The plant nuclear envelope as a multifunctional platform LINCeD by SUN and KASH

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Abstract

The nuclear envelope (NE) is a double membrane system enclosing the genome of eukaryotes. Besides nuclear pore proteins, which form channels at the NE, nuclear membranes are populated by a collection of NE proteins that perform various cellular functions. However, in contrast to well-conserved nuclear pore proteins, known NE proteins share little homology between opisthokonts and plants. Recent studies on NE protein complexes formed by Sad1/UNC-84 (SUN) and Klarsicht/ANC-1/Syne-1 Homology (KASH) proteins have advanced our understanding of plant NE proteins and revealed their function in anchoring other proteins at the NE, nuclear shape determination, nuclear positioning, anti-pathogen defence, root development, and meiotic chromosome organization. In this review, we discuss the current understanding of plant SUN, KASH, and other related NE proteins, and compare their function with the opisthokont counterparts.

Key words: KASH, lamin, LINC, meiotic chromosome organization, nuclear envelope, nuclear positioning, nuclear shape, SUN.

Introduction

In eukaryotes, genomic DNA is enclosed by the nuclear envelope (NE) which consists of the inner nuclear membrane (INM) and outer nuclear membrane (ONM). The luminal space enclosed by the two membranes (namely the perinuclear space; PNS) and the ONM are continuous with the endoplasmic reticulum (ER). Embedded in the NE, nuclear pore complexes form channels connecting the nucleoplasm and cytoplasm. However, the NE is neither simply an extension of the ER nor merely a nucleocytoplasmic barrier, but rather a multifunctional platform, thanks to a collection of NE proteins. Studies in animals and yeast have shown that the NE is populated by proteins that play important roles in nuclear structure, mechanotransduction, genome organization, gene regulation, cell polarization, and cell migration (Wang et al., 2009; Mekhail and Moazed, 2010; Zuleger et al., 2011; Gundersen and Worman, 2013; Isermann and Lammerding, 2013; Jevtic et al., 2014; Wong et al., 2014). Unlike nucleoporins, which are conserved among eukaryotes, limited homologue assignments have been made to known NE proteins (excluding nucleoporins) between plants and opisthokonts (Wilson and Dawson, 2011; Zhou and Meier, 2013; Devos et al., 2014). The INM Sad1/UNC-84 (SUN) proteins are among the few known protein families whose homologues exist in plants. In this review, we focus on plant SUN proteins and their ONM-binding partners, the Klarsicht/ANC-1/Syne-1 homology (KASH) proteins, which were recently identified in plants (Evans et al., 2014; Tatout et al., 2014). We discuss their functions and relationship to other plant NE proteins.

The opisthokont NE bridges formed by SUN and KASH proteins

SUN proteins are proteins that contain a conserved SUN domain, which were first identified at the C-termini of Schizosaccharomyces pombe Sad1 and Caenorhabditis elegans
SUN proteins are widely conserved in eukaryotes. Performing a BLAST search against the NCBI non-redundant protein database using the SUN domain of \textit{S. pombe} Sad1 and the expected threshold of 1e-06 revealed that SUN domain proteins are present in animals (metazoa), fungi, green plants (including green algae), and other eukaryotes, such as heterokonts, alveolates, and amoebozoa (Supplementary Fig. S1 available at JXB online). Interestingly, this search also revealed a prokaryotic hypothetical protein (GenoInfo number 655447074; see Supplementary Fig. S1).

