REVIEW PAPER

The molecular architecture of the plant nuclear pore complex

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Abstract

The nucleus contains the cell’s genetic material, which directs cellular activity via gene regulation. The physical barrier of the nuclear envelope needs to be permeable to a variety of macromolecules and signals. The most prominent gateways for the transport of macromolecules are the nuclear pore complexes (NPCs). The NPC is the largest multiprotein complex in the cell, and is composed of multiple copies of ~30 different proteins called nucleoporins. Although much progress has been made in dissecting the NPC structure in vertebrates and yeast, the molecular architecture and physiological function of nucleoporins in plants remain poorly understood. In this review, we summarize the current knowledge regarding the plant NPC proteome and address structural and functional aspects of plant nucleoporins, which support the fundamental cellular machinery.

Key words: Arabidopsis thaliana, nuclear envelope, nuclear pore complex, nucleoporin, nucleus, proteomics.

Ultrastructure of the plant NPC

Because the nuclear pore complexes (NPCs) are the largest macromolecular complexes in cells, early studies of the complexes in plants were performed using electron microscopy. More than 40 years ago, Yoo and Bayley (1967) reported that nuclear pores in the pea plant resembled those described in various animal cells, and comprised 1–3 central granules (or possibly tubules), which were surrounded by an annulus. It was estimated that nuclear pores occupied a maximum of 38% of the nuclear envelope area. Roberts and Northcote (1970) used a freeze-etch technique to reveal the high-resolution structure of NPCs in sycamore and bean. They showed that, as in other organisms, the plant NPC is octagonally symmetrical around its cylindrical axis. They also determined that the plant NPC (1150 × 640 Å) is larger than its yeast counterpart (960 × 350 Å), but smaller than that in vertebrates (1450 × 800 Å). These studies provided the first model of higher plant NPCs, which was based on observation of vertical sections and a rough three-dimensional structure.

Fiserova et al. (2009) used an in-lens field emission scanning electron microscope (fSEM), a type of high-resolution SEM, to gain further insight into NPC structure. Using cultured tobacco cells, the NPC structure of the nuclear membrane was visualized on both the cytoplasmic and nucleoplasmic sides. They clearly demonstrated that, in both logarithmic and stationary phase cells, NPCs are non-randomly distributed over the nuclear envelope (as previously observed in other higher eukaryotes). The density of the NPCs was largely unchanged during cell growth, but NPCs became predominantly organized into rows in stationary phase cells. Interestingly, different NPC conformations, which were related to different cell stages, were observed. Logarithmic phase cells, which are metabolically active and undergo rapid cell division, contained NPCs with a larger inner pore diameter, which may be capable of rapid and effective transport. In contrast, stationary phase and senescent cells contained NPCs with smaller inner pore diameters; internal filaments were observed within the pores, which emerged from the base of each subunit and were directed toward the NPC centre. These results suggest that the NPC may differentially regulate transport activity and specificity by changing its component parts. Importantly, such a conformational change was also observed in Xenopus (Goldberg et al., 1997) and Drosophila (Kiseleva et al., 2001), suggesting that the mechanisms underlying NPC differentiation are conserved across eukaryotes.

Abbreviations: FG, phenylalanine–glycine; Nup, nucleoporin; NPC, nuclear pore complex.

