Nuclear envelope proteins and their role in nuclear positioning and replication

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Abstract
Controlled movement of the nucleus is important in a wide variety of plant cellular events. Positioning involving intact nuclei occurs in cell division, development, tip growing systems such as the root hair and in response to stimuli, including light, touch and infection. Positioning is also essential in the division and replication of nuclear components, ranging from chromosome attachment to the breakdown and reformation of the nuclear envelope. Although description and understanding of the processes involved have advanced rapidly in recent years, significant gaps remain in our knowledge, especially concerning nuclear proteins involved in anchoring and interacting with cytoskeletal and nucleoskeletal elements involved in movement. In the present review, processes involving the movement and positioning of nuclei and nuclear components are described together with novel proteins implicated in nucleoskeletal and cytoskeletal interactions.

Introduction
Although micrographs of many plant cell types suggest that the nucleus is positioned passively, squeezed between the vacuole and cell wall in a thin layer of cytoplasm, there is considerable evidence that the positioning of the nucleus is active and important in cell function and in development. In addition to the localization of entire nuclei, physical positioning of chromatin is involved in the regulation of gene expression, and movement of other nuclear components is vital in successful cell division. In the present review, we consider nuclear movement and positioning in plants and describe recent advances in the description of underlying structural protein complexes. The proteins of the NE (nuclear envelope), which provide major points of physical attachment through which forces needed for movement are delivered, are the major subject of the present review.

Mobility of entire plant nuclei
Nuclear migration occurs in a wide variety of circumstances, related to both developmental processes and external signalling. For example, migration occurs before the unequal cell division that results in the formation of guard cells [1] and of root hair cells [2]. Nuclear migration is also required at the tissue level, being essential as part of the processes during development that establish the planes of division required for an organized plant body [3,4]. Root hairs provide a good example of the range of nuclear movements observed. It has been know for some time that their nuclei migrate before the unequal cell division that results in hair formation, but also undertake more rapid movement in the developing hair, first to the point of bulging, where the hair will form, and subsequently within the hair itself, where movement of the nucleus appears essential for tip growth and requires the presence of actin [5–9]. Using fluorescent protein constructs with an NLS (nuclear localization signal), Chytilova et al. [7] obtained remarkable images of the pleomorphic state of the Arabidopsis root hair nucleus, sometimes breaking up into sub-structures as it migrated within the hair. Thus, whereas the migration of the nucleus involves actin, presumably associated with attachment to the ONM (outer nuclear membrane), the underlying structure of the nucleus appears to be less rigidly organized than in animal cells.

Movement of the nucleus may also result from external stimuli. Nagai [10] and Williamson [11] described the movement of nuclei to a wound site (termed traumotactic nuclear migration), related directly to wound repair and defence, whereas Qu and Sun [12] observed that movement was rapid and sensitive to repeated stimulation. Using application of external mechanical stimuli to cells in vitro, Lintilhac and Vesecky [3] observed nuclear migration resulting in alignment of division planes with the force applied.

Migration of the nucleus also occurs in response to light and was first shown in the prothallus of the fern Adiantum capillus-veneris L. [13]. Arabidopsis nuclei undergo reversible repositioning in response to blue light irradiance of greater than 50 μmol/m2 [14], believed to be important in avoiding DNA damage. This system also appears to involve...
actin-based movement, through thick actin filaments associated with the nucleus at the base of the cell [14].

**Mobility and positioning of nuclear components**

**Chromatin positioning and gene silencing**

One of the remarkable features of the eukaryotic nucleus is the density of packing of chromosomal DNA, with concomitant issues for the expression of individual genes. It is now recognized in mammalian cells that levels of transcription of regions of chromatin depend to a great extent on their physical arrangement in the nucleus [15]. Gene-poor regions of chromosomes tend to be adjacent to the NE, whereas gene-rich regions are normally in the interior of the nucleus [16]. This localization is not static; for instance, gene-poor regions move from the nuclear periphery to the interior and back during the cell cycle in mammalian cells [17,18] and evidence has been obtained for microdomains at the nuclear periphery likely to associate with different genomic regions with different effects on expression [19]. In mammalian systems, highly active genes cluster at the nuclear pores, whereas inactive genes associate with the proteins of the lamina [20,21]. It has been suggested that localization of genes to the nuclear lamina is important in the epigenetic propagation of gene silencing [22]. That interaction with the nuclear periphery is an active, rather than a passive, mechanism of regulating gene activity, is suggested by the fact that experimental movement of a gene to the nuclear periphery, by tethering it to the lamina, results in a reduction in transcriptional activity [23–25]. Interaction with the nuclear periphery may not only involve association with lamins; experiments with yeast, which, like plants, lack lamins; show that the interaction may be directly with lamins; experiments with yeast, which, like plants, lack lamins; show that the interaction may be directly

