

The Plant Nuclear Envelope

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Abstract The nuclear envelope is an important but poorly studied dynamic membrane system in plants. In particular, surprisingly little is known about the proteins of the higher plant nuclear envelope and their interactions. While structurally similar to the nuclear envelope of other kingdoms, unique properties suggest significant differences. For instance, plants lack sequence homologues of the lamins and instead of centrosomes the entire nuclear envelope surface acts as a microtubule-organising centre. This chapter reviews the structure of the nuclear envelope in relation to its protein domains, namely the inner and outer membrane, and the pore domain. Recent advances in the characterisation of novel proteins from these domains are presented. In addition, new insights into mechanisms for the targeting and retention of nuclear envelope proteins are discussed. The nuclear envelope is of importance in cell signalling and evidence for physical nucleo-cytoskeletal linkage and for the nucleoplasm and periplasm as calcium signalling pools are considered. Finally, the behaviour of inner nuclear membrane proteins during the breakdown and reformation of the nuclear envelope in mitosis is discussed.

1 Introduction

The nuclear envelope (NE) is a complex structure separating cytoplasm from nucleoplasm and is a defining characteristic of eukaryotic cells. In electron micrographs it appears as a two-membrane system with the lumen perforated by nuclear pores, but static images do not convey its complexity and dynamic interactions with the nucleoplasm and nucleoskeleton and with the cytoplasm and cytoskeleton.

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Such interactions require an array of proteins with complex binding interactions as well as mechanisms for transport through the pores, traffic and targeting within the membrane, breakdown and reformation during cell division and for positioning the nucleus within the cell.

Detailed study of the proteins of the plant NE has only recently been undertaken and this chapter will consider advances in knowledge and understanding of its protein constituents and their binding partners largely achieved over the last five years. Large gaps in knowledge remain; in particular key “missing” links whose presence is suggested by current data. This means that it remains an important and under-researched area. There are a number of unique features of the plant NE; hence knowledge from other organisms cannot be generalized to plants. In particular, plants lack structures analogous to centrosomes and the entire outer NE serves as a microtubule-organising centre (MTOC) (Shimamura et al. 2004). The plant NE is associated with the cytoskeleton and nucleoskeleton, but plant nuclei lack sequence homologues of the nuclear lamins (see Morena Diaz de la Espina 2008; and Brandizzi et al. 2004). Thus, understanding the interactions of the plant NE with elements of both cytoskeleton and nucleoskeleton is a particularly important area.

2 Nuclear Envelope Domains

The nuclear envelope is a double membrane surrounding the nuclear material. In it, three separate but linked domains have been identified; the outer nuclear membrane (ONM), the inner nuclear membrane (INM) and, the membrane connection between them, the pore membrane (POM) within the nuclear pore complex (NPC). In addition, invaginations collectively called the nucleoplasmic reticulum (see Prunuske and Ullman 2006) or transnuclear strands (Fricker et al. 1997; Collings et al. 2000) penetrate the nucleoplasm and greatly increase the surface area of the INM and its proximity to chromatin in certain cell types. The nuclear pore complexes are involved in maintaining the individual composition of the three membrane domains as proteins can only diffuse into the INM through them (Mataj 2004). There is an increasing body of evidence to suggest that INM proteins require a nuclear targeting sequence recognised for passage through the pore (Lusk et al. 2007). In addition, protein binding also strongly influences the constituents of each of the domains with protein–protein interactions acting to anchor and thereby enrich proteins within them.

3 The Outer Nuclear Membrane

The ONM is in close association with perinuclear endoplasmic reticulum (PNER) and is linked to it through junctional regions that allow traffic of PNER proteins into the ONM and vice versa (Staehelin 1997). The ONM is also frequently decorated

with ribosomes and functions in protein synthesis (Gerace and Burke 1988). The ONM appears to share most of its protein constituents with the PNER, but the two proteomes, while over-lapping, are not identical and proteins predominantly located at the ONM have been identified (Schirmer and Gerace 2005). The abundance of proteins also differs between the two domains, though their proximity and close connection makes separating them for biochemical quantification difficult if not impossible. It is suggested that the presence of physical constrictions of 25–30 nm in diameter in the junctional regions restrict protein movement from endoplasmic reticulum (ER) to ONM (Craig and Staehelin 1988; Staehelin 1997).

The proteins of the ONM play a vital role in connecting the nucleus to the cytoskeleton, for nuclear positioning, maintaining the shape of the nucleus, signalling and cell division. The bridge between the INM and ONM described in animal systems involves proteins of the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex (Crisp et al. 2006). In the LINC complex, proteins possessing a transmembrane domain spanning the ONM interact via a conserved binding domain with partners that span the INM and interact with nucleoskeletal proteins. These interactions prevent the ONM protein from moving back into the PNER thereby retaining it in the ONM. The LINC complex is considered in detail later in the chapter.

