriction site and an ATG start codon, and JR008 (reverse GGGGACCACTTTACACGTCAGTTGGCCTTTTGCC) to remove the TAG stop codon and add a BamHI restriction site (266 bp). These two PCR fragments were digested and subcloned into pGEM-3Z (Promega) to add a SacI restriction site (788 bp). These two PCR fragments were digested and subcloned into pGEM-3Z (Promega) to make pGEM-3Z CX-PAGFP. The CX-PAGFP fragment was PCR amplified for Gateway cloning (Invitrogen) using the primers JR024 and JR025 (reverse GGGGACCACTTTACACGTCAGTTGGCCTTTTGCC) to add forward and reverse aatB recombination sites, respectively, as well as a Kozak consensus sequence (AAA AAA) immediately upstream of the start codon (1119 bp). This PCR fragment was recombined with the Gateway pDONR207 vector in a BP reaction to produce pENTR207 CX-PAGFP. Recombination of this vector with the Gateway plant binary vector pMDC32 (Curtis and Grossniklaus, 2003) in an LR reaction produced pMDC32 CX-PAGFP. Expression of CX-PAGFP is under control of a single CaMV 35S promoter in this vector.

Marking Golgi bodies with mRFP

In the plant binary vector pVKH18En6 ST-mRFP (provided by Federica Brandizzi), the GFP of pVKH18En6 ST-GFP (Saint-Jore et al., 2002) is replaced with monomeric RFP (provided by Roger Tsien). ST-mRFP expression is under the control of 6x tandemly-repeated CaMV 35S promoters. ST-mRFP marks not only the Golgi bodies but the excess is secreted and fills the apoplastic space between leaf epidermal cells, therefore outlining them, as well.

Plant transformation

4–6-week-old plants of Nicotiana tabacum SR1 cv. Petit Havana were transiently transformed with fluorescent constructs following the protocol of Batoko et al. (2000). Briefly, Agrobacterium tumefaciens GV3101-pMP90 were transformed by heat-shock with the binary vectors described above. Colonies were grown to mid-log phase in 10 ml YEB medium at 28 °C with 100 mg l⁻¹ kanamycin selection. The culture was pelleted and washed twice in infiltration medium [50 mM MES, pH 5.6, 2 mM Na₃PO₄, 0.5% d-glucose (w/v), and 100 μM acetosyringone (Aldrich)]. Resuspended bacteria were diluted to an optical density (∼600 nm) of 0.1 and 0.05 for CX-PAGFP and ST-mRFP, respectively. Small needle marks were made in the leaves and diluted bacteria were injected into the leaf lamina through the lower epidermis using a 1 ml syringe (without needle) and gentle pressure. When co-expression of the PAGFP and mRFP containing constructs was desired, the bacteria were mixed in appropriate volumes of infiltration buffer prior to injection into the leaf. Fluorescent protein expression was studied in lower epidermal cells between 2–6 d after transformation.

Confocal microscopy

Confocal imaging and activation of photoactivatable GFP was done with a Zeiss LSM 510 META system. PAGFP emits very little fluorescence at 510 nm by excitation with the 488 nm line of a argon laser prior to activation. A 25 mW blue diode 405 nm laser was used at fairly high output (25–50% transmission) to target small regions (2 μm diameter) of the endoplasmic reticulum using the photobleaching function of the Zeiss software in time-lapse mode. Generally, 3–5 pulses of the 405 nm laser were sufficient to activate PAGFP so that it produced very bright fluorescence emission which was detected by excitation at 488 nm using a 500–530 nm band pass filter. The 488 nm line of the argon laser was used at a very low transmission percentage (3.2%) for time-lapse imaging of the photoactivated GFP and in tests on fully activated cells this resulted in no appreciable photobleaching during the time-course (1–2 min) of most experiments. Golgi body mRFP fluorescence was excited with a 543 nm HeNe laser and emission was detected using a 565–615 band pass filter. Most of the images used for subsequent analyses were made using a 63x 1.4 NA oil immersion objective. Pieces (5–10 mm²) were cut out of the transformed leaf lamina and mounted under a 50 mm long coverslip in water so that the lower epidermis was facing upwards. The majority of time-lapse imaging of the cortical ER in this study was done in two dimensions, i.e. time resolved x–y. Movement of the photoactivated ER membrane was analysed for 15 cells in which the actin cytoskeleton was functioning and for 5 cells in which it had been depolymerized by treatment with latrunculin B (see below).

