The plant nuclear envelope in focus

Katja Graumann and David E. Evans
School of Life Sciences, Oxford Brookes University, Headington Campus, Oxford OX3 0BP, U.K.

Abstract
Recent progress in understanding the plant NE (nuclear envelope) has resulted from significant advances in identifying and characterizing the protein constituents of the membranes and nuclear pores. Here, we review recent findings on the membrane integral and membrane-associated proteins of the key domains of the NE, the pore domain and inner and outer NEs, together with information on protein targeting and NE function.

Introduction
The NE (nuclear envelope) is an important but poorly studied dynamic membrane system in plants. Structurally, it is similar to those of other kingdoms; however, few of its functional protein components have been identified so far. The lack of homologues of well-studied animal and yeast NE proteins [such as LEM1 (lamin–emerin–man1) domain proteins, LBR (lamin B receptor), nesprins and laminas] as well as plant-specific features such as the MT (microtubule)-nucleating activity of the plant ONM (outer nuclear membrane) and lack of centrosomes suggest that knowledge of other organisms cannot be generalized to plants [1–3]. The present review focuses on current knowledge of the properties and features of membrane-intrinsic plant NE proteins as well as their NE targeting mechanisms.

Pore membrane components
Despite the physical continuity of the NE membranes [INM (inner nuclear membrane), pore membrane and ONM], their functions and properties differ due to the presence of domain-specific proteins. The pore membrane is a highly curved membrane that anchors the NPCs (nuclear pore complexes) to the NE. The composition of animal and yeast NPCs has been well studied and it has been found that membrane-intrinsic nucleoporins such as gp210 and pom121 reside in the pore membrane and facilitate the anchorage of the NPC [4]. Research on the composition and function of plant NPC is still in its infancy, and so far only a few functional homologues of animal and yeast nucleoporins have been characterized in plants [5–8]. Putative plant homologues of the pore membrane nucleoporins gp210 and yeast ndc1 (Figure 1) have been identified in silico, suggesting similar organization of the pore membrane and NPC anchorage in plants [9]. In addition, a plant-specific protein family of tail-anchored membrane proteins have recently been found to be located in the pore membrane and ONM and to function in RanGAP (RanGTPase-activating protein) anchorage [10,11]. The role of the three WIPs [WPP (Try-Pro-Pro) domain-interacting proteins; WIP1, WIP2a and WIP3] and the two WITs [WPP domain-interacting tail-anchored proteins; WIT1 and WIT2] in plant-specific NE targeting mechanisms is discussed below.

Calcium signalling at the NE
One established function of the plant NE is in calcium signalling [12]. For instance, perinuclear calcium spiking in legumes is essential for the formation of symbiotic relationships causing root nodulation [13,14]. In both animal and plant cells, the periplasm acts as a Ca2+ store with calcium channels and pumps located in both INM and ONM involved in the regulation of nuclear calcium levels independently of the cytosol [1,12,15]. Downie et al. [16] first reported the presence of the SERCA (sarcoplasmic/endoplasmic reticulum Ca2+ -ATPase) type Ca2+ pump homologue LCA (Lycopersicon calcium ATPase) at the NE in tomato cells. More recently, it was shown that LCA is located in the ONM (Figure 1) and functions to replenish the calcium pool in the NE lumen [15]. Putative INM-localized calcium channels, including P-type calcium ATPases, nucleotidedominated channels and chloride channels, are also predicted to exist and contribute to nuclear calcium homoeostasis [15]. NE-localized ion channels involved in perinuclear calcium spiking include the Lotus japonicus homologues CASTOR and POLLUX and their Medicago truncatula homologue DM1 (Doesn’t make infection 1) [13,14,17]. Their specific functions remain unresolved but it has been shown that they are permeable to cations [18]. It is hypothesized that they either act as counter channels allowing influx of cations into the periplasm in response to Ca2+ efflux or alter the membrane potential of the NE to open voltage-gated Ca2+ channels [18]. It is hypothesized that CASTOR
Figure 1 | Plant NE-intrinsic and -associated proteins
Membrane-intrinsic plant proteins characterized to date include AtSUN1, AtSUN2, LCA, DMI1, CASTOR and POLLUX, as well as WIPs and WITs. Although γ-TuRC proteins and F-actin (filamentous actin) are associated with the outer surface of the NE, it is not known what proteins either directly or indirectly tether them there. Likewise, NMCP1, UNC1, LeMF1 and LeFFPs associate with the inner side of the NE but their anchoring mechanisms are also unknown. Histone H1 was shown to be involved in MT nucleation at the ONM but how the nucleoplasmic localized protein is connected to this cytoplasmic event remains to be established.