While definite protein constituents of the plant ONM still remain elusive, one possible candidate is DMI1 (doesn't make infection 1). This putative ion channel of *Medicago truncatula* was found in a screen for proteins essential in establishing symbiotic relationships between plants and nitrogen-fixing bacteria (Riely et al. 2006). It was shown to be involved in generating perinuclear calcium oscillations in response to Nod factor signalling. Nod factor is released by nitrogen-fixing bacteria and the corresponding cellular ion fluxes, calcium spikes, lead to the activation of early nodulation genes, which result in root hair nodulation and thus the symbiotic interaction between the plant root and the bacteria (Riely et al. 2006; Peiter et al. 2007). Although DMI1 is an ion channel it is not thought that Ca^{2+} from the periplasmic space uses this channel to efflux into the cytoplasm. Instead, DMI1 has a C-terminal RCK (Regulator of the Conductance of K^+) domain, which when deleted abolishes calcium spikes and interferes with nodule formation. A Green Fluorescent Protein (GFP) fusion to DMI1 was used to localise the protein to the NE. Intriguingly, *Lotus japonicus* homologues CASTOR and POLLUX are localised to plastid membranes in onion and pea roots (Riely et al. 2006; Peiter et al. 2007).

In addition to ONM intrinsic proteins there are also a number of peripheral and soluble proteins that associate with the cytoplasmic face of the outer nuclear membrane. For instance, RanGAP (see Rose 2008) has been shown to decorate the plant ONM. While it has been long known that the entire surface of the plant NE has microtubule (MT) nucleating activity (Stoppin et al. 1996) only recently two proteins were identified to be essential for this function. While animal cells have one MTOC that nucleates MTs, plant cells have so far been shown to have at least three distinct regions with such a function – the NE surface, the cortex underlying the plasma membrane and branching points of pre-existing MTs (Starr and Han 2003). Intrinsic to eukaryotic MT nucleation sites is the gamma-tubulin ring complex (gamma-TuRC), which consists of five gamma-tubulin complex proteins (GCP) and

gamma-tubulin itself (Fava et al. 1999; Murphy et al. 2001). The plant proteins AtGCP2 and AtGCP3 are homologues of *Drosophila* and yeast GCP2 and 3, and have been shown to form a soluble complex with gamma-tubulin that associates with the plant ONM (Seltzer et al. 2007). Intriguingly, both have NE targeting domains that are thought to target the gamma-TuRC to the NE, which is then retained there by associating with an as yet unknown ONM intrinsic protein (Seltzer et al. 2007).

4 The Pore Membrane

The proteins of the nuclear pore and the mechanisms of transport through it have been discussed in detail elsewhere in this volume (Rose 2008). The discussion here will therefore be limited to the proteins and role of the pore membrane. The pore membrane is the only membrane connection between INM and ONM and therefore all proteins destined for the INM must pass through it. It is also the point of anchorage of the proteins of the pore and is important in nuclear pore (and therefore NE) formation.

A number of pore domain proteins have been identified in metazoans and yeast. In a comparative genomic study, Mans et al. (2004) identified five pore membrane proteins (POMS); Pom34, Pom152, Pom 210, Ndc1p and Pom121. They observed that Pom34 and Pom152 were restricted to fungi, Pom121 was unique to vertebrates and only Pom210 and Ndc1 were present in animals, fungi and plants.

Pom34 and Pom121 contain two transmembrane domains that span the pore membrane. Both the N-terminus and the C-terminus of Pom34 reach into the pore and are involved in the molecular organisation of the pore complex (Miao et al. 2006). On the other hand, Pom121 is required for NE formation. Depletion of Pom121-containing membrane vesicles does not affect vesicle binding to chromatin but fusion to form a closed NE does not occur (Antonin et al. 2005). Thus, it appears that Pom121 links formation of the nuclear pore complex with the reassembly of the NE.

Glycoprotein 210 (Pom210/gp210) is a major component of the nuclear pore complex (Courvalin et al. 1990). It contains a large perinuclear N-terminal domain that comprises 95% of its mass and contains an Ig-fold domain, which allows interactions between gp210 and other NE components (Greber et al. 1990; Mans et al. 2004). A single transmembrane (TM) segment anchors the protein into the pore membrane and is followed by a short C-terminal cytoplasmic tail. The TM domain is thought to contain a nuclear targeting signal as it is essential and sufficient for correct localisation of gp210 (Wozniak and Blobel 1992). For it to properly function, gp210 forms homodimers (Mans et al. 2004). Homologues of gp210 are present in *Arabidopsis* and are predicted to be structurally similar to the mammalian and *Caenorhabditis elegans* proteins, with a C-terminal transmembrane region (Cohen et al. 2001).

Ndc-1, a 74-kDa protein with six transmembrane domains, has been identified in the pore membranes of animals and yeast (Winey et al. 1993; Chial et al. 1998).

Stavru et al. (2006) demonstrated that it is also present in spindle pole bodies of *S. cerevisiae*. Ndc1 interacts with soluble Nups such as Nup53 and is therefore essential for anchoring the nuclear pore complex (NPC) to the membrane (Mansfeld et al. 2006). It also plays a role in NPC assembly, in particular in correct assembly of Nups with FG repeats that are implicated in cargo trafficking through the pore (Stavru et al. 2006). On the basis of homology, Mans et al. (2004) suggest that an Ndc1 homologue is present in plants; its localization and interactions, however, are as yet unknown.