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**Nuclear positioning and NE dynamics in cell division**

Higher plants, like animals, undergo open mitosis. The NE breaks down in prophase and reassembles in anaphase/telophase around the decondensing chromatids. NEBD (NE breakdown) commences with tearing of the NE, which in animals is mediated by MTs (microtubules) and dynein [34], whereas in plants MTs and the PPB (pre-prophase band) array of MTs are implicated [35]. NE tearing appears to commence at regions where the NE and PPB are in close proximity and precedes the disintegration of the PPB [35–38]. Disassembly of the NPCs (nuclear pore complexes) accompanies NEBD. In animals, the soluble components of the NPC and NE usually associate with the chromosomes or parts of the mitotic apparatus and are involved in maturation of the mitotic spindle [34]. So far, the location of the plant nucleoporins in cell division remains unknown, apart from the Arabidopsis homologue of Tpr (translocated promoter region), which appears to be associated with the mitotic spindle [39–40]. Similarly, Arabidopsis RanGAP (Ran GTPase-activating protein) is also present at the spindle and at later stages associated with the phragmoplast and cell plate [41]. Proteins of the NE migrate into the mitotic membranes in both animals and plants [36,38,42,43]. Whether specific NE domains remain in the mitotic membranes is unclear as evidence is limited. When the LBR (lamin B receptor)–GFP (green fluorescent protein) construct, based on a non-plant protein, is expressed in plants, it distributes throughout the mitotic membranes [42]. Experiments in our laboratory localizing the tomato Ca$^{2+}$ pump LCA (Lycopersicon Ca$^{2+}$- ATPase), showed localization in specific regions of the membrane network, possibly related to its function in regulating Ca$^{2+}$ signalling in mitotic events [36,44].

In animal cells, both lamins and NE integral proteins are required for NE reformation in anaphase/telophase [43]. For instance, the mammalian INM protein SUN1 associates with chromatin at an early stage and interacts with histone acetyltransferase, resulting in further chromatin decondensation [45]. SUN1 is present in membrane tubules of the mitotic membranes [45], which attach to chromatin, mediated by NE intrinsic proteins, and subsequently expand and flatten to form the NE sheet. Changes in the shape of the membranes are mediated by reticulons; in particular, RTN4A (reticulon 4A) is required for NE expansion [46]. Although it remains unknown how the plant NE reforms, it can be speculated that processes similar to those in animals occur. Support for this comes from experiments that have shown that complete NE
and NPC reform when plant cell extract is incubated with nuclear lamina and vice versa [37]. In addition, plant homologues of SUN domain proteins [31] and reticulons [46,47] have been identified. Finally, it has been shown that the plant NE marker LBR–GFP as well as NE proteins such as the WIPs [WPP (Trp-Pro-Pro)-interacting proteins] and NTIs (WPP-interacting tail-anchored proteins) are present in the reforming NE. Apart from WIP3, they also localize at the cell plate [36,42,48]. This suggests that the NE membrane and cell plate membranes have similar protein compositions [36,42,48].

The events of cell division, including NEBD and NE reformation, involve positioning of nuclear components mediated through binding to proteins associated with nuclear structures and are regulated by various kinases including CDKs (cyclin-dependent kinases) and Aurora kinases, which mediate attachment and detachment events. In animal cells, hyperphosphorylation of INM proteins abolishes their binding interactions with chromatin and other proteins and is essential for NEBD [43]. How CDKs and Aurora kinases regulate plant NEBD and reformation remains to be established. Aurora kinases are associated with the plant NE in interphase and localize to the mitotic spindle, centromeres and cell plate in division. They phosphorylate histone H3 and are implicated in regulating chromosome segregation and cytokinesis [49,50]. Plant cyclins are implicated in chromatin condensation and NE reformation, and plant B1 cyclins have been found to accumulate at the NE [37]. In addition to kinases, RanGAP plays a critical role in NEBD and NE reformation [51]. As well as localizing to the NE, Arabidopsis RanGAP labels the PPB and cortical division zone and is involved in maintaining the division plane and cell file organization in root tip cells. Depletion of the protein arrests dividing cells in metaphase/anaphase and results in cell death in the root tip meristem [51].

**Proteins of nuclear movement and attachment**

Movement of nuclei and nuclear components depends on effective force transmission between the cytoskeleton and nuclear components, mediated through the NE. This involves attachment of elements of the cytoskeleton to the ONM, linkage across the periplasm of the NE and attachment of proteins of the INM to the nucleoskeleton and chromatin.