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Direct involvement of the inner nuclear envelope protein in NPC anchoring and positioning was suggested by studies in vertebrate cells (Lenz-Bohme et al., 1997; Liu et al., 2000; Maeshima et al., 2006). The distribution of NPCs on the nuclear envelope in vertebrates correlates with the distribution of laminains. NPCs in tobacco are closely linked to a filamentous structure on the inner nuclear membrane (Fiserova et al., 2009; Fiserova and Goldberg, 2010), and the organization and dimensions of these filaments resemble the arrangement of the nuclear lamina in Xenopus oocytes (Goldberg et al., 2008). Although no lamin homologues have been identified in plants, the plant NPC might be anchored on the nuclear envelope in the same way as in vertebrates. Nuclear matrix constituent protein1 (NMCP1), which is a long coiled-coil protein localized at the nuclear rim, is considered to be the best candidate of plant lamin-like protein (Masuda et al., 1997; Boruc et al., 2012). Mutants of two NMCP1 homologues in Arabidopsis, little nuclei1 (line1) and line2, show reduced nuclear size and an altered nuclear structure (Dittmer et al., 2007). Thus, NMCP1/LINC is thought to determine nuclear organization in plants (Dittmer et al., 2007). Moreover, Arabidopsis SUN (Sad-1/UNC-84)-domain proteins, which are inner nuclear envelope proteins, have been isolated and characterized (Graumann et al., 2010; Oda and Fukuda, 2011). A mammal SUN-domain protein is known to interact with the NPC and probably regulates NPC distribution across the nuclear surface (Liu et al., 2007). It will be necessary to determine how plant NPCs interact with these proteins on the inner nuclear envelope if we are to better understand NPC function, positioning, assembly, and disassembly.

Attempts to identify NPC components using proteomics

Yeast and vertebrate NPC proteomes

The first comprehensive proteomics study of NPCs was performed in yeast (Saccharomyces cerevisiae) (Rout et al., 2000). There are several advantages to working with yeast nuclei: yeast have the highest NPC/nuclear volume ratio of any organism (Maul, 1977) and, unlike the nuclei in mammalian cells, they do not have a lamina connecting the NPCs to other structures and/or protein complexes. Rout and Blobel (1993) prepared a highly enriched NPC fraction from yeast spheroplasts after several rounds of sucrose density gradient centrifugation. The fraction was then subjected to three different HPLC separation techniques followed by SDS–PAGE to identify the individual proteins associated with the NPC (Rout et al., 2000). Matrix-associated laser desorption ionization time-of-flight (MALDI-TOF) and MALDI-ion trap tandem mass spectrometry identified a total of 174 proteins, of which 34 were previously uncharacterized open reading frames (ORFs). The uncharacterized ORFs and putative nucleoporins were epitope tagged and their subcellular localizations were analysed by immunofluorescence and immunoelectron microscopy to determine the position and stoichiometry of each nucleoporin within the NPC. In all, 29 nucleoporins and 11 NPC-associated proteins, which serve as transport factors, were identified.

Two years after this yeast NPC proteomics study, Cronshaw et al. (2002) reported the first vertebrate NPC proteomics data. They developed a fractionation procedure that yielded highly enriched nuclear envelopes from rat liver nuclei. After removing the chromatin, the nuclear membranes and their associated proteins were extracted by incubation in Triton X-100 and SDS. This procedure yielded intact NPCs embedded in the laminae. Then, a zwitterionic detergent was used to solubilize the NPCs specifically and release the monomeric nucleoporins. The solubilized proteins were separated by high-performance liquid chromatography (HPLC) and SDS–PAGE, and analysed by using both MALDI-quadrupole-quadrupole TOF (MALDI-QqTOF) and MALDI-ion trap spectrometry. The uncharacterized proteins were then expressed as green fluorescent protein (GFP) fusions in HeLa cells to investigate their localization. This work identified and classified 29 nucleoporins and 18 NPC-associated proteins (Cronshaw et al., 2002).