Apart from gp210 and Ndc1, plants also possess a family of unique pore membrane proteins. Two of the three WIPs (WPP interacting protein) localise to the ONM/pore membrane in *Arabidopsis* and homologues have also been found in other plant species (Xu et al. 2007). The WPP domain and its importance in RanGAP targeting has been discussed elsewhere (see Rose 2008). Similar to gp210, WIPs1-3 contain a TM domain and nuclear targeting signal that ensure correct localisation to the ONM and pore membrane. A coiled-coil domain at the cytoplasmic side is thought to mediate both dimerisation and association with the WPP domain of RanGAP. Whether the WIPs are also involved in the assembly, anchorage and maintenance of NPC is as yet unsolved. While all three WIPs seem to functionally overlap, a triple knockout abolishes NE anchorage of RanGAP. Curiously, this only occurs in *Arabidopsis* root tips and does not affect the development and growth of the plant. Xu et al. (2007) therefore speculate that RanGAP may be dispensable in root tip nuclear transport and suggest that the members of the WIP family are fairly newly evolved plant ONM/pore membrane proteins. It certainly demonstrates that RanGAP anchorage to the NE can be cell-type specific, a phenomenon that has not been shown in animals so far (Xu et al. 2007).

Thus, there appears to be a divergence between kingdoms in the protein composition of the pore membrane and therefore the anchorage of pore proteins. In plants, only Pom210 and Ndc1 have thus far been identified as components of this ring, though others may yet be discovered. This places one protein with six transmembrane domains and a second single pass protein with a very large luminal domain into this region; it is suggested that they are arrayed in a ring beneath the nucleoporins. As referred to above, all inner nuclear membrane proteins have to traffic through this domain to either enter or leave this membrane and mechanisms for this traffic are therefore of particular interest.

5 The Inner Nuclear Membrane

A number of proteins of the inner nuclear membrane have been characterised in animal cells. Excitement in this field stems, at least in part from the growing number of human diseases such as muscular dystrophies, lipodystrophy, skeletal defects and epilepsy stemming from INM protein mutations (e.g. Wilkie and Schirmer 2006). The variety and function of INM proteins is significant. For

instance, LEM domain proteins (Lamin Associated Proteins (LAPs), Emerin, Man) interact with BAF (barrier to autointegration factor) and are involved in gene silencing (Gruenbaum et al. 2005). In addition, emerin associates nuclear actin to the INM and Man plays an antagonising role in the TGF-beta (transforming growth factor beta) signalling cascade (Gruenbaum et al. 2005; Bengtsson 2007). AS11-3 (amino acid sensor independent) are also signal transducing proteins that prevent inappropriate expression of amino acid permeases (Zargari et al. 2007). Another well-studied mammalian INM protein is the lamin B receptor (LBR). It has its own multimeric protein complex and apart from a sterol reductase activity binds to lamin B, chromatin and is involved in RNA splicing (Chu et al. 1998; Nikolakaki et al. 1997). Other INM proteins connect with the nucleoskeleton, in particular the lamina, and SUN (Sad1/Unc84) proteins even link the nucleoskeleton with the cytoskeleton via the LINC complex (Crisp et al. 2006; see below). Overall, the INM and its proteins are implicated in nucleic acid metabolism, signal transduction, NPC spacing and the tethering of nuclear matrix and chromatin. Most of these functions also involve the lamina as the lamina is tightly associated with the animal INM and is essential for its integrity (Gruenbaum et al. 2005). As no plant homologues of lamins seem to exist (Irons et al. 2003; Rose et al. 2004; Graumann et al. 2007; also see Moreno Diaz de la Espina 2008) it is not surprising that many of the well-characterised animal INM proteins do not appear to have plant homologues either – apart from two notable exceptions (Table 1).

Firstly, two *Arabidopsis* homologues of the yeast SUN protein Sad1 were identified by Van Damme et al. (2004) in a screen for proteins implicated in plant cytokinesis and phragmoplast formation. GFP fusions of AtSad1a and AtSad1b showed them to be localised to the NE in interphase and a putative bipartite nuclear localisation signal suggests that both may be present in the INM portion of the NE (van Damme et al. 2004; Graumann K, unpublished observations). Van Damme et al. (2004) also used the GFP fusions to examine for the first time the fate of plant NE proteins in mitosis. Prior to NE breakdown AtSad1a was observed in dots associated with the nuclear rim and close to the plasma membrane. After NE breakdown the construct surrounded the spindle and phragmoplast. Interestingly in metaphase the proteins had accumulated in a few bright dots at both ends of the spindle, which resembled yeast Sad1 bodies. From this van Damme et al. (2004) argue that AtSad1a and AtSad1b may be involved in MT nucleation during mitosis as well as other functions that the SPB and MTOC fulfil in dividing yeast and animal cells respectively, such as telomere clustering. Our findings that AtSad1a and AtSad1b contain the highly conserved SUN domain (see LINC complex) would support such a hypothesis (Graumann and Evans, unpublished results).