Within the nucleus of animal cells, the nuclear lamina, a meshwork of coiled-coil type V intermediate filament proteins lying just beneath the NE, anchors chromatin to the NE via a variety of protein attachments [52]. Anchorage of the lamina to the NE is then achieved by interactions with a number of INM proteins including the LBR and members of the LEM [LAP (lamina-associated polypeptide)–emerin–Man1] domain family of proteins, including emerin, Man1, LAPs and otefin. The importance of these attachments between chromatin, which they bind via BAF (barrier-to-autointegration factor), and the LEM domain proteins is indicated by their involvement in a wide range of processes including cell-cycle control, nuclear reassembly, chromatin organization, gene silencing and signalling, as well as in genetic diseases termed laminopathies often associated with nuclear damage and premature death [52,53].

In spite of their importance in mammalian cells, plants lack sequence homologues of the lamins and of the proteins for their attachment to the INM, including LBR and LEM domain proteins. They do, however, possess long coiled-coil proteins localized to the nuclear periphery that represent structural homologues of the lamins. NMCP1 (nuclear matrix constituent protein 1) was the first member of the family to be identified in carrot protoplasts [54]. Four homologues have been identified in *A. thaliana* and named Little Nuclei. Little Nuclei mutants show morphological defects of the nucleus and are suggested to be involved at a key differentiation step following nuclear formation [33]. Electron microscopy studies reveal that a filamentous meshwork is present, but is much less ordered than that in animal cells [55]. The role of these plant nuclear-localized lamin-like proteins in movement processes, including attachment of chromatin, gene positioning and silencing and attachment of the NE, has yet to be described in detail.

**Nucleocytoskeletal bridging components**

Non-plant systems possess one further major linkage between the nucleoskeleton and cytoskeleton: a bridge formed by the interaction of membrane intrinsic proteins of the SUN domain family in the INM and KASH (KLarsicht/Anc-1/SYNE-1 homology) domain proteins in the ONM [56–58]. Together, these two proteins form a complex termed LINC (linker of nucleoskeleton and cytoskeleton) [56]. Work in our laboratory has recently identified and characterized plant SUN domain proteins providing a first indicator for the presence of putative LINC complexes in plants [31]. Sequence homologues of SUN domain family proteins have been found in the dicotyledon *A. thaliana*, the vine (*Vitis vinifera* L.), and in the monocotyledons rice (*Oryza sativa* L.) and corn (*Zea mays* L.), all of which contain two homologues (Figure 1), which in *Arabidopsis* are located on chromosomes 3 and 5. The moss *Physcomitrella patens* contains one homologue only [31].

There is considerable evidence that suggests that the two *Arabidopsis* homologues AtSUN1 and AtSUN2 are functional SUN domain proteins [31]. First, they show the classical domain structure of family members, including the highly conserved SUN domain found in animals and fungi at the C-terminus. Other conserved features include coiled-coil domains and a transmembrane domain towards the N-terminus, which anchors the proteins into the INM [31]. The proteins show INM localization with the presence of a functional bipartite NLS at the N-terminus (Figure 1). Like animal and yeast SUN domain proteins, they form multimeric complexes facilitated by their coiled-coil domains with both AtSUN1 and AtSUN2 shown to form homomers and heteromers [31]. Further evidence for their function within macromolecular anchoring structures comes from mobility studies of fluorescent protein fusions of AtSUN1 and AtSUN2, which revealed them to be highly immobile at the
domain proteins: Sad1 in clustered at the NE as a bouquet, which is facilitated by SUN to interphase nuclei. In prophase 1 of meiosis, telomeres are associated with the INM is via SUN and LEM domain proteins [59–61]. which also lack lamins, chromatin positioning interaction constituents in the absence of lamins; for instance, in yeast, proteins may provide direct binding partners for nuclear binding interactions [31]. As indicated above, SUN domain domain proteins of other kingdoms, it is involved in these plant NE. Deletion of the N-terminus increased the mobility to be more closely related then the two monocotyledon SUN domain proteins.

