

# The plant nuclear envelope

I. Meier

Plant Biotechnology Center and Dept. of Plant Biology, Ohio State University, 210 Rightmire Hall, 1060 Carmack Rd., Columbus (Ohio 43220, USA), Fax: + 1 614 292 5379, e-mail: meier.56@osu.edu

**Abstract.** This review summarizes our present knowledge about the composition and function of the plant nuclear envelope. Compared with animals or yeast, our molecular knowledge of the nuclear envelope in higher plants is in its infancy. However, there are fundamental differences between plants and animals in the structure and function of the nuclear envelope. This review will com-

pare and contrast these differences for nuclear pore complexes, nuclear transport, inner nuclear envelope proteins and the role of the nuclear envelope during mitosis. In some cases, seemingly ‘novel’ aspects of plant nuclear envelope function may provide new insight into the animal cell nucleus.

**Key words.** Plant nucleus; lamina; nuclear pore complex; Ran signaling; RanGAP; centrosome; MFP1; MAF1; nuclear envelope; microtubules; MTOC.

## Introduction

The nucleus is the most prominent compartment of any eukaryotic cell, and home to its chromosomes. The chromosomes are surrounded by a double-membrane system, termed the nuclear envelope. The outer membrane is a simple continuation of the endoplasmic reticulum in its protein composition. In contrast, the inner membrane has a distinct protein composition and specialized functions. Also located at the nuclear envelope are nuclear pore complexes (NPCs), which occupy pores where the inner and outer membranes are fused together. NPCs are large protein conglomerates responsible for the selective import and export of macromolecules traversing the envelope [1, 2]. The nuclear envelope has several main functions. It separates the biochemical environment of the nucleus from that of the cytoplasm, and mediates and regulates the selective exchange of molecules between the nucleus and cytoplasm (nucleocytoplasmic transport) [3]. The nuclear envelope also acts as an anchoring surface for some chromatin (e.g. heterochromatin), and in higher organisms, plays a still-enigmatic role in the highly complex dissociation and re-formation of the nucleus during cell division [4, 5]. Although nuclei are typically depicted as spheres, the shape of the nuclear envelope can diverge greatly from this image. Significant grooves and invaginations, both static and dynamic in na-

ture, have been found in both animal and plant nuclei [6, 7]. These structural features increase the interaction surface between the nucleus and cytoplasm, and suggest that nuclear and cytoplasmic activities may be more structurally linked than was previously anticipated.

Plants have finally reached center stage as a unique new model for the molecular structure of the nucleus. The first investigations of the plant nucleus revealed some similarities and a surprising number of differences in the nuclear envelope biology of animals and plants. This review will focus on evidence that nuclear organization is fundamentally different in the ‘other kingdom’.

## NPCs

Macromolecules enter and exit the nucleus by trafficking through NPCs in the nuclear envelope. Although the structure of the plant NPC was described 3 decades ago [8], there is virtually no information about its molecular constitution [9]. However, several candidate nuclear pore proteins (‘nucleoporins’) have been detected in plants. There is a 100-kDa carrot nuclear matrix protein that associates closely with the NPC, and is recognized by antibodies against mammalian and yeast nucleoporins [10], but this protein has not been identified. Eight proteins associated with the tobacco NPC are covalently

modified by *N*-acetylglucosamine (GlcNAc), a glycosylation found in many animal nucleoporins [11]. Interestingly, whereas vertebrate nucleoporins are modified by single O-linked GlcNAc residues, each tobacco glycan consists of more than five GlcNAc residues [12]. The function of O-GlcNAc modification of nucleoporins is not known, but it is interesting that yeast nucleoporins are not glycosylated, and that the glycosylation moiety differs between plants and vertebrates. One plant GlcNAc-modified protein has been cloned, and has ~30% similarity to prokaryotic aldose-1-epimerases [13]. This protein is localized at the nuclear rim, but its association with the plant NPC and potential function remain to be tested.