The second family of plant INM proteins is in fact not membrane intrinsic but strongly associates with the NE very much like the earlier described components of the Ran cycle. The Aurora kinase family members regulate mitotic processes and two of the three *Arabidopsis* homologues have been found to localise to the NE (Kawabe et al. 2005). GFP fusions of these were followed throughout mitosis and were found to be present at the mitotic spindle, centromeres and the cell plate (Demidov et al. 2005; Kawabe et al. 2005). As serine-threonine kinases they have

Table 1 Proteins of the nuclear envelope and their putative plant homologues

Protein	Organisation	Function	Plant homologue	Reference
LAP1 and LAP2 (lamin associated proteins)	LAP1A/B/C isoforms LAP2β/γ/δ/ε/ξ isoforms All have LEM (Lamin, Emerin, Man) domain	LEM interacts with BAF (barrier to auto integration factor), which cross links DNA with LEM domain proteins DNA replication and expression NE expansion after mitosis	None LEM domain restricted to metazoa	Foisner (2001); Gruenbaum et al. (2005)
Emerin	LEM domain	Interacts with nuclear f-actin, lamins and BAF Transcriptional regulator, death promoting repressor	None	Gruenbaum et al. (2005)
Man1	LEM domain	Binds BAF and Smads and transcriptional regulators Antagonises TGF β signalling	None	Bengtsson (2007)
LBR (lamin B receptor)	Eight TM with sterol reductase activity N' terminal binds lamin and chromatin	N-terminus associates with b-type lamins, chromatin and other components of its own multimeric complex and is involved in RNA splicing and chromatin organisation	None	Chu et al. (1998)
Nurim (nuclear rim)	Six TM	Tightly associates with nuclear matrix Is hypothesised to have enzymatic action on lamins	None	Hofemeister and O'Hare (2005)
Nesprins	Nesprins-1/2/3 Predominately ONM proteins but isoforms present in INM	In ONM part of the LINC complex In INM interacts with emerin and lamins Speculated involvement in lamina organisation	None	Wilhelmsen et al. (2006)
RFBP (ring finger binding protein)	Ring finger binding domain Type IV p-type ATPase	Phospholipid transporting ATPase Sub nuclear trafficking of transcription factors with Ring domain	Four putative <i>Arabidopsis</i> homologues – ALA4/5/11/12	Mansharamani et al. (2001) Graumann K. (unpublished observation) ^a
SUN (Sad1/UNC-84 homology)	SUN1 and SUN2 C-terminal SUN domain	Interact with lamins on nucleoplasmic side and nesprin at periplasmic side Involved in nucleio-cytoskeletal bridging	Two putative <i>Arabidopsis</i> homologues AtSad1a and AtSad1b	Tzur et al. (2006b); Van Damme et al. (2004) Graumann et al. (unpublished results)
NCX – sodium calcium exchangers		Facilitate transport of Ca ²⁺ from nucleoplasm to perinuclear space Interactions with GM1 potentiate exchange	None	Ledeer and Wu (2007)

^aBLAST search of rabbit RFBP identified hits in *Medicago truncatula*, *Oryza sativa* and *Arabidopsis thaliana*; four of the six *Arabidopsis* homologues have putative bipartite nuclear localisation signals (Motif Scan) and one (ALA5) is predicted to be nuclear (WoLF PSORT)

been shown to phosphorylate histone H3 and in view of their cellular locations they have been implicated in chromosome segregation and cytokinesis (Demidov et al. 2005; Kawabe et al. 2005).

So far AtSad1a, AtSad1b and the Aurora kinases are the only identified plant INM components. Clearly with the plant INM implied in a variety of important nuclear and cellular functions there is great potential for discovering more of its components. A lack of lamins in plants suggests that other lamin-like proteins functionally replace lamins in plants (see Moreno Diaz de la Espina 2008) and that the plant NE proteome may vary considerably from its animal and yeast counterpart (Meier 2001; Brandizzi et al. 2004).

6 Targeting and Retention of INM Proteins in Plants: Studies with the Lamin B Receptor

Bi-directional transport of RNAs and soluble proteins through the NPC are explained in detail in the chapters by Merkle (2008) and Rose (2008). This highly regulated process enables the cell to control the type and amount of molecules that enter and exit the nucleus and therefore DNA and RNA metabolism as well as translation and protein synthesis. This ultimately affects all aspects of nuclear and cellular function. It seems rational to argue that transport of INM proteins would also be regulated as they play crucial roles in chromatin organisation (Lusk et al. 2007). A basic model for correct INM protein localisation is the targeting-retention model (Mattaj 2004). It suggests that INM proteins are co-translationally inserted into the ER membrane and from there diffuse through the ER-ONM-pore membrane continuum into the INM, where they are tethered by protein interactions and thus accumulate. Other membrane intrinsic proteins such as ER and ONM proteins would diffuse out of the INM and are not retained there due to a lack of interaction partners. This model has since been expanded by growing research that points to a more controlled approach of INM protein targeting similar to that of soluble proteins (Lusk et al. 2007). It is thought that INM protein targeting already commences during co-translational insertion into the ER membrane. Both mammalian INM proteins LBR and nurim as well as viral INM proteins were shown to have a sorting signal in their first TM domain, which is recognised by the components of the translocation channel and results in active targeting of the INM proteins to the NE mediated by importin beta (Saksena et al. 2004; Saksena et al. 2006). How the INM proteins pass the pore membrane is still not clear but it has been demonstrated that the process requires energy and results in restructuring of the NPC (Ohba et al. 2004; King et al. 2006; Lusk et al. 2007). Lusk et al. (2007) suggest a bimodal system in which membrane proteins with a cytoplasmic/nucleoplasmic domain smaller than 25 kDa may diffuse through the pore but proteins with larger domains are actively transported. Nuclear localisation signals (NLS) previously found in soluble proteins have also been identified in INM proteins such as LBR and SUN2