Latrunculin B treatment

Leaf pieces cut out as described above were placed into 25 μM latrunculin B (Calbiochem) in an Eppendorf tube for 1 h prior to imaging to depolymerize the actin cytoskeleton. The working solution of latrunculin B was made immediately prior to use from a frozen stock solution (10 mM in DMSO).

Image analysis—photoactivated ER membrane

Time-lapse series of the photoactivated GFP were analysed using the Radial Profile Plot plug-in of ImageJ (freeware available from the
NIH at http://rsb.info.nih.gov/ij/). This plug-in produces a profile plot of normalized integrated intensities around concentric circles as a function of distance from a point in the image. Movement of activated ER membrane away from the activation spot was recorded as an increase in intensity at a distance from the centre spot or as a decrease in intensity at the centre spot in time. Intensity at a given diameter was used to plot intensity decay curves for the time period from peak intensity after activation. For this report, the curve that contained the highest post-activation normalized intensity (see below) was used in the analysis of each activation. Fitting these curves using SPSS software (SPSS Inc.) enabled the dynamic nature of ER membrane movement to be estimated. As a first approximation, a decay curve that is well fit by an exponential equation:

$$f(t) = b_0 \exp(-b_1 t)$$

where $b_0$ and $b_1$ are parameters of the curve, can represent diffusive movement. When the decay is better fit by other curves, for example, the inverse curve:

$$f(t) = b_0 + (b_1 / t)$$

it is likely that other factors, for example, energetic processes, are involved in the movement. Raw intensity data was normalized across all data sets using the following equation:

$$I_n = [(I_t - I_{min})/(I_{max} - I_{min})] \times 100$$

where $I_n$ is the normalized intensity, $I_t$ is the intensity at time $t$, $I_{min}$ is the minimum intensity in the data set, and $I_{max}$ is the maximum intensity in the data set.

Various curve-fitting models were used to fit the normalized intensity data and statistics were generated from the equation with the highest $r^2$ value in each case. The speed of CX-PAGFP movement was estimated by calculating the half-time of intensity decay ($t_{1/2}$) using different equations based on the best curve fit in each case:

1. **Exponential curve fit:** $t_{1/2} = \left[\ln(I_{1/2}) - \ln(b_0)\right]/b_1$
   where $I_{1/2} = (I_{max} - I_{min})/2$

2. **Inverse curve fit:** $t_{1/2} = b_1/(I_{1/2} - b_0)$

Finally, the mobile fraction ($A$) of fluorescent protein in the membrane was estimated by calculating the intensity difference between photoactivation and the end of the time series:

$$A = I_a - I_n$$

where $I_a$ is activation intensity (normalized to 100%), $I_n$ is intensity at the final time point.

**Image analysis-tracking Golgi body movement**

Movement of individual fluorescent particles in time-resolved 2-, or 3-D. In 5/15 cases where photoactivation of the PAGFP in the ER membrane was analysed, Golgi body movement was also analysed. All Golgi bodies within a large region of interest, that extended at least in a 12 μm radius beyond the activation spot, were analysed. Time series images were filtered to remove background and thresholding was used so that the software could reliably track moving Golgi bodies. Various relevant statistics about Golgi movement including velocity, track length, and an index of movement pattern (the ‘meandering’ index) were generated automatically. In addition, the Volocity software was used to generate vectorial maps of Golgi movement within the region of CX-PAGFP activation so that comparisons were possible between the direction of GFP movement and Golgi body movement.

**Results**

Supplementary movies showing the photoactivation of PAGFP in the ER membrane can be found at JXB online. Movie 1: rapidly remodelling ER membrane (Fast ER membrane (B019-6).avi). Actual elapsed time 40.9 s (4.6 Mb). Movie 2: slowly remodelling ER membrane (Slow ER membrane (B022-8).avi). Actual elapsed time 97.1 s (3.3 Mb). Movie 3: non-remodelling ER membrane after treatment with latrunculin B (Latrunculin B ER membrane (B024-9).avi). Actual elapsed time 120.1 s (7.2 Mb).

