

RESEARCH PAPER

The first 238 amino acids of the human lamin B receptor are targeted to the nuclear envelope in plants

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Abstract

In plants, the nuclear envelope (NE) is one of the least characterized cellular structures. In particular, little is known about its dynamics during the cell cycle. This is due to the absence of specific markers for *in vivo* studies. To generate such an *in vivo* marker, the suitability of the human lamin B receptor (LBR) was tested. When the first 238 amino acids of the LBR, fused to the green fluorescent protein (GFP), were expressed in tobacco plants, fluorescence accumulated only at the NE of leaf epidermal cells. This was confirmed by electron microscopy. The protein was shown to be membrane-integral by phase separation. Distribution of fluorescence was compared with two ER markers, GFP-calnexin and GFP-HDEL. While co-localization of all three markers was noted at the NE, only LBR-GFP was specific to the NE, while the other two also showed fluorescence of the cortical ER. These results suggest that common targeting mechanisms to those in animals and fungi exist in plants to direct and locate proteins to the NE. This chimaeric construct is the first available fluorescent integral membrane protein marker to be targeted exclusively to the plant NE and it provides a novel opportunity to investigate the dynamics of this membrane system *in vivo*. With it, the cell cycle was followed in tobacco BY-2 cells stably expressing the fusion protein. The interphase labelling of the NE altered in metaphase into an ER-like meshwork, suggesting the dispersal of the NE to ER as in animal cells. Finally, the meshwork of fluorescent membranes was lost and new fluorescent NE formed around the daughter nuclei.

Key words: GFP, lamin B receptor, membrane targeting, *Nicotiana tabacum*, nuclear envelope.

Introduction

The nuclear envelope (NE) is a unique feature of eukaryotic cells and comprises a concentric double membrane, perforated by nuclear pores. The outer NE (ONE) is a functional continuum with perinuclear endoplasmic reticulum (ER), through 'junctional' regions and it has therefore been suggested that the components of this membrane and of the lumen are likely to be identical with it. However, while many proteins are present in both the ER and the ONE, there are functional and physical distinctions between the two membranes, not least because of the highly specialized function of the NE (Franke *et al.*, 1981; Gerace and Blobel, 1982). The inner NE (INE) contains a functionally distinct group of proteins, which includes those involved in maintaining the structure of the nucleus by their interaction with the nuclear lamina (Schuler *et al.*, 1994; Ye and Worman, 1994). To date, knowledge of plant NE organization and protein composition is very limited (see Meier, 2001, for a review). Moreover information on the dynamics of the plant NE during progression through the cell cycle imaged with specific markers in living cells is limited. Progress in this area has been hindered by the absence of a marker uniquely localized to the NE for use in *in vivo* studies. Markers directed to the NE but also localized with other subcellular structures (e.g. RanGAP, MAF1, MFP1; Rose and Meier, 2001; Gindullis and Meier, 1999) have been constructed as GFP-chimaeras, but lack the specificity needed for exclusive analysis of the properties of the NE.

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Abbreviations: NE, nuclear envelope; ONE, outer nuclear envelope; INE, inner nuclear envelope; GFP, green fluorescent protein; LBR, lamin B receptor, PLT, progressive lowering of temperature.

Recent work using RanGAP-GFP fusions in *Arabidopsis* showed a discontinuous distribution of fluorescence, suggestive of nuclear pore association rather than NE membrane (Pay *et al.*, 2002). Immunofluorescence labelling during mitosis showed RanGAP co-localizing with microtubules. Immunolabelling of the protein degrading 26S proteasome showed NE labelling, in addition to other structures (Yanagawa *et al.*, 2002). During mitosis the proteasome labelling colocalized with the microtubules of the mitotic spindle.

To date, the dynamics of the mammalian NE have been successfully investigated using a GFP-fusion with the N-terminal lamin-B receptor domain (Ellenberg *et al.*, 1997). The LBR is a constitutively expressed, 58 kDa integral membrane protein of the INE (Worman *et al.*, 1990; Holmer *et al.*, 1998). It is present in animal, but not plant or fungal cells. The protein has eight transmembrane domains, and a large N-terminus in the nucleoplasm to which the lamins and chromatin bind (Ye and Worman, 1994; Schuler *et al.*, 1994; Takano *et al.*, 2002). These protein-protein and protein-DNA interactions are responsible, in part, for retention in the INE (Soullam and Worman, 1993, 1995). The carboxyl-terminal domain binds to B-type lamins and HP1-type chromatin proteins (Ye and Worman, 1994, 1996; Ye *et al.*, 1997). It also shares similarity in sequence to yeast and plant sterol reductases (Schuler *et al.*, 1994; Ye *et al.*, 1997). Studies using truncated LBR indicate that the N-terminus contains a bipartite nuclear location signal (NLS) and that the first transmembrane domain is necessary and sufficient for protein targeting to the INE (Soullam and Worman, 1993, 1995; Smith and Blobel, 1994). The NE targeting of human LBR has also been demonstrated in yeast (Smith and Blobel, 1993).