and it is thought that NLS and karyopherin-mediated transport is advantageous and even essential for INM protein import (Lusk et al. 2007). In addition to karyopherins, interactions with certain Nups might also be crucial. For instance, deleting Nup170 disrupts INM protein but not soluble protein import (Lusk et al. 2007). Once INM proteins arrive at their destined membrane, binding interactions with lamins, chromatin and other INM proteins are thought to retain them in the INM.

Using a truncated LBR fused to GFP the authors have also studied INM protein targeting in plants (Irons et al. 2003, Graumann et al. 2007). The full length LBR protein has eight transmembrane domains but the N-terminal nucleoplasmic domain and first transmembrane domain are sufficient for INM localisation in animal cells (Ellenberg et al. 1997). When this truncated mammalian LBR containing a bipartite NLS and single transmembrane domain is expressed in a plant system, it localises to the NE (Irons et al. 2003; Fig. 1). Our recent studies suggest that the mechanisms for targeting LBR to the INM are conserved between kingdoms and traffic through the plant pore membrane occurs in the same way as in mammalian cells (Graumann et al. 2007). In addition, it has revealed that strong binding interactions in the INM are not necessary for retention. In mammalian cells the N-terminal domain of LBR binds to lamin B and chromatin, and thereby becomes immobilised at the INM. However, lamin B homologues do not exist in plants and FRAP analysis showed that LBR-GFP is highly mobile in the plant INM (Graumann et al. 2007). The authors favour a model in which LBR is trapped in the INM as so far no nuclear export signal (NES) could be identified in its entire sequence. Building on the theory that INM proteins require NLS and karyopherins for import like soluble proteins it can be hypothesised that an NES and exportins may be involved in export of the protein. Instead, the INM protein remains in the INM and may eventually be degraded by a proteasome pathway (Graumann et al. 2007).

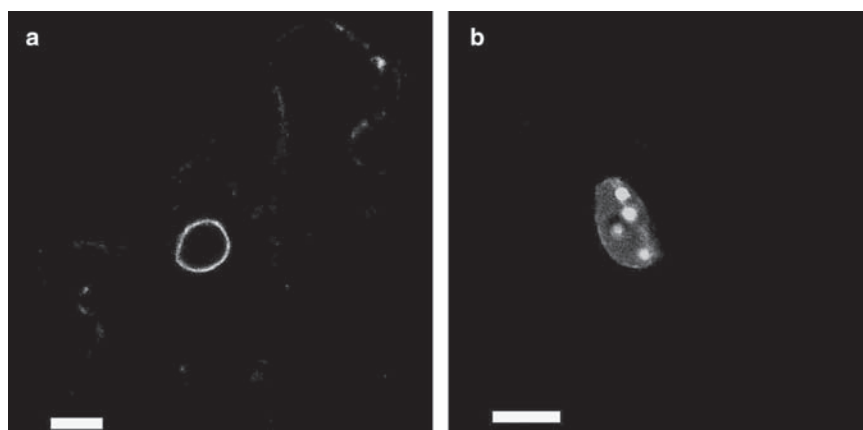


Fig. 1 LBR-GFP localises to the NE when transiently expressed in tobacco leaf epidermal cells (a); when a putative binding domain is mutated the protein is relocated to inclusions inside the nucleus (b). Scale bar = 10 μ m. Graumann, K., unpublished

7 The Lumen and the LINC Complex

Although the NE separates the chromatin from the cytoplasm, the nucleus is not isolated from the rest of the cell. While NPCs provide aqueous channels for molecules to be exchanged between nucleus and cytosol, LINC complexes connect the cytoskeleton with the nucleoskeleton thereby presenting a further route of communication between the nucleus and the cell (Crisp et al. 2006). The composition and function of these bridging complexes have been subject to recent investigations in animal and yeast systems (Starr and Han 2003; Starr and Fischer 2005; Tomita and Cooper 2006; Tzur et al. 2006b; Wilhelmsen et al. 2006; Worman and Gundersen 2006), however very little is known about their existence in plants.