The plant NPC proteome

Knowledge of the individual components and overall structure of NPCs in plants largely lagged behind that of vertebrate and yeast NPCs. With a few exceptions, nucleoporin homologues could not be identified in plants using homology-based approaches (Meier, 2006). Several nuclear proteomics studies were reported, but they only identified a few nucleoporins (Pendle et al., 2005; Aki and Yanagisawa, 2009). It was largely unknown which nucleoporins comprised plant NPCs. Because a protocol for the biochemical isolation of plant NPCs has not been developed, it is difficult to perform studies similar to those done in yeast and vertebrates. To overcome these problems, interactive proteomics was used to identify plant NPC components (Tamura et al., 2010). Transgenic plants were generated, which expressed GFP-tagged mRNA export factor1 (RAE1), a known nucleoporin in Arabidopsis. The nucleoporins were then purified by immunoprecipitation from extracts of transgenic plants with an anti-GFP antibody. A linear ion trap mass spectrometer (LTQ-Orbitrap) was used to identify a total of 200 proteins in the immunoprecipitates. By comparing these with a database containing metazoan nucleoporins and performing expression studies of GFP fusions, 24 proteins were classified as nucleoporins (Tamura et al., 2010).

To obtain more information about plant NPCs, Tamura et al. (2010) selected other nucleoporins identified in the RAE1–GFP immunoprecipitates and used them as bait for further rounds of NPC purification. Finally, five cycles of interactive proteomic analysis were performed. This procedure identified at least 30 putative nucleoporins, 22 of which had not been previously annotated. This work also demonstrated that the interactive proteomic approach is a very powerful technique, which can be used to identify the individual components of macromolecular complexes in plants comprehensively.

Domain architecture

Proteomic analysis and X-ray crystallography revealed the detailed protein structure of individual nucleoporins. It was estimated that 38% of all nucleoporin amino acid residues contain an
α-solenoid fold, 29% contain phenylalanine–glycine repeats (FG repeats), and 16% contain β-propeller folds. Other individual fold types accounted for <5% of the total nucleoporin pool (Devos et al., 2006). The small number of predicted fold types within the nucleoporin proteins and their similar internal symmetries suggest that the bulk of the NPC structures evolved through a series of gene duplications and divergences from a simple precursor set of only a few proteins. The predicted structures of individual Arabidopsis nucleoporins are summarized in Fig. 1.

**Phenylalanine–glycine repeats**

There are an estimated 128 FG domains, which harbour thousands of total FG repeats, within any given yeast NPC (Rout et al., 2000). The FG repeats interact with nuclear transport receptors, providing a selective barrier to the diffusion of macromolecules (Radu et al., 1995; Patel et al., 2007). The FG repeats appear to be localized toward the inside of the NPC (Rout et al., 2000). It is therefore reasonable to suggest that nucleoporins rich in FG repeats (FG nucleoporins) coat the central pore surface, providing interaction domains for transport receptors within the central pore. Several studies investigated how these FG repeats function as a transport barrier. It appears that the FG repeats are intrinsically unfolded, and contain short clusters of hydrophobic amino acids (e.g. FXFG or GLFG) separated by hydrophilic spacers. Because of the flexibility of FG repeat domains, it was proposed that phenylalanine-mediated inter-repeat interactions cross-linked the FG repeat domains into elastic and reversible hydrogels (Frey et al., 2006). The hydrogel-like properties of the nuclear pores were thought to function as a molecular sieve. Systematic and large-scale analyses of the FG nucleoporins in yeast revealed that two forms are present in NPCs (Patel et al., 2007). FG nucleoporins anchored at the NPC centre (central FG nucleoporins) form a cohesive meshwork of filaments via hydrophobic interactions. In contrast, FG nucleoporins anchored at the NPC periphery (cytoplasmic and nucleoplasmic FG nucleoporins) are generally non-cohesive. Therefore, a two-gate model of NPC architecture is proposed, which comprises a central diffusion gate formed by a meshwork of cohesive FG nucleoporins and a peripheral gate formed by repulsive FG nucleoporins (Patel et al., 2007; Strambio-De-Castillia et al., 2010).