The N-terminal domain of the LBR, comprising the nucleoplasmic N terminal region and one transmembrane domain, has been fused to the enhanced GFP and localized to the NE, and to a lesser extent the ER of COS-7 cells (Ellenberg *et al.*, 1997). The LBR-GFP chimaera has allowed the *in vivo* dynamics of interphase and mitotic cells in mammalian cells to be followed (Ellenberg *et al.*, 1997; Gerlich *et al.*, 2001; Beaudouin *et al.*, 2002).

Extensive searching of the higher plant protein and DNA sequence databases indicates that there is no plant homologue to the N-terminal domain of the mammalian LBR (SL Irons and DE Evans, unpublished results), although nuclear lamin-like proteins have been reported to exist in plants (Beven *et al.*, 1991; McNulty and Saunders, 1992; Minguez and Moreno Diaz de la Espina, 1993; Gindullis *et al.*, 2002).

To obtain an *in vivo* marker for studying the dynamics of the plant NE, the suitability was tested of the human LBR-GFP chimaera (Ellenberg *et al.*, 1997) optimized for plant expression. The protein fusion was expressed in stably transformed tobacco plants and cells and followed the

subcellular localization of the LBR-GFP₅ fusion. It was found that it localizes at the membrane of the NE in interphase. This chimaeric LBR construct is the first specific fluorescent marker for the study of the dynamics of the plant NE *in vivo*. With it, it has been possible to follow the *in vivo* dynamics of the NE in actively dividing cells. The fate of components of the NE during NE breakdown and re-formation is of particular importance and has not previously been investigated in living plant cells. In this paper, the migration of the LBR-GFP₅ construct is described during both NE breakdown and re-formation. Together, these results suggest that plants may share common signals for NE targeting with animal and yeast cells, and/or that the LBR may have structural and functional plant homologues.

Materials and methods

Molecular cloning

Standard molecular biology techniques were adopted (Sambrook *et al.*, 1989). To generate a lamin B receptor-GFP fusion with plant optimized expression, the coding region of the first N-terminal 238 amino acids of the human LBR (Ellenberg *et al.*, 1997) was fused to GFP₅ (Haseloff *et al.*, 1997), which lacks the aberrant cryptic intron, and then spliced into pVKH18EN6 (Batoko *et al.*, 2000) at the *Bam*HI-*Sac*I sites. To do so, PCR was performed using oligonucleotides SI16 (5'-GTCGGCGGATCCATGCCAAGTAGGAAATTTGCC) and SI13 (5'-CCAGTCGACGTGGGATCTTTCTGTTTACACATCAACAGC) to amplify LBR and SI17 (5'-GCGTCCGAGCTCTTATTTGTATAGTTTCATCCGCG) and SI14 (5'-CAGAAAGATCCACGTCGACTGGAGAACTTGTTTCAAATGG) to amplify GFP₅. The overlapping PCR was finally performed using the amplified DNA sequences as template using the oligonucleotides SI16 and SI17. The overlapping PCR product was designed to contain a glycosylatable region (N-glyc.; Batoko *et al.*, 2000). Therefore the final LBR-GFP₅ fusion was generated as follows: *Bam*HI-LBR-Nglyc.-GFP₅-*Sac*I.

A GFP₅-calnexin fusion (spGFP₅CX) was generated by overlapping PCR. GFP₅ fused at the 5' end to a sporamin signal peptide and bearing a glycosylatable region (Batoko *et al.*, 2000) was amplified with primers FB60 (5'-CGAGACGGATCCATGAAAGCCTTCACACTCGCTCTCTTCTTAGC) and FB78 (5'-CTCTTTCTCAACATCTAGATCTAGAGTTTCTGCTCCTTTG). The last 236 base pairs of arabidopsis calnexin (Huang *et al.*, 1993) were amplified with oligonucleotides FB80 (5'-GCGCCGGAGCTCCTAATTATCACGTCTCGGTTGCC) and FB79 (5'-GAAACTCTAGATCTAGATGTTGAGAAAGAGAAACAAAGGCAGAAGAGG). A spacer of seven amino acids was inserted between the GFP₅ and the calnexin sequence. The overlapping amplification was performed with oligonucleotides FB60 and FB80. The overlapping PCR product was designed to contain a glycosylatable region between the signal peptide and the coding region of calnexin (Batoko *et al.*, 2000). The construct was inserted between the *Bam*HI and *Sac*I sites of pVKH18En6 (Batoko *et al.*, 2000).