### Nuclear transport receptors

Proteins to be imported into the nucleus generally contain a nuclear localization signal (NLS), which typically consists of a cluster of basic amino acid residues. Three types of animal NLS (SV40-like NLS, bipartite NLS, and Mata2-like NLS) can function in plant cells [14]. Plant nuclei specifically and reversibly bind all three types of NLSs [15–17]. In animals and yeast, importin  $\alpha$  and importin  $\beta$  are the main nuclear import receptors. Importin  $\alpha$  (the adapter) binds the NLS-containing cargo protein. Importin  $\beta$  (the receptor) then binds to importin  $\alpha$  and Ran, which is necessary for nuclear import and mediates docking of the complex to the NPC [18, 19]. Homologs of both importin  $\alpha$  and importin  $\beta$  have been identified in plants. The *Arabidopsis* importin  $\alpha$  homolog binds in vitro to all three types of animal NLS [20]. In contrast to mouse importin  $\alpha$ , *Arabidopsis* importin  $\alpha$  recognizes the SV40 large tumor-antigen NLS, the bipartite NLS of the *Xenopus laevis* nuclear factor N1N2, and the yeast GAL4 NLS with high affinity in the absence of importin  $\beta$  [21]. In a mammalian reconstituted in vitro nuclear import system, *Arabidopsis* importin  $\alpha$  mediates nuclear import in the absence of mouse importin  $\beta$ , comparable to the level obtained with the mouse importin  $\alpha/\beta$  complex [21]. Together, these data suggest that an importin- $\beta$ -independent nuclear import pathway exists in plants.

In contrast to *Arabidopsis* importin  $\alpha$ , rice importin  $\alpha$  binds only to monopartite and bipartite NLSs [22–24], suggesting that plants express importins that are specialized for particular NLSs. Rice importin  $\alpha$  assembles *in vitro* with mouse importin  $\beta$ , and rice importin  $\beta$  mediates nuclear envelope docking and translocation into HeLa cell nuclei in vitro [25, 26]. Future characterization of the different systems (e.g. the identification of *Arabidopsis* importin  $\beta$  and the functional analysis of additional *Arabidopsis* importin- $\alpha$  family members [27, 28]) will be necessary to determine whether nuclear import mechanisms differ between plant species. Alternatively,

plant importin  $\alpha$  might act both as an adapter and as a receptor within one species, similar to mammalian importin 7, which serves as an adapter for importin  $\beta$  to import histone H1, but can also serve as the direct receptor to import ribosomal proteins [29, 30].

One difference between plant and animal nuclear import was that permeabilized plant protoplasts could not be depleted of cytoplasmic factors involved in nuclear import [31]. This experimental difference, which caused plant importins to be analyzed in mammalian in vitro import systems rather than plant systems [21], might point to fundamental differences in the organization or mechanisms of plant nuclear import receptors in vivo. Interestingly, *Arabidopsis* importin  $\alpha$  co-localizes in the cytoplasm with microtubules and microfilaments, and is redistributed when these cytoskeletal structures are depolymerized [32]. In the presence of an NLS-containing peptide, but not an NLS mutant, *Arabidopsis* importin  $\alpha$  binds in vitro to microtubules and microfilaments [32]. These data suggest that importin  $\alpha$  interacts with the cytoskeleton in plants. Further research is needed to determine whether the cytoskeleton might play any role in transporting nuclear cargo to NPCs in animals or yeast, in vivo.

Significantly less is known about nuclear export in plants. Exportin 1, the transport receptor for leucine-rich nuclear export signals (NES) in animals, has also been cloned from *Arabidopsis* [33]. *Arabidopsis* exportin 1 interacts with the Rev NES and with an endogenous plant NES, both of which function in nuclear export in plants. Exportin 1 also binds to RanBP1 and Ran1 from *Arabidopsis*. Based on these results, the plant nuclear export machinery appears to be very similar to that in animals and yeast.