**Coiled-coils**

The nuclear basket within the NPC is generally constructed from large coiled-coil proteins: NUCLEAR PORE ANCHOR (NUA) in plants (Xu et al., 2007a), MYOSIN-LIKE PROTEIN1/2 (Mlp1/2) in yeast (Rout et al., 2000), and TRANSLocATED PROMoter REGION (Tpr) in vertebrates (Cordes et al., 1997). Coiled-coils mediate protein–protein interactions, implying that the nuclear basket serves as a recruitment platform that brings various factors together within the nucleus. The yeast FG nucleoporin complex, Nsp1–Nup82–Nup49 complex (Nup62–Nup58–Nup54 complex in plants), is held together by coiled-coil interactions (Bailer et al., 2001) and tethered to the NPC scaffold via the N-terminal coiled-coil region of NUCLEOPORIN-INTERACTING COMPONENT OF 96kDa (Nic96). The FG nucleoporins in plants also possess a coiled-coil domain and are

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**Fig. 1. Summary of Arabidopsis nucleoporins.** The structural motifs that appear next to each nucleoporin refer to the yeast protein folds predicted by Devos et al. (2006). Protein fold illustrations were derived from PDB ID: 1D7M, 1DXZ, 1KXS, 2Q5X, 2RFO, and 3T97. AGI code: Arabidopsis genome initiative code.
thus assumed also to form a complex (Nup62–Nup58–Nup54 complex). However, Nup93, a plant homologue of yeast Nic96, has no obvious coiled-coil motif (Tamura et al., 2010), implying that the plant FG nucleoporin complex is tethered to the NPC scaffold via other interactions.

α-Solenoids

The α-solenoid fold comprises a two- or three-α-helix unit, which is repeatedly stacked to form an elongated domain with the N- and C-termini at opposite ends of the molecule (Brohawn et al., 2009). Approximately one-third of nucleoporins contain α-solenoid domains (Devos et al., 2006). The outer and inner rings (scaffold nucleoporins) are dominated by an evenly distributed meshwork of α-solenoid domains, which is expected to facilitate the formation of a flexible fold. This allows large conformational changes without breaking protein–protein interactions, accommodates nucleocytoplasmic transport cargoes of different sizes, and promotes malleability of the nuclear envelope (Alber et al., 2007a). α-Solenoid folds are also common in large protein assemblies, such as those found in clathrin-, COPI-, and COPII-coated vesicles (Devos et al., 2004). In coated vesicles, clathrin-like and adaptor proteins form the structural scaffold of the protein coat, which surrounds the membrane of the vesicle in a lattice-like fashion (Fotin et al., 2004a, b), a role that may be similar to that played by the scaffold nucleoporins in the NPC. Therefore, it was proposed that the core structures of NPCs and vesicle-coating complexes have a common evolutionary origin (Devos et al., 2004, 2006).

β-Propellers

The β-propeller is a disc-like structure assembled from structural modules, known as blades, circularly arranged around a central channel (Chen et al., 2011). In general, each blade consists of 4–10 repeats of a four-stranded antiparallel β-sheet motif. The central channel of the propeller fold is usually funnel-like in shape, with a wider bottom opening, which serves as the entry point to the active site. A set of nucleoporins were initially identified as β-propellers based on sequence analysis. In yeast, only SECRETORY13 (SEC13) and SEC13 HOMOLOGUE1 (SEH1) contain the signature WD-40 repeat motif, and were among the very first β-propellers to be identified (Pryer et al., 1993). Since then, other nucleoporins containing WD-40 repeats have been identified as β-propeller nucleoporins. Berke et al. (2004) experimentally determined the crystal structure of the N-terminal domain of human Nup133. They revealed that it consists of a seven-bladed β-propeller, which provides a molecular platform that mediates multiple interactions with other proteins (Berke et al., 2004). After this structure was solved, additional non-canonical β-propeller domains within the NPC were identified. The β-propellers are widely assumed to serve as protein–protein interaction sites (Chen et al., 2011). Peripheral β-propeller domains function to recruit accessory proteins, whereas those located centrally within the NPC bind subcomplexes (Strambio-De-Castilla et al., 2010; Grossman et al., 2012).