The ER-targeted yellow fluorescent protein (spYFP-HDEL) was generated by amplification of a c-myc tagged EYFP (Clontech) with oligonucleotides FB116 (5'-CGCCAGGCAACGTCGACTGGCGAGGAGCTGTTACCGGGGTGG) and FB90A (5'-GCGCCGAGCTCCTAAAGCTCATCATGCAATCCTCCTCAGAGATAAGTTTCTGC). The amplified product was inserted downstream of a sporamin signal peptide at a *Sal*I/*Sac*I site of an existing sporamin

signal peptide-GFP₅-HDEL construct cloned into pVKH18En6 binary vector.

Stable expression

Stably transformed plants were generated via *Agrobacterium tumefaciens*-mediated transformation as described by Hadlington and Denecke (2001). Stable BY-2 cell transformation was achieved as described in Saint-Jore *et al.* (2002). Double transformation was achieved as in Saint-Jore *et al.* (2002) with the following amendment: wild type BY-2 cells were incubated with 50 µl each of LBR-GFP₅ and spYFP-HDEL transformed agrobacteria for 2 d, the cells were washed and plated onto antibiotic plates, and selected as previously described (Saint-Jore *et al.*, 2002).

Synchronization

Stationary phase cells, maintained in 20 ml volumes at 27 °C on an orbital shaker at 130 rpm, were transferred (1 ml) into 20 ml fresh BY-2 medium, supplemented with 5 µg ml⁻¹ aphidicolin in DMSO and 40 µg ml⁻¹ hygromycin (final concentrations). Cells were returned to the shaker at 130 rpm for 24 h at 27 °C. After 24 h the cells were washed by gentle agitation in a sterile fine mesh filter in fresh BY-2 medium containing no aphidicolin or antibiotics and the washing medium was changed several times (final volume 500 ml). The washed cells were resuspended in 20 ml fresh medium plus hygromycin and shaken for 11 h prior to viewing with a confocal microscope.

Imaging

Confocal imaging was performed using an inverted Zeiss LSM 510 Laser Scanning Microscope with a 40× oil immersion objective. For imaging expression of GFP constructs alone or in combination with YFP, the single- and multi-track facilities of the confocal microscope were used, respectively, as previously described (Brandizzi *et al.*, 2002b). For imaging GFP and ethidium bromide (EtBr), the 488 nm excitation line of an argon ion laser (GFP) and the 543 nm excitation line of the helium laser (EtBr) were used alternately. Fluorescence was detected using a 488/543 nm dichroic beam splitter and 505–530 nm band pass filter for GFP and 560 nm long pass filter for EtBr. Post-acquisition image processing was with an LSM 5 Image Browser (Zeiss) and Adobe Photoshop 5.5 software.

Ethidium bromide staining involved the incubation of leaf tissue or BY-2 suspension cultures with EtBr (50 µg ml⁻¹) and 50 µg ml⁻¹ RNase for 30 min (Brandizzi and Caiola, 1998).

For imaging expression in leaves, roughly 1 cm² of leaf tissue was mounted in water on a slide. For imaging BY-2 cells, 50–100 µl of cells were taken from a suspension culture and put on a slide prior to confocal observations. Samples were analysed at room temperature.

Electron microscopy

Leaf material was prepared for electron microscopy using the progressive lowering of temperature (PLT) technique as described by Gunawardena *et al.* (2001) with the exception of the fixative used. For this study, leaf material was fixed for 1 h in 1% paraformaldehyde/1% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 6.9).

For immunogold labelling, sections were treated as described in Gunawardena *et al.* (2001) using as primary antibody anti-GFP (Molecular Probes, Leiden, The Netherlands) diluted 1:3000 in PBS BSA (1%). Control grids were incubated in the absence of primary antibody. Sections were then washed (3×10 min) in PBS BSA 1% fish gelatin before incubation for 1 h at room temperature in secondary antibody (10 nm gold conjugated goat anti-rabbit secondary antibody, British Biocell, Cardiff, UK) diluted 1:20 with 1% fish gelatin in PBS BSA (1%). Sections were then viewed using a JEOL 1200 EXII transmission electron microscope. Sections

were then post-stained using uranyl acetate and lead citrate (Reynolds, 1963) before examination.