### Ran GTPase and nuclear transport

The small GTP-binding protein Ran is a crucial component of nuclear import and export [18, 19]. GTPase-activating protein (RanGAP) and Ran-binding protein 2 (RanBP2) are localized to the cytoplasmic side of the NPC, whereas the nucleotide exchange factor for Ran, named RCC1, is localized inside the nucleus. These localizations are thought to establish a gradient of high RanGDP in the cytoplasm and high RanGTP in the nucleus, which determines the directionality of nucleocytoplasmic transport. RanGTP dissociates imported cargo from import receptors, but stabilizes complexes between export receptors and their cargo. After an export receptor/cargo complex reaches the cytoplasm, it in turn is dissociated due to hydrolysis of RanGTP to RanGDP [18]. Ran has been cloned from several plant species [34–36]. Plant Rans suppress the *pim1* mutation in *Schizosaccharomyces pombe*, demonstrating that they

are indeed functional. However, their function has not yet been demonstrated in a plant nucleocytoplasmic transport assay. *Arabidopsis* RanBP1 and RanBP2 are about 60% similar to mammalian and yeast RanBPs [37] and sequences for putative RanGAPs from *Arabidopsis*, alfalfa and rice are also now available in GenBank (see below). No plant homologs of RCC1 have been identified yet.

### Do plants have nuclear lamins?

A mesh of intermediate filament proteins, the nuclear lamins, lines the mammalian inner nuclear membrane. Lamins bind DNA and chromatin, and are depolymerized during mitosis in response to phosphorylation. Lamins are thought to provide scaffolding structures within interphase nuclei, and to mediate the attachment of chromatin to the nuclear envelope during interphase and chromatin detachment during mitosis [38]. In the early nineties, several groups reported lamin-like proteins in different plant species [39–41]. A purification protocol adapted from animal lamins to pea nuclei was used to purify four proteins between 49 and 66 kDa [40]; these proteins were recognized by antibodies against mammalian intermediate filament proteins, and by antibodies against a peptide derived from lamin B. By immunofluorescence and immunogold labeling, these antigens were located mostly in the internal nuclear matrix, and not predominantly at the nuclear rim. Similar results were obtained by other investigators [41]. Although these early reports were promising, no lamin ortholog has been identified molecularly from plants. In a recent search of all publicly available plant sequences, including the full *Arabidopsis* genome, no homologs of lamins were found ([42] and I. Meier, unpublished results). Similarly, despite earlier reports of lamin-like proteins in yeast [43], the fully sequenced *Saccharomyces cerevisiae* genome [44] also contains no lamin genes. It is therefore likely that nonanimal eukaryotes have a distinct set of nuclear envelope proteins that functionally replace the lamins.

The number of known inner-membrane proteins from animals is growing, in part due to the newly awakened interest in such proteins as targets of human genetic disease [45]. Such proteins include lamin B receptor (LBR), lamina-associated polypeptide-1 (LAP1), LAP2, emerin, MAN1, otefin and nurim (reviewed in [45]). None of these proteins have an identifiable homolog in the plant databases. These findings, though negative, strongly underscore the possibility that the plant nuclear membrane has a unique protein composition, and that plants evolved unique solutions to nuclear architectural problems such as chromatin organization and nuclear structure.

### Proteins associated with the plant nuclear envelope

Which nuclear envelope proteins might substitute functionally for lamins in plants? Masuda et al. [46] identified a 134-kDa carrot nuclear matrix protein (NMCP1), with sequence similarity to myosin, tropomyosin and intermediate filaments. Immunofluorescence and immunoelectron microscopy experiments have shown that NMCP1 is located at the nuclear envelope. NMCP1 has a central coiled-coil domain flanked by a nonhelical short head and larger tail domain and a pI of 5.6–5.8, similar to lamins [46]. Although the protein is roughly twice the size of animal lamins, it is currently the best candidate for a potential lamin-like molecule in plants. The phylogenetic relationship between NMCP1 and lamins is not resolved, and important questions for the future include testing whether NMCP1 forms filaments, associates with nuclear membrane proteins or binds to DNA, histones or chromatin.