The α-solenoid and β-propeller nucleoporins are thought to act as the structural scaffold of the NPC. Both the α-solenoid and the β-propeller folds provide extensive solvent-accessible surfaces, which appear well suited for binding other proteins. Moreover, the α-solenoid and β-propeller folds seem to be quite robust and can tolerate significant variation in amino acid sequence while still retaining the core structure, thereby allowing optimization interactions with many different partners (Devos et al., 2006).

NPC architecture

Electron microscopy studies have dissected the NPC structure (Callan and Tomlin, 1950; Yoo and Bayley, 1967; Roberts and Northcote, 1970). These studies show that the general morphology of the NPC is conserved among eukaryotes. Using a different approach, Alber et al. (2007a, b) performed a computational analysis to determine a detailed architectural map of the yeast NPC. They combined a diverse set of immunoelectron microscopic, crystallographic, and proteomic data to generate the map with an estimated 5 nm resolution, which agreed with a large body of complementary data for both vertebrates and yeast (Alber et al., 2007a, b; Brohawn et al., 2009). Although the primary sequence homology between nucleoporins from different model organisms is low, the high conservation of the overall shape and predicted fold types suggests that this NPC map can be applied to the NPCs of other organisms. Therefore, we compared a data set derived from each plant nucleoporin with those from yeast and vertebrate nucleoporins and generated an architectural map of plant NPCs (Fig. 2). According to this model, nucleoporins can be subdivided into five classes: transmembrane ring, core scaffold (inner ring, outer ring, and linker), cytoplasmic filaments, nuclear basket, and central FG (Alber et al., 2007a, b; Brohawn et al., 2009; Grossman et al., 2012).

Transmembrane ring nucleoporins

Transmembrane nucleoporins are thought to anchor the NPC to the pore membrane and bind the assembled complex to the nuclear envelope. In plants, two transmembrane nucleoporins, GLYCOPROTEIN OF 210 kDa (Gp210) (Pom152 in yeast) (Gerace et al., 1982; Greber et al., 1990) and NUCLEAR DIVISION CYCLE1 (NDC1) (Wozniak et al., 1994), constitute an outer transmembrane ring. In addition to these proteins, yeast and vertebrates possess their own unique membrane proteins, PORE MEMBRANE PROTEIN OF 34 kDa (Pom34) (Rout et al., 2000) and Pom121 (Hallberg et al., 1993), respectively. In vertebrates, Gp210 serves a fundamental role in NPC disassembly and is phosphorylated during nuclear envelope breakdown (Galy et al., 2008). An Arabidopsis gpo210 knockout mutant was identified as an embryo defective mutant (EMB3012) (http://www.seedgenes.org/index.html) (Meinke et al., 2008), which arrested at the proglobular stage during embryogenesis. This suggests that plant Gp210 plays an essential role in embryonic development. Human NDC1 is involved in NPC assembly and is required for targeting the heritable disease-associated nucleoporin, Aladin, to the NPC (Kind et al., 2009; Yamazumi et al., 2009). It would be interesting to determine whether a plant Aladin also tethers to the NPC via NDC1.
Cytoplasmic filaments and the nuclear basket

Two characteristic peripheral NPC components, the cytoplasmic filaments and the nuclear basket, are localized asymmetrically within the cytoplasm and nucleoplasm of the NPC, respectively. These structures play a role in specific interactions and can serve as docking sites for transport complexes at both the cytoplasmic and nucleoplasmic sides. The cytoplasmic filaments are composed primarily of two FG nucleoporins, Nup214 and CG1 (Kraemer et al., 1995; Rout et al., 2000). Although these nucleoporins are not well characterized in plants, those in yeast provide sites at which mRNA export factors can mature messenger ribonucleoprotein particles (Folkmann et al., 2011). The biggest nucleoporin in vertebrates (Nup358) is localized in the cytoplasmic filaments, and tethers RanGAP (RanGTPase-activating protein) to the NPC to facilitate transportin-dependent nuclear import (Hutten et al., 2009). In contrast, plants and yeast have no Nup358 homologues in their genomes (Grossman et al., 2012). Plants use the WIP–WIT complex (discussed below) to anchor RanGAP on the nuclear envelope (Xu et al., 2007b; Zhao et al., 2008), whereas yeast RanGAP is localized to the cytosol (Hopper et al., 1990).