Phase partition

The membrane location of the LBR-GFP₅ construct was assessed using Triton X-114 (TX-114) partition as described by Bordier (1981) using 0.2–1.0 mg ml⁻¹ of proteins extracted from leaf tissue and BY-2 cells in 10 mM TRIS-HCl, 150 mM NaCl, 0.5–1.0% TX-114.

Protein concentration

The total, soluble and integral membrane proteins from the phase partition were concentrated by incubation with saturated ammonium sulphate solution (1 ml protein sample to 1.5 ml ammonium sulphate solution) overnight at 4 °C. Tubes were centrifuged at 13 000 rpm in a microfuge for 10 min, the supernatant removed and protein resuspended in TE (50 mM TRIS, 2 mM EDTA). Determination of protein concentration was carried out using the Bio-Rad Protein assay following the manufacturer's instructions.

SDS polyacrylamide gel electrophoresis

Electrophoresis was performed in denaturing conditions using a discontinuous buffer system (Laemmli, 1970). SDS polyacrylamide gels (12%, pH 8.8) with stacking gels (pH 6.8) were prepared using the Bio-Rad Mini Protean II unit. 15 µl of each protein sample, diluted 1:1 with 2× SDS PAGE loading buffer (Sambrook *et al.*, 1989), were loaded on the gels and run at 100 V until the dye front reached the end of the gel. Western blotting (Sambrook *et al.*, 1989) was performed using the Bio-Rad mini-blot system for wet blotting, blotting for 1 h at 100 V onto Schleicher and Schuell 0.45 µm nitrocellulose membrane. The blotted membranes were blocked with PBST 5% skimmed milk powder, then immersed in primary antibody in PBST 5% skimmed milk powder (anti-GFP 1:3000 dilution) overnight at 4 °C. Primary antibody was washed off and a secondary antibody added (goat anti-rabbit conjugated to horseradish peroxidase (HRP) 1:10 000 in PBST 5% milk). Proteins were visualized using an ECL detection system (Amersham Pharmacia, UK) as per the manufacturer's instructions.

Results and discussion

LBR-GFP₅ localizes exclusively to the NE in tobacco leaves

In this study, a GFP₅ (Haseloff *et al.*, 1997) fusion construct of the first 238 amino acids of the mammalian LBR was generated and expressed in tobacco leaves and BY-2 cells under the control of an enhanced 35S promoter.

Tobacco epidermal cells stably expressing LBR-GFP₅ and stained with ethidium bromide showed intense red fluorescence localized at the nucleoplasm (Fig. 1A). When the same plant material was analysed with the imaging settings for GFP fluorescence, bright fluorescence was localized at the rim of the nuclei, strongly indicating labelling of the NE (Fig. 1B, C). No fluorescence was detected in the cortical endoplasmic reticulum (Fig. 1D).

To compare the subcellular distribution of LBR-GFP₅ with ER markers, tobacco leaf epidermal cells expressing a GFP₅-calnexin fusion (spGFP₅CX, Fig. 1E, F) and ER targeted/retained GFP₅ (spGFP₅-HDEL; Fig. 1G, H), which are ER membrane and soluble markers, respect-