The key components of the LINC complex are ONM located proteins with a highly conserved KASH (Klarsicht/ANC-1/Syne Homology) domain and SUN domain proteins of the INM. The KASH domain in the perinuclear space then interacts with the SUN domain to create a bridge across the two membranes. This connection interlinks cytoplasmic microtubules, filamentous actin and intermediate filaments with nuclear lamins (Starr and Fischer 2005; Wilhelmsen et al. 2006; Crisp et al. 2006). To interact with the cytoskeletal components KASH domain proteins have large coiled-coil domains or spectrin repeat regions that can protrude up to 500 nm into the cytoplasm. Indeed, KASH domain proteins are huge molecules with the largest, human nesprin-1, being just over 1 MDa in size (Zhang et al. 2002; Padmakumar et al. 2004). Actin-binding KASH proteins, such as *C. elegans* ANC-1 protein, have two additional calponin domains at the N-terminus to directly associate with f-actin (filamentous actin) (Starr and Fischer 2005; Wilhelmsen et al. 2006; Worman and Gundersen 2006). *Schizosaccharomyces pombe* Kms1, *C. elegans* ZYG-12 and *Drosophila* Klarsicht proteins are examples of KASH domain proteins that associate with components of the MT cytoskeleton, in particular dynein (Mosley-Bishop et al. 1999; Starr and Fischer 2005; Wilhelmsen et al. 2006). Human nesprin-3 is the only KASH domain protein that has so far been shown to interact with intermediate filaments (Wilhelmsen et al. 2005). On the nuclear side of the LINC complex SUN domain proteins interact with lamins and chromatin. The transmembrane domain, nuclear localisation signals and lamin binding domains but not the highly conserved C-terminal SUN domain are necessary for INM localisation of the proteins. SUN domain proteins also have coiled-coils, which are thought to be involved in homo- and heterodimerisation so that multiple cytoskeletal and nuclear components can be cross-linked (Tzur et al. 2006a). Thus, coupling the nucleus to the cytoskeleton allows for controlled movement, positioning and anchorage of the nucleus inside the cell. The LINC complex also associates centrosomes and spindle pole bodies (SPB) to the NE in animal and yeast cells respectively (Starr and Han 2003; Starr and Fischer 2005) and is involved in centrosome duplication (Kemp et al. 2007). The physical connection between cytoskeleton and chromatin via KASH and SUN proteins has been shown to be necessary for clustering of telomeres and the formation and anchorage of the meiotic chromosome bouquet at the NE (Chikashige et al. 2006; Tomita and Cooper 2006; Schmitt et al. 2007).

Components of the LINC complex are also part of signalling pathways (Starr and Fischer 2005), for example during apoptosis (Tzur et al. 2006a). To date SUN and KASH domain homologues have been identified in the model organisms *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus* and *Homo sapiens* (Starr and Han 2003; Starr and Fischer 2005; Tomita and Cooper 2006; Tzur et al. 2006a; Wilhelmsen et al. 2006; Worman and Gundersen 2006). However, very little is known about their existence in plants. Our recent investigations suggest that while KASH domain proteins may be less conserved in plants, SUN domain homologues exist (Graumann and Evans, unpublished observations). Their identification and characterisation should allow the first insights into the workings of a possible plant LINC complex.

8 The Nucleoplasmic Reticulum and Nucleoplasmic Signalling

The nucleoplasmic reticulum exists in many cells as invaginations of both inner and outer nuclear envelope, often traversing the nucleus. As their lumen is connected directly to the cytoplasm, they greatly increase the surface area of the nucleus and provide regions within the nucleus where nucleoplasm and cytoplasm are in close proximity (Fricker et al. 1997; Broers et al. 1999; Echevarria et al. 2003). They may be branched and contain nuclear pore complexes and are surrounded by the nuclear lamina. The nucleoplasmic reticulum is involved in nucleoplasmic signalling and has been shown to be a site of calcium release (Lui et al. 1998; Echevarria et al. 2003). It may also be involved in the organisation of chromatin via lamin-DNA contacts. Broers et al. (1999) and Lagace and Ridgway (2005) showed that proliferation of the nucleoplasmic reticulum in a number of animal cell types was stimulated by CTP: phosphocholine cytidyltransferase- α , a key enzyme in the CDP-choline pathway for phosphatidylcholine synthesis that shows fatty acid-stimulated localisation to the NE.

The NE and nucleoplasmic reticulum play a key role in intranuclear signalling involving calcium and the inositol trisphosphate pathway. The nucleoplasm acts as a separate signalling domain from adjacent cytoplasm with the NE achieving attenuation of signalling (Al-Mohanna et al. 1994). Calcium signals in the nucleus influence DNA repair and gene transcription and the nuclear envelope and nucleoplasmic reticulum as a calcium signalling pool (Marius et al. 2006); several Ca-responsive proteins have been identified in the nucleus including annexin, transcription factors, calmodulin, and calcium-dependent protein kinases and phosphatases (Bouche et al. 2005; Kalo et al. 2005).

In animals the nuclear envelope contains a number of key components of Ca-signalling pathways: inositol 1,4,5-trisphosphate receptors, ryanodine receptors and Ca-ATPase activity. Inositol 1,4,5-trisphosphate receptors initiate localised nuclear InsP3-mediated calcium signals and the Ca signal generated results in movement of nuclear protein kinase C to the nuclear envelope (Santella and Kyoizuka

1997; Bootman et al. 2000; Marius et al. 2006). In plants, the nucleoplasm has been shown to act as a separate Ca-signalling domain (Pauly et al. 2000). Nuclear Ca responds to temperature and to mechanical stimulation and transient receptor potential (TRP)-like Ca channels have been suggested to be present (Xiong et al. 2004). Patch-clamping isolated red beet nuclei show that non-selective voltage-dependent Ca-channels are present (Grygorczyk and Grygorczyk 1998). Mathematical analysis of Ca transport in the nucleus suggests a model in which the lumen of the NE acts as a Ca-signalling pool into which Ca is accumulated by active transport and released by Ca channels (Briere et al. 2006). In this model, channels in the inner nuclear membrane release Ca into the nucleoplasm. A calcium transporter located in the inner membrane is predicted to pump Ca back into the lumen. The author's laboratory provided immunocytochemical evidence for a Ca-ATPase localised at the nuclear envelope (Downie et al. 1998) but it has not been possible to prove an INM location and the pump may be restricted to the ONM. Further evidence for such a pump at the ONM has been provided (Bunney et al. 2000).