The nuclear basket is composed of eight elongated filaments, which protrude ~60–80 nm from the nuclear face of the NPC into the nucleoplasm and converge on a distal ring structure (Strambio-De-Castillia et al., 2010). Each nuclear basket consists of one nucleoporin (NUA in plants), which has long coiled-coil domains, and two FG nucleoporins (Nup136/Nup1 and Nup50 in plants). Human Tpr (NUA in plants) is thought to constitute the central architectural element that forms the scaffold of the nuclear basket, whereas Nup153 (Nup136/Nup1 in plants) binds to the nuclear coaxial ring linking the NPC core structures to Tpr.
It is reported that both Arabidopsis NUA and Nup136 are involved in mRNA export (Xu et al., 2007a; Tamura et al., 2010), as suggested by a study of vertebrate Tpr (Shibata et al., 2002). Importantly, Arabidopsis nua and nup136 mutant plants display similar phenotypes, including early flowering and low fertility, indicating that NUA and Nup136 function together during plant development. In Xenopus, Nup153 (Nup136/Nup1 in plants) is the only nucleoporin that interacts with lamin B, which supports the nuclear structure. Although no lamin homologue has been identified in plants, it is reported that Arabidopsis nucleoporins can change shape, depending on the amount of accumulated Nup136 (Tamura et al., 2010; Tamura and Hara-Nishimura, 2011). This implies that Nup136/Nup1 is the nucleoporin that links the NPC to the nuclear lamina in plants.

Central FG nucleoporins

Central FG nucleoporins, also known as barrier nucleoporins, show a transverse orientation with respect to the central channel of the NPC and form a selective barrier that mediates nucleocytoplasmic transport. Native FG repeat regions have an unfolded structure, allowing multiple low-affinity and high-specificity interactions with transport factors (Strawn et al., 2004). In fact, in vitro studies show that all FG nucleoporins appear to have the ability to interact with at least one transport receptor (Ryan and Wente, 2000). Bayliss et al. (2000) determined the crystal structure of the human transport receptor, importin β, and identified its two FG repeat binding sites. It is estimated that there are ~160 transport factor binding sites within each NPC (Grossman et al., 2012), which allow the simultaneous binding of multiple transport factors within a single NPC.

Five central FG nucleoporins (Nup98, Nup62, Nup58, Nup54, and Nup35) have been identified in plants and are well conserved in other organisms. Of these, only Nup62 has been characterized in Arabidopsis (Zhao and Meier, 2011). Arabidopsis Nup62 interacts with nuclear transport factor2 (NTF2), as previously observed in yeast (Nsp1) (Clarkson et al., 1997) and vertebrates (Nup62) (Percipalle et al., 1997). Knockdown of Nup62 transcripts results in a severe dwarf phenotype and early flowering, indicating an important function for Nup62 at different stages of plant development. Further analysis of central FG nucleoporins will be required to identify how the different FG nucleoporins function during the plant life cycle. This will increase our understanding of the specific interactions between transport factors and NPCs in plants.

Outer ring, inner ring, and linker nucleoporins (scaffold nucleoporins)

Scaffold nucleoporins are composed of three groups of nucleoporin subcomplexes: the outer ring, the inner ring, and the linker. They connect membrane nucleoporins to central FG nucleoporins, thereby bridging the anchoring transmembrane layer and the barrier layer of the NPC. Because scaffold nucleoporins form the rigid skeleton of the NPC, most remain incorporated within the NPC during the entire life of a cell (D’Angelo et al., 2009). Scaffold nucleoporins are thought to play a key role in maintaining the stability of the nuclear envelope by ensuring co-planarity of the outer and inner surfaces (Alber et al., 2007a). Therefore, it is proposed that a major function of the outer rings is to facilitate the smooth transition of the pore membrane into the inner and outer nuclear envelopes (Alber et al., 2007a).