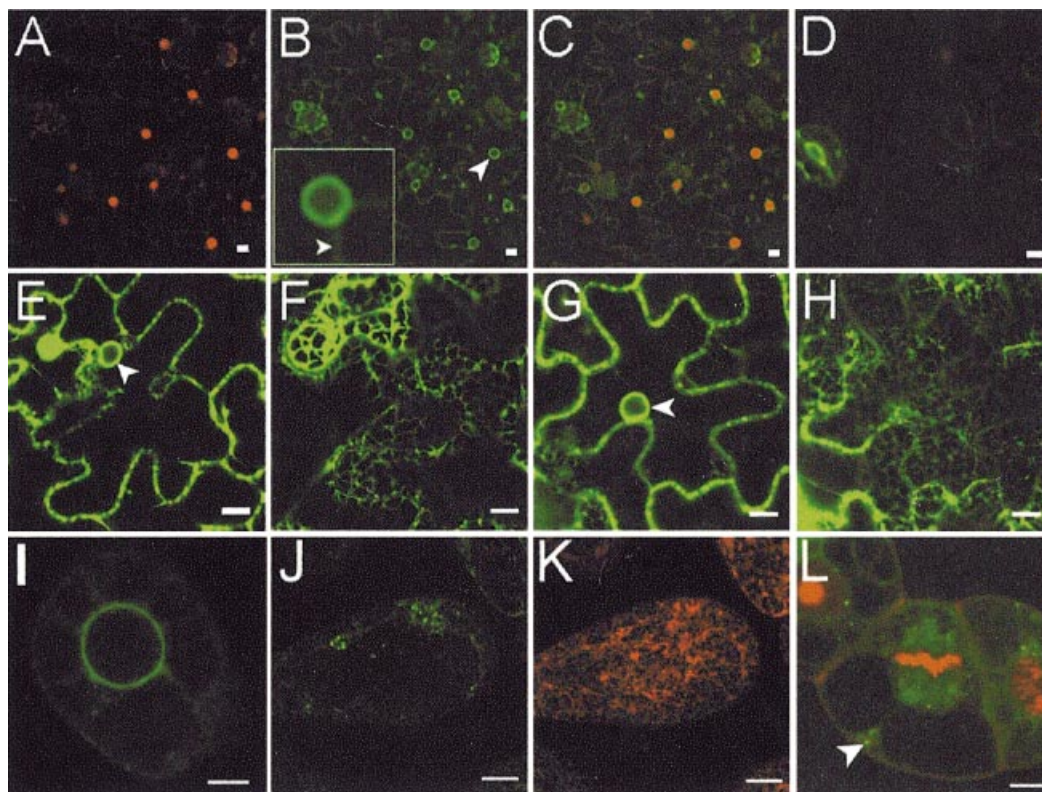


Fig. 1. (A–D) Ethidium bromide-stained stably expressing LBR-GFP₅ tobacco leaf epidermal cells. (A) ethidium bromide staining of chromatin; (B) LBR-GFP₅ fluorescence localized to the nuclear rim in leaf epidermal cells (arrow). Inset: magnified nucleus, adjacent auto-fluorescent cell wall (arrow); (C) merged image of (A) and (B); (D) view of the cortex of stably transformed leaf epidermal cells showing no ER fluorescence from LBR-GFP₅. (E) Transiently expressing sGFP₅CX tobacco leaf epidermal cell showing bright NE and ER fluorescence; (F) view of the cortex of the leaf epidermal cell shown in (E), sGFP₅CX shows intense fluorescent labelling of cortical ER. (G) stably expressing spGFP₅-HDEL tobacco leaf epidermal cell, bright NE and ER fluorescence; (H) view of the cortex of the leaf epidermal cell shown in (G), spGFP₅-HDEL high ER fluorescence. (I) interphase tobacco BY-2 cell stably expressing LBR-GFP₅, 14 d in suspension. (J, K) Tobacco BY-2 cell stably co-expressing LBR-GFP₅ and spYFP-HDEL, 2 d in suspension. (J) LBR-GFP₅ labelling of cortical ER. (K) The same cell as in (J), imaged for the expression of YFP, spYFP-HDEL heavily labels the cortical ER. (L) Ethidium bromide-stained metaphase tobacco BY-2 cell stably expressing LBR-GFP₅. Arrow indicates bright punctate structure. Scale bar=10 µm.

ively, were analysed. spGFP₅CX and spGFP₅-HDEL accumulated at the NE. However, strong labelling was easily detectable at the cortical ER (Fig. 1F, H). These results indicate exclusive fluorescence location at the NE, unlike spGFP₅CX and spGFP₅-HDEL.

In order to investigate further the subcellular localization of the LBR-GFP₅ chimaera, an ultrastructural study was undertaken by electron microscopy. Antibodies to GFP and immunogold immunocytochemistry were used to detect the location of the expressed protein in the transformants. Gold particles were localized at the NE in cells expressing the construct (Fig. 2A), but not in non-transformed controls (Fig. 2B). It was not possible to discriminate between INE, ONE and NE luminal staining because the indirect immunostaining technique used a secondary antibody for detection. The GFP domain of the construct is anticipated to be NE luminal, anchored to the membrane by the LBR domain and the combined size of

the primary and secondary antibodies limit the resolution of the technique.