9 The Nuclear Envelope and Mitosis

Plants in common with most eukaryotes, with the notable exception of fungi, undergo open cell division, whereby the NE breaks down at the onset of mitosis and reforms around the nuclei of the new daughter cells. Through the use of fluorescent protein fusions and live cell imaging it has been shown that some NE proteins relocate to the ER during NE disassembly in cultured animal (Ellenberg et al. 1997) and plant cells (Irons et al. 2003; Fig. 2). In the animal cells it was shown that the NE marker (LBR-EGFP) exhibits a significant change in mobility between interphase and mitosis, with LBR being predominantly immobile within the intact NE in contrast to showing a high diffusion rate within mitotic membranes (Ellenberg et al. 1997). The presence of NE proteins in the ER during mitosis has also been shown by immunolabelling of native proteins in mammalian cells (Yang et al. 1997). In plant cells the NE/ER mitosis story remains to be fully described as, despite the clear specific location of the protein to the NE, recent photobleaching data suggests that LBR-GFP shows a high level of mobility within the NE membranes at interphase, and apparently lacks the significant fraction of immobilised protein present in mammalian cells (Graumann et al. 2007).

The possible mechanisms responsible for NE breakdown (NEBD) have been well described in animal cells and to some extent in plants. In animal cells NEBD is the result of microtubule-dependent stretching of the nuclear lamina which causes deformation of the NE and changes in nuclear pore distribution (Beaudouin et al. 2002; Salina et al. 2002). Initial tearing of the NE occurs on the opposite side of the nucleus where the tensile forces are at their greatest; the tearing is possibly linked to the localised disassembly of nuclear pores creating a focal point for the membrane perforation. After the initial tearing of the nuclear membrane, structure of the NE is lost rapidly. In tobacco BY-2 cells co-expressing a microtubule marker (GFP-MBD)

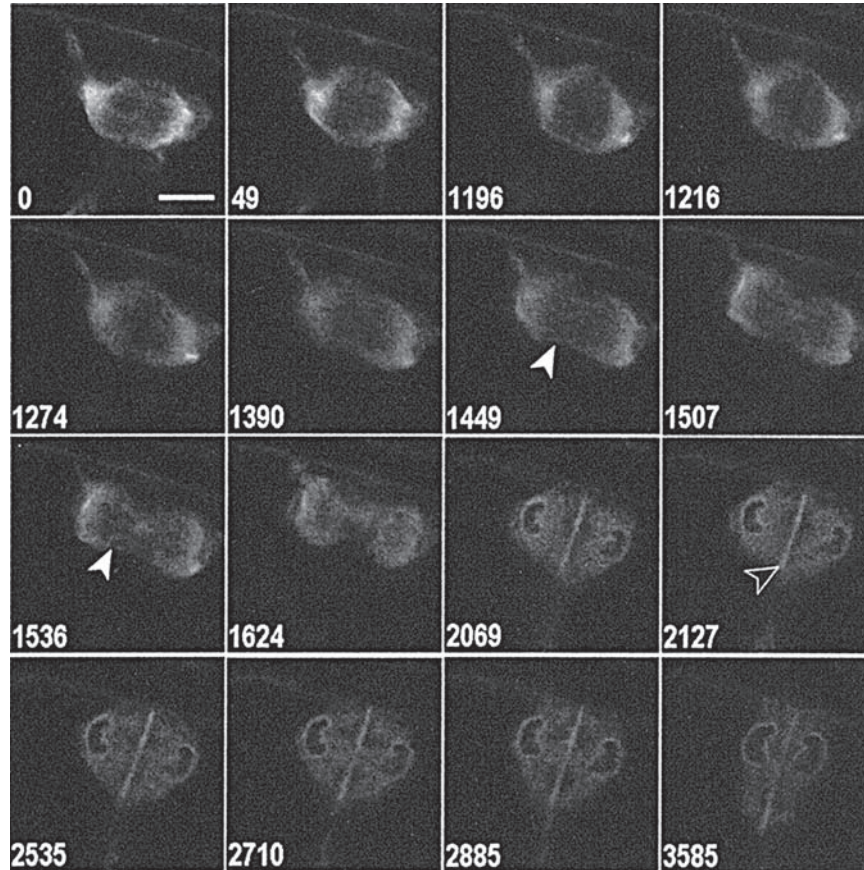


Fig. 2 Cell cycle in BY-2 cells expressing LBR-GFP as a nuclear envelope marker. Cell cycle progression is indicated in seconds as elapsed time from the start of the sequence. A metaphase BY-2 cell transformed with LBR-GFP (Irons et al. 2003) shows fluorescence distributed throughout the ER (time = 0–1,216 s). During the cycle progression, tubular membranous structures form through the MA (*arrowhead*, time 1,274–1,507 s). Subsequently, the ER membranes encircle the newly formed daughter nuclei (1,624 s) and the NE forms around each nucleus (2,069 s). The phragmoplast (*empty arrowhead*) forms between the nuclei and grows across the cell as more wall is assembled (2,069–3,585 s). Scale bar = 20 μm . Reproduced from Irons et al. (2003) with permission