The largest and most evolutionarily conserved subcomplex, comprising several nucleoporins, is located at the outer rings and is known as the Nup107/160 subcomplex in plants and vertebrates and the Nup84 subcomplex in yeast. In plants, it is composed of eight nucleoporins: Nup160, Nup133, Nup107, Nup96, Nup85, Nup43, SEC13, and SEH1 (Fig. 2) (Xu and Meier, 2008; Tamura et al., 2010; Wiermer et al., 2012). Electron microscopic and biochemical analyses revealed that the yeast Nup84 subcomplex has a 40 nm long, Y-shaped, triskelion-like structure (Simossoglou et al., 2000; Kampmann andBlobel, 2009). This structure has two hinge regions that are conformationally flexible, which allows the NPC scaffold component to change its structure to enable large cargoes to pass through the central channel (Kampmann and Blobel, 2009). The scaffold nucleoporins located in the central part of the main channel are called inner ring nucleoporins and linker nucleoporins. In plants, the inner ring consists of Nup205, Nup188, Nup155, and Nup35 (Fig. 2) (Tamura et al., 2010). Similar to the outer ring nucleoporins, they contain α-solenoid and β-propeller folds and exhibit the typical structural scaffolding motif. The linker nucleoporins are attached between the outer and inner rings (Alber et al., 2007a), and include Nup93 and Nup88. They act as a bridge between the core scaffold and the FG nucleoporins.

Genetic studies have characterized several plant scaffold nucleoporin mutants. Arabidopsis nup96 and nup88 mutants were isolated during a genetic screen aimed at identifying downstream components responsible for resistance (R) protein activation (Zhang and Li, 2005; Cheng et al., 2009). Both mutants showed deficiencies in innate immunity and, possibly, an impairment in nuclear import of proteins, which is involved in the plant response to pathogens. Furthermore, Wiermer et al. (2012) isolated knockout mutants for each component of the Nup107/160 complex and investigated plant immune responses. Of the seven mutated nucleoporins, only nup160 and seh1 caused impaired immune responses. These results suggest that Nup160, Nup96, and SEH1 within the Nup107/160 subcomplex are important for defence signalling (Wiermer et al., 2012). The other Nup107/160 subcomplex proteins, Nup133 and Nup75, are required for fungal and rhizobial colonization in Lotus japonicus (Kanamori et al., 2006; Saito et al., 2007). Moreover, Arabidopsis nup160 and nup96 mutants show altered hormonal and temperature responses and an early flowering phenotype (Dong et al., 2006; Parry et al., 2006). Further work is required to identify the underlying molecular mechanisms and functions of the plant Nup107/160 subcomplex in various signalling pathways.