MFP1 is a second coiled-coil protein located at the plant nuclear envelope. It was identified as a matrix attachment region-binding protein from tomato [47], and is located in small speckle-like structures at the nuclear rim [48]. MFP1, like NMCP1, has similarity to myosin, tropomyosin and intermediate filament proteins. MFP1 is an 81-kDa protein, 85% of which is predicted to be a coiled-coil domain, preceded by a short non- $\alpha$ -helical N-terminus with two hydrophobic, putative transmembrane domains. This N-terminus is required to target MFP1 to the nuclear rim, suggesting that it might be anchored in the nuclear membrane. Considering their structural similarity and overlapping locations, it will be worth testing for direct interactions between MFP1 and NMCP1.

MFP1 is a candidate for a plant protein that mediates chromatin interactions with the nuclear membrane, because of its C-terminal domain that binds to 'MAR' (matrix attachment region) DNA [47]. Immunogold labeling is first needed to determine whether MFP1 is indeed located at the inner nuclear membrane. Because MFP1 has been biochemically linked to the plant nuclear matrix, it will be interesting to understand how it attaches to this structure. A novel binding partner that interacts with the central coiled-coil domain of MFP1 was identified in a yeast two-hybrid screen [49]. MFP1-associated factor 1 (MAF1) is a small serine/threonine-rich protein which is, like MFP1, widely conserved among higher plants, but has no homologs in animals or yeast [50]. Based on immunocytochemistry and the localization of GFP fusion proteins, MAF1 is located at the nuclear rim [49], but its function is not known.

### A link between Ran signal transduction and plant nuclear envelope proteins

Surprisingly, MAF1 is related to the N-terminal domains of four putative plant RanGAPs [42]. All known plant RanGAPs contain a unique N-terminal domain not found in yeast or mammalian RanGAPs (fig. 1A). An alignment of MAF1 sequences from six different higher plant species and the four putative RanGAPs shows a clear relatedness of this plant-specific N-terminal domain with MAF1 (fig. 1B). The 'WPP' domain defined by the consensus sequence shown in figure 1B is unique to these two types of proteins. Molecular modeling of *Arabidopsis* RanGAP onto the crystal structure of the *S. pombe* RanGAP Rna1p indicates a close fit of the plant sequence, except for the WPP domain (fig. 1C). This suggests that the WPP domain is an N-terminal extension of a molecule with otherwise close structural similarity to Rna1p. Mammalian RanGAP associates with the nuclear rim via a SUMOylated C-terminal domain [51], which is not present in yeast and plant RanGAPs (fig. 1A). However, the subcellular distribution of an *Arabidopsis* RanGAP-GFP fusion protein is strikingly similar to that of human RanGAP, with a predominant location at the nuclear envelope ([51] and I. Meier, unpublished results). Together, these data suggest that the WPP domain could be a plant-specific protein-protein interaction domain involved in targeting plant RanGAP to the nuclear rim. Further investigation is needed into the targeting and functions of Ran and RanGAP in plants, and the connection between these signaling molecules and the probably plant-specific nuclear rim proteins MFP1 and MAF1.

### Functions of the plant nuclear envelope during mitosis

Several inner-membrane proteins in animals, which appear to have no homologs in *Arabidopsis*, bind to chromatin and are proposed to mediate chromatin-nuclear envelope interactions during interphase [52]. Their apparent absence from plants implies that plants use a completely different set of nuclear membrane proteins to attach to chromatin. While this may seem surprising, it is interesting to consider that many nuclear membrane proteins may function primarily to disassemble and reassemble the nuclear envelope around chromatin, during mitosis. Both multicellular plants and animals go through an 'open' mitosis, whereas unicellular eukaryotes such as yeast go through mitosis with their nuclear envelope intact [38]. If the primary function of inner-membrane proteins has evolved around the orchestration of open mitosis, and if open mitosis has independently evolved in plants and animals, it is conceivable that these proteins –

and possibly some aspects of the process itself – differ significantly between the kingdoms. Indeed, the pattern and timing of relocalization of NMCP1 during mitosis in carrot cells is similar to that of animal nuclear lamins [53]. Much more information is needed on the mitotic dynamics of other plant nuclear envelope proteins, and the effects of knockout and conditional mutants of these proteins on the plant cell cycle.