and an ER/Golgi marker (Nag-DsRed) to highlight the NE it was observed that NEBD occurred before the disappearance of the pre-prophase band (PPB), a MT structure characteristic of plant cell division (Dixit and Cyr 2002). The breakdown of the NE followed ruffling of the nuclear membranes in the area of the NE in closest proximity to the PPB. The spatio-temporal link between the loss of the PPB and breakdown of the NE in plant cells suggests that plant NE breakdown is initiated by membrane tearing and involves the attachment of MTs to the surface of the NE (Dixit and Cyr 2002).

A current topic of recent interest in the animal NE field is the process of NE re-formation at the end of mitosis. It has been known for some time that the NE assembles in a stepwise manner, with recruitment of INM proteins preceding that of pore proteins (Chaudhary and Courvalin 1993; Haraguchi et al. 2000). The finer mechanisms of this reassembly are now being elucidated. The INM protein SUN1 has been shown to be recruited at a very early stage to the condensed chromatin of the daughter nuclei (Chi et al. 2007). The protein was shown to associate with a histone acetyltransferase, in addition, when SUN1 expression was reduced, hyper acetylation of histones and delayed chromosome decondensation was observed. As such it suggests a possible functional role for SUN proteins in NE assembly.

Two recent publications using *Xenopus* egg extracts to study NE reassembly have dealt with the physical aspects of membrane recruitment and expansion to produce the new NEs. The recruitment of NE membranes around chromatin has been shown to arise from the intact tubular ER network in vitro (Anderson and Hetzer 2007). The ends of ER tubules were shown to bind directly to DNA and it is proposed that by virtue of the highly dynamic and mobile nature of the ER the membranes can quickly spread over the surface of the chromatin, with the membranes expanding and forming flattened sheets (Anderson and Hetzer 2007). In another study, Baur et al. (2007) demonstrated that, as for other membrane binding and fusion events in the cell, NSF and SNARE proteins are necessary for NE formation and successful pore complex assembly. As with many aspects of the plant NE in cell division there has been very little work undertaken on the process of plant NE reassembly; once again lack of native plant marker proteins is a clear problem.

Examples of location of native plant NE and NE-associated proteins during mitosis are gradually increasing. As previously mentioned immunolabelling of a tomato Ca-ATPase gave a strong NE signal, when studied in dividing cells the protein was found to localise in discrete domains within the mitotic membranes (Downie et al. 1998). This could suggest a possible role for the LCA-1 Ca-ATPase in regulating the calcium signalling pool in the mitotic apparatus (MA). Such targeting to specific regions of the MA raises interesting questions regarding the organisation of discrete domains within mitotic membranes.

Plant RanGAPs, the proteins that activate RanGTPase allowing the conversion of RanGTP to RanGDP, thus aiding the maintenance of the Ran gradient and directionality of nuclear import/export, are located at the outer side of the NE during interphase (Rose and Meier 2001; Pay et al. 2002). During mitosis these proteins are found associated with MTs within the mitotic apparatus (Pay et al. 2002), and are also associated with the phragmoplast at the end of division (Jeong et al. 2005). A specific N-terminal WPP domain in the plant RanGAP mediates the novel location of this protein during mitosis, which differs from that observed for animal cells (Jeong et al. 2005). An antibody raised against the *Arabidopsis* Ran2 protein also differed from the location seen for mammalian cells at interphase, the plant protein being found in the perinuclear and NE region but not in the nucleoplasm whereas the animal Ran proteins are usually present in the nucleus (Ma et al. 2007). These subtle differences in protein location between plant and animal Ran and Ran-associated proteins and kingdom-specific targeting domains highlights the fact that

the plant NE clearly differs from that of its animal counterpart and as such provides a tantalising indication of the novel aspects of the plant nucleus that may be unveiled in the future.

10 Summary and Future Directions

Whilst the importance of the nucleus is well recognised in plant biology, research into its membranes and related protein constituents' remains in its infancy. Some information can be extrapolated from animal and yeast systems, but striking differences, such as the lack of plant homologues to lamins and to the vast majority of known animal nuclear membrane proteins point to the interesting possibility that the plant nuclear membranes contain novel proteins that contribute to an overall nuclear structure similar to that seen in other eukaryotic cells. Approaches using fluorescent proteins to localise novel candidates to the nuclear envelope and to describe their behaviour in processes involving it are proving fruitful in characterising this fascinating and important membrane structure. However, use of multiple approaches, including proteomics, mutagenesis and reverse genetics are needed to further the field. Given the role of the nuclear envelope and its interactions in processes as significant as cell division and in control of gene expression, the field must be considered an important one for future development.

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