The LBR-GFP₅ construct is an integral membrane protein

LBR is an integral membrane protein in mammalian cells (Soullam and Worman, 1993, 1995). As plant cells were used as a heterologous system for LBR-GFP₅ expression, a mislocation of the chimaeric protein in the endomembrane system had to be excluded. Therefore, a phase separation assay (Bordier, 1981) was carried out in order to assess the cellular distribution of the LBR-GFP₅ fusion. The assay is based on partitioning total cellular extracts between an aqueous phase and a detergent phase obtained with extraction in Triton X-114; membrane integral proteins partition into the detergent-enriched phase, while soluble proteins partition with the aqueous phase. As membrane and soluble markers of the endomembrane system

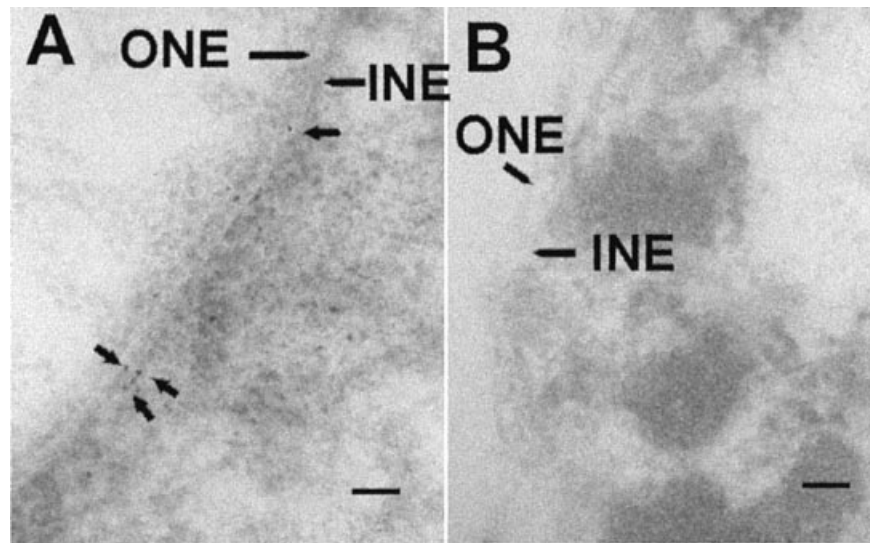


Fig. 2. (A, B) Electron microscope immunocytochemistry of LBR-GFP₅ distribution at the NE of a stably expressing LBR-GFP₅ tobacco leaf epidermal cell. Sections were stained with anti-GFP primary antibody, followed by secondary 10 nm gold antibody (A). Arrows indicate the position of gold particles. The position of the inner NE (INE) and outer NE (ONE) are indicated. A control in which no primary antibody was added is shown in (B). Scale bars represent 100 nm.

spGFP₅CX and sp-GFP₅-HDEL, were adopted respectively. Figure 3 shows that LBR-GFP₅ and spGFP₅CX partitioned in the detergent phase. The spGFP-HDEL fusion partitioned in the aqueous phase, as expected, while LBR-GFP₅ was absent from it. These results indicated that the construct was membrane integral. The lower molecular weight bands seen in the aqueous and Triton fractions may originate from GFP₅ clipping from the LBR-GFP₅ construct. Such GFP cleavage has been observed in other GFP membrane protein constructs expressed in tobacco cells (Brandizzi *et al.*, 2002a) and with soluble protein GFP fusions in tobacco protoplasts (Frigerio *et al.*, 2001).

Taken together, the results from confocal and electron microscopy and biochemical investigations indicate that LBR-GFP₅ fusion is a membrane integral protein that locates at the NE membrane in plant cells as it does in animal cells.

LBR-GFP₅ highlights NE dynamics in BY-2 cells

The LBR-GFP₅ fusion was then used as a vital marker specifically to follow NE dynamics during the cell cycle in plant cells *in vivo*. As far as is known, this has never been achieved before. To do so, BY-2 cells were stably transformed (Fig. 4) and followed through the cell cycle.

In BY-2 cells stably expressing LBR-GFP₅ the presence of bright fluorescence was observed, localizing mainly at the NE (Fig. 1I). Faint ER was also detected. Thus young cultures resembled stably transformed plants with a high specificity of NE staining. Similar ER labelling with LBR-GFP₅ was also found in mammalian cells (Ellenberg *et al.*, 1997). The ER labelling intensified in BY-2 cultures that had been repeatedly transferred.