Proteins of the animal INM have received attention for their involvement in diseases termed laminopathies [19,20]. This has revealed that in addition to separating nucleoplasm and cytoplasm, the NE, in particular the INM and its protein constituents, are actively involved in various nuclear and cellular processes such as chromatin organization, transcription, RNA maturation and signalling [19–23]. Interestingly, key players involved in these processes such as the LBR and LEM domain proteins have no homologues in plants. Apart from possibly DMI1 and POLLUX, the only other two putative INM-intrinsic proteins identified so far are AtSUN1 [Arabidopsis thaliana SUN1 (Sad1/UNC-84)] and AtSUN2 (Figure 1), putative members of the SUN domain protein family [3,24]. Originally identified as homologues of yeast Sad1 [25], we have shown that the two proteins contain a conserved C-terminal SUN domain [24] and other conserved features including a similar domain structure of an N-terminal transmembrane domain followed by coiled-coil domains as well as the ability to form heteromers and homomers [24]. Their NE localization and presence of a functional bipartite NLS suggest that AtSUN1 and AtSUN2 are components of the plant INM (Figure 1; [24]). In animals and yeast, SUN domain proteins are part of nucleo-cytoskeletal bridging complexes. The INM-localized SUN domain proteins associate with lamins in the nucleoplasm and ONM-localized KASH (Klarsicht/ANC-1/Syne homology) domain proteins in the periplasm that interact with cytoskeletal components including actin, SPBs (spindle pole bodies), MTOCs (MT organizing centres), MT motor proteins and intermediate filaments [23,26–28]. These nucleo-cytoskeletal bridging complexes are essential for a variety of functions including nuclear positioning in the cell, anchorage of telomeres and decondensation of chromatin in cell division and apoptotic signalling [21,23,29,30]. The presence of putative plant SUN domain proteins and the observation that nuclear histone H1 is involved in MT organization at the plant ONM (Figure 1) [31] are the only two indicators that structures similar to the animal and yeast nucleo-cytoskeletal bridging complexes may also be present in plants. If they are, however, then their composition and probably function will differ because plants lack sequence homologues of lamins and KASH domain proteins and have different NE–cytoskeletal associations (see below; [24,32]).

Plant-specific lamin-like elements
In animals, lamins assemble to form a structural lattice closely associated with the INM that maintains the overall structure and shape of the nucleus and is involved in various nuclear processes. Despite the lack of lamin homologues in plants, recent investigations using field scanning SEM (scanning electron microscopy) revealed that tobacco nuclei
also have a filamentous lattice that is similar in structure to the lamina [33]. It consists of thick and thin filaments and interconnects NPC. While the identity of the constituents of the meshwork remains to be established, likely candidates are plant-specific, nuclear filamentous proteins shown to localize to the NE periphery (Figure 1). These include the tomato MAR (matrix attachment region)-binding filament-like protein 1 (LeMFPI), tomato and Arabidopsis filament-like plant proteins (LeFPPIs), carrot NMCP1 (nuclear matrix constituent protein 1) and its Arabidopsis homologues LINC1 (little nuclei 1) and LINC2 (Figure 1) [34–37]. Tomato LeMFPI associates with MARs of DNA to link DNA to the NE and co-localizes with PCNA (proliferating-cell nuclear antigen), which suggests that it is involved in DNA replication [38]. In addition, it binds to LeFPPI, of which over seven genes are present in the tomato genome as well as homologues in other plant species including Arabidopsis [36]. Carrot NMCP1 [34] and its Arabidopsis homologues LINC1 and LINC2, on the other hand, are required for maintaining overall nuclear structure as their deletion reduces the size of nuclei and alters nuclear morphology [37].

**Cytoskeletal associations with the NE**

The plant NE not only associates with nucleoskeletal structures but has also been shown to link to cytoskeletal elements. However, a different organization of the cytoskeleton in plants and the lack of plant KASH domain sequence homologues suggest different NE–cytoskeleton associations. For instance, plants lack centrosomes and instead have several MT nucleating sites: the NE surface, branching points of pre-existing MT and the cortex underlying the plasma membrane [39]. Central to animal, yeast and plant MT of pre-existing MT and the cortex underlying the plasma membrane, several MT nucleating sites: the NE surface, branching points of pre-existing MT and the cortex underlying the plasma membrane, several MT nucleating sites: the NE surface, branching points of pre-existing MT and the cortex underlying the plasma membrane, several MT nucleating sites: the NE surface, branching points of pre-existing MT and the cortex underlying the plasma membrane.