**Photoactivation of the ER membrane: control**

GFP-HDEL highlights the reticulate nature of the cortical ER in cells of the lower leaf epidermis of N. tabacum (Fig. 1a). Reticulate-patterned areas of ER remodel relatively slowly while trans-vacuolar strands that are seen traversing the cell flow quickly. Golgi bodies associate with the ER reticulae and strands (Fig. 1a). Overexpression of CX-PAGFP in the ER membrane resulted in a change in ER morphology such that flat sheet-like regions of ER were observed (Fig. 1b). Sheets of ER were connected by tubules that appeared similar to those of the normally reported ER network. Because the ER is not visible prior to photoactivation of the PAGFP in its membrane, only about 75% of the cells that were targeted yielded analysable results. The other cells either had not been transformed with the CX-PAGFP construct or the ER was not in the focal plane before the scanning began. Photoactivated GFP in the ER membrane was found to move away from the site of laser activation at various speeds (Fig. 1c–j). $t_{1/2}$ values of intensity decay ranged from 5.12 to 18.57 s (mean $t_{1/2} = 9.28 \pm 5.39$ s, n=15) (Table 1). Visual inspection of the ER morphology in the activated region indicated a strong correlation between ER morphology and $t_{1/2}$. Fast intensity decay occurred when the targeted area of membrane was within a fast-moving strand of cytoplasm and slow intensity decay occurred in areas of tubular and planar ER that appeared static. The salient fact arising from this observation is that the ER membrane is a fluid structure in which CX-PAGFP is mobile. More importantly, the type of curve equation used to derive $t_{1/2}$ values gave clues as to the mechanism of membrane movement. When PAGFP in the ER membrane was fast moving, the intensity decay curve was always fit better by the inverse equation (e.g. $r^2$ range 0.82–0.97) than by the exponential decay equation (Fig. 2a) (n=13). Only in two cases of the slowest moving PAGFP, did the exponential decay curve provide better fit ($r^2 = 0.96$ and 0.97) (Fig. 2b). The mobile fraction ($A$) of PAGFP in the activated region (i.e. the amount of activated GFP that moved out of the activated region during the course of observation) ranged from 88.97% to 100.00% (mean $A = 94.32 \pm 4.52$, n=15).
Photoactivation of the ER membrane: latrunculin B

When cells were treated with latrunculin B to depolymerize the actin cytoskeleton prior to photoactivation of CX-PAGFP, the exponential decay equation always gave better fit to intensity data than the inverse equation (Figs 1k–n, 2c). $t_{1/2}$ of intensity decay values ranged between 17.94 and 33.33 s (Table 1) ($n=5$). The mean latrunculin B $t_{1/2}$ value was significantly longer than in the control case (23.81 ± 8.32 versus 9.28 ± 5.39 s, respectively, $P <0.05$). The mobile fraction ($A$) of PAGFP in the activated region after latrunculin B treatment ranged from 79.36% to 95.46% (mean $A=88.67±6.74\%$). There was no difference in the mobile fraction between the control and latrunculin B treatment.

Golgi body movement

When all of the ER membrane in a cell was photoactivated at once, individual Golgi bodies were observed to move in close association with the ER tubules or along the edges of ER sheets. Golgi bodies were often enclosed within lacunae of the ER sheets (Fig. 1b), but were mobile as the lacunae remodelled quickly. Golgi bodies within a region centred...
on the activation spot were tracked with Volocity version 3.0 (Fig. 3a) (n=5 cells). Several important statistics were generated including: total number of tracks (178±66), track length (4.01±1.24 μm), velocity (0.25±0.39 μm s⁻¹), displacement, i.e. linear distance between start and end point (2.34±0.80 μm), and Meandering Index, i.e. displacement/track length (0.55±0.10) (Table 1). Large variation in the speed of different Golgi bodies within the same cell was responsible for the high standard deviation of velocity. The Meandering Index is derived as a function of particle displacement (i.e. linear distance between first and last measured position) and track length. High values indicate straight tracks and low values indicate a high degree of direction changing along a track. The software also rendered movement of individual Golgi bodies pictorially such that relative direction and speed were represented by vectors of varying length (Fig. 3b, c). In each plot, Golgi tracks are shifted in x–y so that they appear to share a common origin. It was possible to inspect the actual track shape (Fig. 3b) or a directional vector (Fig. 3c) of Golgi movement visually to correlate the direction and speed of Golgi movement with movement of the bulk of photoactivated GFP in the ER membrane (Fig. 3a) in each case. Golgi bodies were always seen to behave in a similar manner to the PAGFP. In particular, when the PAGFP moved very quickly (low \( t_{1/2} \)) away from the activation spot, Golgi bodies moved quickly in the same direction and with high Meandering Index. Low Meandering Index Golgi tracks were associated with slowly moving areas of PAGFP in those cases where flow within the ER membrane was best fit by an exponential decay equation. When cells had been treated with latrunculin B, Golgi bodies did not move (Fig. 1k–n); they became stationary at triple junctions of ER tubules or within lacunae of the planar sheets.