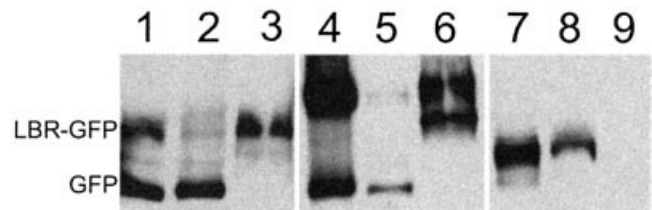


Fig. 3. Western blot of GFP₅ protein constructs extracted from plant material, visualized using ECL (see materials and methods). Lane 1, LBR-GFP₅ total protein extract; 2, LBR-GFP₅ aqueous fraction; 3, LBR-GFP₅ Triton X-114 fraction. LBR-GFP₅ partitions to the Triton fraction confirming it as an integral membrane protein. The lower molecular weight bands seen in the aqueous and Triton fractions are GFP₅ cleaved from the LBR-GFP₅ construct. The dual bands in LBR-GFP₅ total and Triton fractions are likely to be the result of incomplete glycosylation (lanes 1, 3). Lane 4, SpGFP₅-CX total protein extract; 5, SpGFP₅-CX aqueous fraction; 6, SpGFP₅-CX Triton X-114 fraction. The two bands in the SpGFP₅-CX total and Triton fraction lanes are due to incomplete glycosylation of the expressed proteins. SpGFP₅-CX, a known membrane protein therefore partitioned to the Triton fraction as expected. Lane 7, SpGFP₅-HDEL total protein extract; 8, SpGFP₅-HDEL aqueous fraction; 9, SpGFP₅-HDEL Triton X-114 fraction. SpGFP₅-HDEL is present in the aqueous fraction only, concurrent with its luminal location *in vivo*. Double bands seen in HDEL total and aqueous lanes are a result of degradation.

BY-2 cells co-expressing LBR-GFP₅ (Fig. 1J) and spYFP-HDEL (Fig. 1K), a soluble ER marker, show much brighter YFP fluorescence of the ER in comparison to LBR-GFP₅. In addition to the NE, the construct decorated punctate structures (Fig. 1L). In yeast, the chicken lamin B receptor was found to be located at the NE and was also noted in stacks of membrane formed in the transformants. It was suggested that these membrane stacks were the