### In plants, the nuclear envelope functions as MTOCs

In animal cells, microtubules emanate from centrosomes (also known as microtubule organizing centers/MTOCs, or spindle pole bodies in yeast). In contrast, higher plants do not appear to have centrosomes [54]. Instead, there is significant evidence that plant microtubules assemble at the outer surface of the nuclear envelope, and that isolated plant nuclei can organize microtubules in vitro [55]. A monoclonal antibody (mAb6C6) directed against calf thymus centrosomes recognizes an 80-kDa plant protein that colocalizes with microtubule clusters on the plant nuclear surface [55–57]. Interestingly, the mAb6C6 antigen, 'p80', remains at the periphery of extracted plant nuclear matrix preparations, suggesting physical links in plants between the nuclear matrix, outer nuclear membrane and MTOCs [48]. The specific nature of this connection is not known. Schmit et al. [58, 59] localized p80 during mitosis and meiosis in plant cells. At the onset of mitosis (prophase), p80 is located on the periphery of the nucleus. Upon nuclear envelope breakdown, p80 relocates to kinetochores, but then reappears at the reforming nuclear envelope during telophase. A similar pattern is seen during meiosis. These findings should encourage the identification and functional investigation of p80, to better understand plant centrosome identity and the microtubule-organizing role of the plant nuclear envelope.

### Concluding remarks

The investigation of the plant nuclear envelope and its function during interphase and cell division is a journey barely begun. Many other journeys into plant cell biology have been halted by the belief that 'it will be just like in animals'. More and more, however, we are learning that a billion years of separate evolution can lead to different solutions to shared cellular problems. Our limited exploration of the plant nuclear envelope has already illuminated interesting and potentially important differences between plant and animal nuclei. Although the function of glycosylation of plant and animal nucleoporins is not understood, nucleoporins in both kingdoms are glycosylated, albeit with slightly different modifications. In plants, importin  $\alpha$  might act as both an adapter and re-