### Discussion

**ER membrane dynamics**

Photoactivatable GFP fused to the trans-membrane domain of calnexin (CX-PAGFP) marks the ER membrane brightly when activated with 405 nm laser light. Activated GFP moves quickly away from the site of activation by a combination of energetic process and diffusion. The actin cytoskeleton is necessary for remodelling of the ER in plants and in animals (Nebenführ et al., 1999; Saint-Jore et al., 2002; Voeltz et al., 2002; Poteryaev, 2005) and large bundles of actin filaments underlie the rapidly moving strands of ER that traverse areas of tubular ER (Fig. 1) (Quader et al., 1987; Boevink et al., 1998). When actin depolymerizing chemicals are used, remodelling of the ER ceases but the ER retains its form. The implication is that there is a connection between the ER and the actin/myosin system but it is, as yet, unknown how they connect. In the absence of actin/myosin remodelling when cells were treated with latrunculin B, CX-PAGFP in the membrane of the ER continues to flow at a reduced rate and disperses throughout the ER in the cell. Intensity decay curves of activated GFP in the absence of actin were well fit by the exponential equation which, as a first approximation, indicates that the mechanism of PAGFP movement is diffusive. Diffusion speeds of proteins within the ER membrane can be affected by a number of different factors including association with other membrane proteins and protein complexes, and aggregation (Nehls et al., 2000). When the actin/myosin system was functioning, the speed of activated CX-PAGFP dispersal varied from far in excess of that resulting from this diffusion alone down to, in 2/15 cases, not significantly different from the rate of diffusion in latrunculin B-treated cells. This suggests that diffusion of CX-PAGFP occurs even in those reticulate regions where fast, actin-driven remodelling is absent. Fast ER membrane movement curves were better fit by the inverse curve function than by the exponential decay function and this allowed an estimation of the speed of fluorescence movement. Components of the movement mechanism, including effective diffusion coefficients for CX-PAGFP in different ER regions may be better estimated using more advanced models for curve-fitting in future experiments. However, Siggia et al. (2000) describe the large potential for variation in effective diffusion coefficients based on structure, for example, tubular versus sheet-like ER, in the bleached ER region.
As an analogy with the ‘mobile fraction’ calculated in photobleaching experiments, which describes the proportion of bleached membrane protein that is replaceable, it has been calculated that most (~90%) of the photoactivated GFP can diffuse away from the site of activation. This suggests that the calnexin sequence, which targets PAGFP to the membrane, is homogenously distributed and does not associate with the immobile fraction. Photoactivation of a mobile fraction protein will tend to increase total cell fluorescence as it redistributes on a diffusion gradient and this might account for the small, apparent immobile fraction observed.
**Golgi body tracking**

Software for motion tracking greatly simplifies the task of describing the movement of motile organelles. In a short time, enough data can be analysed to yield statistically meaningful results. In this experiment, Golgi bodies were tracked as discrete units moving during dispersal of the photoactivated GFP in the ER membrane. On average, Golgi bodies within a cell move at approximately the same speed as that previously reported (this study 0.25±0.39 μm s⁻¹) (Boevink et al., 1998). Large variation in Golgi body velocity can be accounted for by the types of motion that occur within a cell: some Golgi bodies remain relatively stationary or stop and start while others travel quickly with transvacuolar strands of ER. The fastest Golgi bodies measured moved at 0.47±0.12 μm s⁻¹ which is considerably slower than that reported by Boevink et al. (1998) who observed Golgi bodies moving at 2.2 μm s⁻¹.