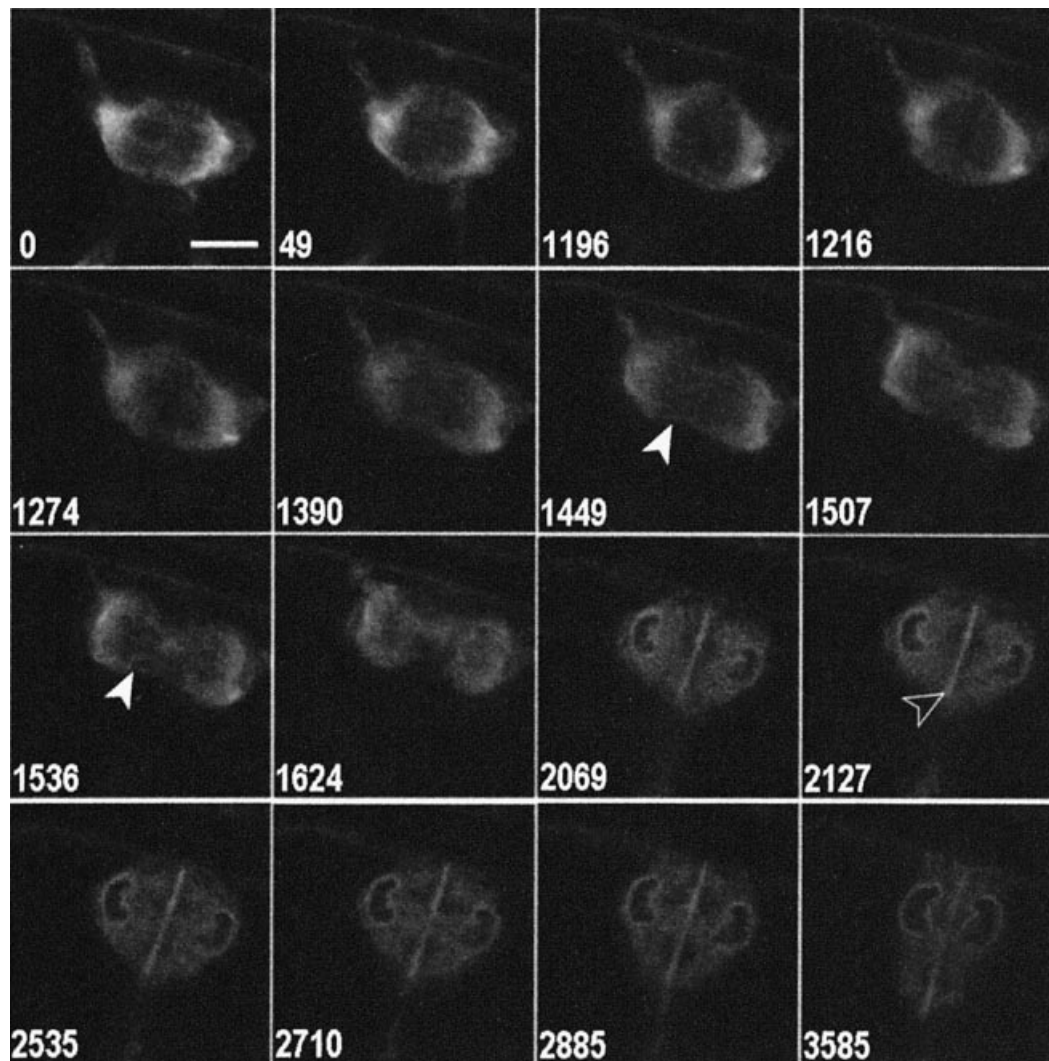


Fig. 4. Numbers refer to time, in seconds, elapsed from the start of images. Cells in late metaphase expressing LBR-GFP₅ show fluorescence distributed through the ER membranes (time 0–1216 s). Tubular membraneous structures form through the mitotic apparatus (arrow, time 1274–1507 s). As division progresses the membranes move towards opposite poles as the chromosomes separate (time 0–1536 s). The ER membranes encircle the newly formed daughter nuclei (1624 s). The NE begins to form around each nucleus (2069 s). The phragmoplast (marked with an empty arrow), which is the basis for the cell wall formation in dividing the cells, forms between the nuclei, this grows across the cell as more wall is assembled (2069–3585 s). Scale bar=20 μ m.

result of accumulation of membrane containing over-expressed LBR when the NE was saturated with the protein (Smith and Blobel, 1993). The fluorescent spot-like structures found in BY-2 cells may be analogous to this. These structures were also present during interphase and cell division (Fig. 1L).

The distribution of the LBR-GFP₅ was then followed during mitosis (Fig. 4). At interphase, specific NE labelling was present. At metaphase the LBR-GFP₅ labels a membraneous meshwork which is continuous with ER as if the NE disperses into the ER as in animal cells (Ellenberg *et al.*, 1997). Later, LBR-GFP₅ fluorescence was observed in tubular structures resembling tubular ER (Fig. 4, arrows) at the division plate between the two

daughter nuclei. Similar labelling of the ER during mitosis has been shown in plant cells with immunolabelled RanGAP and calreticulin (Pay *et al.*, 2002; Denecke *et al.*, 1995). During phragmoplast assembly, fluorescence increases in the re-forming NE that form amongst the membraneous networks and fluorescence of the networks decreases. This resembles the situation in animal cells, where the NE proteins migrate from ER meshwork into the newly forming NE (Ellenberg *et al.*, 1997). The results therefore strongly suggest that components of plant and animal NE migrate to the ER pool after NE breakdown and the new NE of daughter cells re-form from that pool. The possibility of protein breakdown and synthesis contributing to NE breakdown cannot be discounted,

however, the continued high level and steady presence of fluorochrome throughout mitosis would suggest that the original protein pool is present through mitosis, as in animal cells (Ellenberg *et al.*, 1997).

NE re-forms in the middle of the membranous networks partitioned in the two daughter cells and the two new nuclei get closer to the phragmoplast (Fig. 4).

Conclusions

The subcellular distribution of the LBR-GFP₅ raises several questions on the molecular targeting of proteins to NE in plants. Plants do not have identifiable molecular homologues of LBR. This may suggest that plant NE has a unique composition, which could have arisen differently from yeast and animals. However, targeting the amino-terminal 238 amino acids of the human LBR to the higher plant NE suggests that at least one NE protein targeting and anchoring mechanism, as yet unknown, may be common in plants, animals and yeast. Positive protein targeting of heterologous proteins in plants has been previously reported. For example, the last 52 amino acids of the rat sialyl-transferase are sufficient to locate a GFP fusion to the plant Golgi in tobacco plants and BY-2 cells (Saint-Jore *et al.*, 2002; Boevink *et al.*, 1998), despite the absence of such a protein in plants. This example again suggests that proteins may share similar targeting and retention mechanisms in animal and plant cells.

Our studies continue to determine what domains of the N-terminal LBR are necessary and sufficient for NE targeting in plants. Initially, the efforts will concentrate on determining if the LBR-GFP₅ targets the INE or the ONE or both. Finally, the availability of this novel tool will allow completion of the investigations of NE dynamics during mitosis in relation to other subcellular components.

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