Figure 1 | Plant SUN domain proteins
Both AtSUN1– and AtSUN2–CFP (cyan fluorescent protein) fusions (green) transiently expressed in tobacco leaf cells localize to the NE. Chromatin is stained with ethidium bromide (magenta). The phylogram of select plant, animal and yeast members of the SUN domain proteins (created using http://www.phylogeny.fr) shows how the plant members cluster separately. Interestingly, both dicotyledon SUN domain proteins appear to be more closely related then the two monocotyledon SUN domain proteins.

plant NE. Deletion of the N-terminus increased the mobility of the two proteins, indicating that, in common with the SUN domain proteins of other kingdoms, it is involved in these binding interactions [31]. As indicated above, SUN domain proteins may provide direct binding partners for nuclear constituents in the absence of lamins; for instance, in yeast, which also lack lamins, chromatin positioning interaction with the INM is via SUN and LEM domain proteins [59–61]. Chromatin–INM protein interactions are not just restricted to interphase nuclei. In prophase 1 of meiosis, telomeres are clustered at the NE as a bouquet, which is facilitated by SUN domain proteins: Sad1 in Schizosaccharomyces pombe, Mp3 in Saccharomyces cerevisiae and both SUN1 and SUN2 in mammals [59,62–64]. Telomere anchorage at the plant NE occurs during interphase and meiosis. In wheat interphase nuclei, telomeres are associated with the nuclear periphery [28], and in Arabidopsis prophase 1, telomeres first cluster at the nucleoli and then move to the nuclear periphery to remain there throughout prophase 1 [65]. Investigations to establish the presence of AtSUN1 and AtSUN2 at the meiotic NE and their putative involvement in telomere anchorage are ongoing (K. Graumann and S.J. Armstrong, unpublished work).

The second component of the LINC complex, located in the ONM and providing attachment to various cytoskeletal components in non-plant systems, including actin, MT motors kinesin and dynein, centrosomes and SPB (spindle pole body) components are the KASH domain proteins [58,66]. So far, four KASH domain proteins have been characterized in mammals (nesprins 1–4) and in Caenorhabditis elegans (ANC-1, UNCS-83, ZYG-12 and KDP), two in Drosophila (Klarsicht and MSP-300) and in S. pombe (KMS1-2) and one in S. cerevisiae (Cms4) [58,66,67]. Each confers NE anchorage of specific cytoskeletal elements. For instance, nesprins 1 and 2 link to actin, nesprin 3 to intermediate filaments and nesprin 4 to kinesin [58,68]. The requirement of KASH domain proteins for nuclear position and movement has been demonstrated in various systems. For instance, in C. elegans, UNC-83 mediates the movement of the nucleus by cross-linking the nucleus to kinesin [68,69], whereas ZYG-12 interacts with dynein and is involved in MT organization and nuclear positioning in gonad cells [70]. Recently, Zhang et al. [71] have shown that nuclear migration during neurogenesis and neuronal migration in mice involves attachment of the nucleus to centrosomes by dynein/dynactin and kinesin, which in turn is facilitated by nesprins 1 and 2.

In plants, actin, myosin and MT nucleating complexes are known to be associated with the nucleus [7,72,73]. The γ-TURC (γ-tubulin ring complex), required for the attachment of MTs to the NE, comprises two ONM proteins, AtGCP2 (where GCP is γ-tubulin complex protein) and AtGCP3, which are soluble and require anchoring. The anchor has yet to be described, but in animals and yeast, the components of the LINC complex anchor the MTOC (MT-organizing centre) and SPB respectively [72]. Nakayama et al. [74] provided a further indication of connections between the nucleoskeleton and cytoskeleton in plants when they demonstrated that nuclear-localized histone H1 induces the radial organization of MTs at the ONM of tobacco. To date, no KASH domain homologues have been identified in plants and they have thus been suggested to be absent [38,41]. However, as the sequences of KASH domain proteins are poorly conserved, it may be that they are present, but not recognizable by in silico techniques. Alternatively, plants may have evolved KASH-like proteins that are structurally and functionally similar to KASH domain proteins [32]. The identity of the binding partners of AtSUN1 and AtSUN2, both in the nucleoplasm and ONM, remains obscure, but work is ongoing to investigate the function of the two plant SUN domain proteins in nuclei and nuclear component positioning.

Future prospects
Understanding nuclear positioning and movement requires a detailed understanding of the proteins involved both in force generation and in anchorage. Although our understanding of the cytoskeleton and, to a lesser extent, the nucleoskeleton has increased greatly in the last 5 years, key protein components
are yet to be described. Thus, although both MT and actin attachment to the NE has been shown to occur, considerable work remains on the structural anchor proteins of the INM and ONM. Characterization of plant SUN domain proteins points towards a similar anchoring mechanism, via the LINC complex, as observed in other kingdoms; however, this is likely to have plant-specific properties. Work is continuing to characterize other components of the anchor mechanisms at the NE that permit events in nuclear positioning and replication.

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**References**


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