**Targeting of proteins to the plant NE**

The correct targeting of NE proteins, in particular INM proteins, is essential for their proper function and for maintaining overall NE properties. Owing to the continuity of ER (endoplasmic reticulum), ONM, pore membrane and INM, it was thought that INM proteins simply diffuse through the membranes [43]. Comprehensive studies in animal systems have revealed that while passive diffusion may hold true for membrane-bound proteins with nucleoplasmic domains smaller than 25 kDa, it is slow, random and inefficient for accumulating proteins in the INM. Instead, several previous lines of evidence suggest that INM protein import is active, regulated and, similar to soluble protein import, dependent on NLS, karyopherins and the Ran cycle [44,45]. Targeting of INM proteins is thought to start with sorting at the translocon, where in insect cells importin-α-16 associates specifically with INM proteins, which are differentiated from other membrane-intrinsic proteins by their transmembrane domains [46–48]. This event demonstrates that rather than random diffusion of INM proteins through the ER, INM proteins are specifically recognized and are likely to be directly targeted to the INM [45,46,48]. Many INM proteins have been found to have classical NLS, which are recognized by the import machinery and are partly essential for correct targeting to the INM [44,45]. Import of INM proteins was found to be energy dependent [49] and may require changes in NPC structure, for example provided by flexible, sliding nucleoporins [44,45]. In addition, FG nucleoporins are required for INM protein translocation and some FG nucleoporins, such as POM121, are associated with or are in close proximity to the pore membrane [4,45].

Targeting of NE proteins in plants is far less well understood. However, it can be hypothesized that it is similar to that in animal and yeast systems. First, plants show similar mechanisms for the import and export of soluble proteins through NPC [8,32,50]. At least 17 importin-β and eight importin-α homologues as well as three exportin homologues have been identified in plants [1,32]. Most members of the Ran cycle have been characterized [8] and putative nucleoporins with FG repeats have been found in silico [(51); and K. Graumann and D.E. Evans, unpublished work].

Secondly, some plant NE proteins such as DM11, AtSUN1 and AtSUN2 and the WIPs contain putative classical NLS, further indicating the possibility of active protein transport to the plant INM [10,17,24]. Thirdly, our research group showed that the N-terminus and first transmembrane domain of human LBR fused to GFP (green fluorescent protein; LBR–GFP) localize to the INM in tobacco cells, strongly suggesting that mammalian INM targeting signals are correctly recognized by the plant NE targeting machinery [52,53].

Apart from the seemingly conserved INM targeting mechanisms, plants also have a specific NE targeting mechanism for NE-associated proteins, which do not exist in other kingdoms [32]. This system is based on the WPP domain, which is present in LeMFPI, MAF1 (MFPI-associating factor 1) and its Arabidopsis homologues WPP1 and WPP2 as well as Arabidopsis RanGAP [11,32].
Whereas in animals RanGAP is anchored to the ONM by SUMOylation (SUMO is small ubiquitin-related modifier) and binding to RanBP2 (Ran-binding protein 2)/Nup385, in plants the WPP domain is essential for association with the ONM and NPC [32]. A membrane-anchored protein complex consisting of at least one member of the WIPs and WITs (Figure 1) recognizes the WPP domain and anchors the proteins to the NE [11]. The WIPs and WITs in turn are thought to retain their localization at the ONM and pore membrane by interactions with NPC components [11,32]. The RanGAP targeting is specific to undifferentiated Arabidopsis root tip cells, suggesting that this process is linked to development and is more complex than in animal cells [32].

**Conclusions and future prospects**

The work reviewed here reveals significant progress in describing the protein constituents of the plant NE. With this information, the field is rapidly expanding to allow description of fundamental mechanisms. These include the processes of NE breakdown and reformation in mitosis, nucleocytoplasmic interactions, nuclear and chromatin anchorage and the control of gene expression and the processes involved in the formation, function and maintenance of nuclear pores.

**Funding**

We acknowledge support from the Leverhulme Trust [grant number F/00382/H] and Oxford Brookes University.

**References**


69 "References", 310 Biochemical Society Transactions (2010) Volume 38, part 1


Received 30 September 2009
doi:10.1042/BST0380307

©The Authors Journal compilation ©2010 Biochemical Society