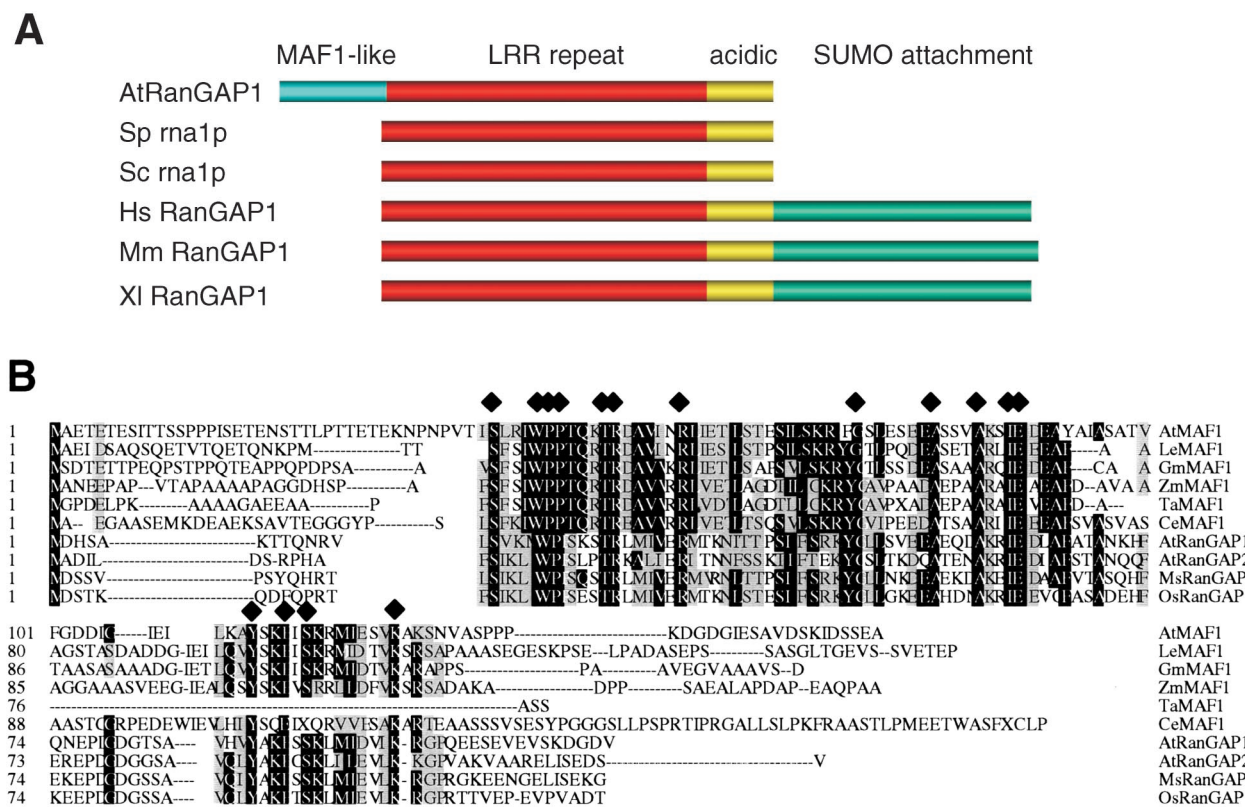


Figure 1. Plant MAF1 and RanGAPs share a unique domain. (A) Domain structure of RanGAP from plants, animals and yeast [42, 62]. At, *Arabidopsis thaliana*; Sp, *Schizosaccharomyces pombe*; Sc, *Saccharomyces cerevisiae*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Xl, *Xenopus laevis*. Proteins are only approximately drawn to scale. (B) Sequence alignment of MAF1 from six higher plant species, compared with the N-terminal domains of RanGAP from three higher plant species. Black and gray shading indicate residues that are identical and similar, respectively, in at least six sequences. Black diamonds indicate residues identical in all sequences. At, *Arabidopsis thaliana*; Le, *Lycopersicon esculentum* (tomato); Gm, *Glycine max* (soybean); Zm, *Zea mays* (maize); Ta, *Triticum aestivum* (wheat); Ce, *Canna edulis*; Ms, *Medicago sativa* (alfalfa); Os, *Oryza sativa* (rice). All GenBank accession numbers are cited in [42]. (C) Modeling of AtRanGAP1 onto the crystal structure of Sp Rna1p [62] using the Cn3D 3.0 program (<http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>). In both the structural representation of Rna1p and the sequence alignment, red indicates structural fit, purple indicates gaps in AtRanGAP1 and yellow marks the position of the Rna1p N-terminus. In the sequence alignment, white indicates additional residues in AtRanGAP1, and cyan marks the extension of the MAF1-like domain shown in (B).

ceptor for nuclear import. The association of importin  $\alpha$  with the cytoskeleton is at present unique to plants, but is provocative in suggesting this possibility for animal eukaryotes. It seems highly unlikely that plants have a nuclear lamina as known in vertebrates. Instead, plants might rely on nuclear envelope-associated coiled-coil proteins of similar structure, which appear to be unique to plants. Further investigation is needed to determine if these proteins are truly the functional equivalents of animal lamins, and which aspects of nuclear or chromatin function they regulate. None of the presently known animal inner nuclear membrane proteins appear to exist in plants. We can only guess at the functional implications of this finding, for example in terms of the higher developmental plasticity of plants compared to mammals. The attachment of plant RanGAP to the nuclear envelope has interesting implications for Ran in animals, since Ran was recently proposed to be involved (directly or indirectly) in membrane fusion events during nuclear assembly [60, 61]. Finally, the unique ability of the nuclear envelope to act as an MTOC in plants may provide significant new insight into what constitutes a functional centrosome. In summary, there appears to be more than one blueprint for a nucleus (and a cell), and it will be exciting to discover new concepts about nuclear structure and function from the green 'aliens' on our planet.

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