Examination of individual Golgi bodies moving within regions of photoactivated GFP, however, revealed that their speed and direction was the same as that of the GFP. In cases of fast dispersing GFP, Golgi velocity was high and directionality, as judged from vector plots and the Meandering Index, was strongly oriented along the GFP movement axis (Fig. 3c). Low Golgi body velocities and Meandering Indices occurred in those regions in which PAGFP was found to be dispersing by diffusion alone, i.e. in the slowly remodelling tubular or planar areas.

**The ER/Golgi connection**

Fluidity and diffusion within the ER membrane are important aspects when considering the existing models of plant Golgi body movement in relation to the ER. Both the ER and the Golgi apparatus are known to move over the actin cytoskeleton (Boevink et al., 1998). Individual Golgi bodies move in close association with the tubules of ER without any apparent gap between the two as judged by confocal microscopy. Nebenführ et al. (1999) proposed the stop-and-go model to explain the movement of Golgi bodies over the ER. This type of motion is indeed what happens. On average, Golgi bodies have a Meandering Index of ~0.5 which means that they travel twice their linear displacement. This type of movement seems to be a function of Golgi location within the ER lattice. Golgi bodies are relatively stationary with oscillating type motion within the slowly remodelling regions of ER and move quickly in straight lines with the CX-PAGFP in the ER membrane along fast transvacuolar strands of ER. Functionally, protein export from the ER to the Golgi is thought to occur at the ER exit sites (ERES: Nebenführ et al., 1999).

Discrete ERESs form within the ER membrane in animal and yeast cells when COPII-coated membrane and/or vesicles concentrate after initiation involving the GTPase Sar1p (Bonifacino and Glick, 2004). In plant cells, most of the COPII vesicle-associated proteins have been identified (Phillipson et al., 2001; Hawes, 2005) and discrete ER membrane regions are marked when Sar1p-GFP is co-expressed with a fluorescent marker of the Golgi body membrane, for example, ST-YFP (Brandizzi et al., 2002; daSilva et al., 2004; Yang et al., 2005). It has been shown recently (daSilva et al., 2004) that plant ERESs co-localize and move with Golgi bodies. Based on this observation, if it is predicted that ERESs are protein complexes within the ER membrane, then these data showing that components can move within the membrane (both by passive and active processes) support the idea of mobile exit sites attached to Golgi bodies. Further, the observation that individual Golgi bodies move along with photoactivated regions of ER membrane make the existence of an ER/ERES/Golgi body physical continuity plausible.

When GFP is fused to the calnexin transmembrane domains and expressed in plant cells (Irons et al., 2003), large sheet-like regions of ER form (Fig. 1b). This surface area increase probably results simply from overexpression of a membrane-targeted construct and such sheets are ideal for observation of the ER/Golgi body interaction. Boevink et al. (1998) reported that the cortical ER network in *Nicotiana* leaf epidermal cells precisely overlaid the actin network. The ER sheets reported here are scaffolded at their margins and throughout on actin filaments (not shown) and the majority of Golgi bodies move around the edges and within the lacunae of the ER. Occasionally, as well, individual Golgi bodies are seen that traverse the sheets but in almost no cases do Golgi bodies appear to be disconnected from the ER. This spatial continuity is under investigation in more detail following the observations of Brandizzi et al. (2002) that Golgi bodies can, on occasion, break free of the ER and move along actin filaments ahead of newly forming ER tubules. It would seem that either the connection of Golgi bodies to ERES within the ER membrane has the ability to be transient or that the ERES/Golgi body complex can disconnect from the ER membrane and transit along the actin network.

**Supplementary material**

Movies that illustrate photoactivation of PAGFP in the ER membrane can be found at JXB online. They illustrate three different cases: (i) fast remodelling ER, (ii) slowly remodelling ER, and (iii) non-remodelling ER after treatment of cells with latrunculin B.

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