NPC-associated proteins

TREX-2

The TREX-2 (transcription-coupled export 2) complex comprises SUPPRESSOR OF ACTIN3 (SAC3), Tho2/Hpr1 PHENOTYPE1 (THP1), SL GENE UPSTREAM OF ySa1 (SUS1), and CELL DIVISION CYCLE31 (CDC31) proteins,
and is involved in mRNA export in yeast (Kohler and Hurt, 2007). The TREX-2 complex anchors at the inner side of the NPC via Nup1 (Nup136/Nup1 in plants) and Nup60 (Nup50 in plants) (Fischer et al., 2002). SUS1 also interacts with the SAGA (Spt, Ada, Gcn5, and acetyltransferase histone acetyltransferase) complex, a large transcription initiation complex that catalyses histone acetylation and de-ubiquitylation. The TREX-2 complex is thought functionally to couple SAGA-dependent gene expression to mRNA export on the inner side of the NPC (Rodriguez-Navarro et al., 2004). Lu et al. (2010) identified TREX-2 components in Arabidopsis, including THP1, SUS1, SAC3s, and CDC31s, and showed that the Nup136/Nup1–THP1 interaction links the TREX-2 complex to the nuclear basket (Fig. 3). Using yeast two-hybrid assays and a bimolecular fluorescence complementation assay, they also showed that THP1 interacts with DSS1, a subunit of the 26S proteasome regulatory particle (Lu et al., 2010). Consistent with this finding, yeast SDD1 was identified as a functional component of the TREX-2 complex (Mannen et al., 2008), and is required for nuclear export of specific sets of mRNA. These results suggest that plants utilize an evolutionarily conserved system for mRNA export through the NPC.

**ESD4**

In *Arabidopsis*, EARLY IN SHORT DAYS4 (ESD4) functionally interacts with NUA, a major component of the nuclear basket (Xu et al., 2007a). Genetic analysis indicates that NUA and ESD4 might act via a shared pathway involved in various aspects of plant development. ESD4 encodes a SUMO (small ubiquitin-related modifier conjugates)-specific protease and is localized on the nuclear envelope (Murtas et al., 2003) (Fig. 3). In both *esd4* and *nua* mutants, the accumulation of SUMO conjugates increases, suggesting that ESD4 and NUA are involved in SUMO homeostasis and in regulating nucleocytoplasmic transport in plants. The nuclear basket components, Mlp1/2 in yeast and Nup153 in humans, tether the SUMO-conjugating enzymes, UBIQUITIN-LIKE PROTEIN1 (Ulp1) and SENTRIN-SPECIFIC PROTEASE2 (SENP2), respectively (Zhang et al., 2002; Panse et al., 2003). The yeast *ulp1* mutant displays altered protein sumoylation patterns and increased pre-mRNA leakage into the cytoplasm. These results suggest that desumoylation within the NPC might be a key regulatory event that prevents inappropriate pre-mRNA export in different species (Lewis et al., 2007).

**WPP DOMAIN-INTERACTING PROTEIN (WIP) and WPP DOMAIN-INTERACTING TAIL-ANCHORED PROTEIN (WIT) complexes**

In vertebrates, RanGAP, which plays an important role in nucleocytoplasmic transport, is anchored to the NPC via Nup358 (Strambio-De-Castillia et al., 2010). This interaction is mediated by a unique C-terminal domain within RanGAP, and is dependent upon sumoylation. In contrast, plant NPCs lack a Nup358 homologue, and all known plant RanGAPs contain a unique N-terminal domain called the WPP domain (a highly conserved tryptophan–proline–proline motif), which is necessary and sufficient for nuclear envelope targeting (Meier, 2000). Two types of plant-specific nuclear envelope proteins, WIPs and WITs, were isolated and identified as RanGAP-anchoring proteins (Xu et al., 2007b; Zhao et al., 2008). Although there is no direct evidence that WIP and WIT proteins physically interact with the NPC, it is assumed that they have a functional connection (Fig. 3). Recently, *Arabidopsis* WIP was reported to function as a KASH (Klarsicht/ANC-1/Syne Homology)-domain protein, which interacts with a SUN-domain protein (Zhou et al., 2012). This SUN–KASH bridge is necessary for maintaining the elongated nuclear shape of epidermal cells, indicating that WIPs are versatile and play different roles.
Conclusion

Since the identification of the plant NPC proteome, much progress has been made in clarifying the molecular components and architecture of the NPC. Despite conserved functional similarities between NPCs from plants and other organisms, structural differences exist. It is necessary to determine how these structural variations and protein sequence differences contribute to the functional organization of the plant NPC. Clearly, reverse genetic studies and high-resolution imaging techniques are needed if we are to understand the function and structure of each nucleoporin in detail.

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