Besides Sad1 and UNC-84, well-studied opisthokont SUN proteins include mammalian SUN1, SUN2, SUN3, SPAG4/SUN4, and SPAG4L/SUN5, \textit{Drosophila} Klaroid and SPAG4/Giacomo, \textit{C. elegans} SUN-1/matefin, and \textit{Saccharomyces cerevisiae} Mps3 (Razafsky and Hodzic, 2009; Starr and Fridolfsson, 2010). These SUN proteins have a similar domain organization: an N-terminal region and a C-terminal region, separated by one or more transmembrane domains (TMDs). Although the N-terminal region of SUN proteins is not conserved, the C-terminal region contains the well-conserved SUN domain. Many SUN proteins have coiled-coil domains (CCDs) N-terminal of the SUN domain. Multiple pieces of evidence have suggested that SUN proteins are INM proteins with their C-termini positioned in the PNS: (i) SUN1 and SUN2 have been biochemically shown to be localized at the INM (Hodzic et al., 2004; Padmakumar et al., 2005; Crisp et al., 2006); (ii) the N-terminal domain of SUN1, SUN2, and UNC-84 interacts with lamins, components of the nucleoskeleton (Lee et al., 2002; Crisp et al., 2006; Haque et al., 2006; Bone et al., 2014); (iii) SUN1, SUN2, Sad1, and Mps3 tether chromosomes to the nuclear periphery (Bupp et al., 2007; King et al., 2008; Morimoto et al., 2012; Link et al., 2014). However, SPAG4/SUN4, SPAG4L/SUN5, and SPAG4/Giacomo may not be localized at the INM (Shao et al., 1999; Kracklauer et al., 2010; Frohnert et al., 2011; Shoji et al., 2013). Studies of mammalian SUN1 and SUN2 suggest that the PNS SUN domains trimerize, facilitated by the CCD N-terminal to the SUN domain (Sosa et al., 2012; Z.C. Zhou et al., 2012). The SUN domain trimer can bind three KASH domains of KASH proteins in the PNS (Sosa et al., 2012; Z.C. Zhou et al., 2012).

KASH proteins are ONM transmembrane proteins with their C-terminal KASH domains positioned in the PNS and their N-terminal domains positioned in the cytoplasm (Zhang et al., 2001; Starr and Han, 2002; Zhen et al., 2002; Padmakumar et al., 2005). The KASH domain of most KASH protein families terminates in a conserved four amino acid motif, which is essential for their NE localization (Razafsky and Hodzic, 2009; Starr and Fridolfsson, 2010). In contrast, KASH proteins vary in their N-terminal cytoplasmic domains, which are usually linked to the cytoskeleton or to motor proteins (Razafsky and Hodzic, 2009; Starr and Fridolfsson, 2010; Mellad et al., 2011). As the nucleoplasmic domain of some SUN proteins interacts with lamins, the SUN–KASH NE bridges have been given the name ‘linkers of the nucleoskeleton and the cytoskeleton (LINC) complexes’ (Crisp et al., 2006). LINC complexes transfer cytoplasmic forces to the nucleus and are essential for nuclear migration or anchorage (Gundersen and Worman, 2013; Razafsky et al., 2014). SUN proteins can also be associated with telomeres and centromeres, and in these cases the SUN–KASH NE bridges are involved in chromosome pairing and synapsis (Kracklauer et al., 2013; Yamamoto, 2014).

Identification of plant SUN proteins

Plant SUN proteins were identified by homology searches using opisthokont SUN domains (Moriguchi et al., 2005; Graumann et al., 2010; Oda and Fukuda, 2011), and two types of SUN proteins were revealed (Fig. 1A): classical SUN proteins, with SUN domains at the C-terminus (Cter-SUN proteins; Murphy et al., 2010), and SUN proteins harbouring an internal SUN domain (mid-SUN proteins; Murphy et al., 2010). Plant Cter-SUN proteins are similar in size to yeast SUN proteins and smaller than mammalian SUN proteins (Graumann et al., 2010). Not only do they contain the highly conserved SUN domain, but the overall protein structure (N-terminal TMD, and PNS CCD and SUN domain) appears conserved across kingdoms (Graumann et al., 2010; Murphy et al., 2010; Oda and Fukuda, 2011). \textit{Arabidopsis} SUN1 (AtSUN1) and AtSUN2 belong to this Cter-SUN group (Fig. 1A). Plant Cter-SUN proteins also have a conserved region N-terminal to the SUN domain (Fig. 1A, green colour; Zhou and Meier, 2013). Native promoter-driven fluorescent protein fusions (Oda and Fukuda, 2011) and immunogold labelling (Graumann et al., 2010) have demonstrated that AtSUN1 and AtSUN2 are located at the NE. Cumulative evidence—the presence of a nuclear localization signal and interactions of the N-terminus with a nucleoplasmic protein—further indicates the INM localization of AtSUN1 and AtSUN2 (Graumann et al., 2010; Graumann, 2014). Similarities between plant and opisthokont Cter-SUN proteins also include functional aspects—such as their ability to interact with KASH proteins and nucleoskeletal components, as well as meiotic functions (see below).

The second group of SUN proteins—the mid-SUNs—are far less well understood than the Cter-SUNs in both plants and opisthokonts. Three mid-SUN proteins each have been described in \textit{Arabidopsis} (AtSUN3, AtSUN4, and AtSUN5; see Fig. 1A for AtSUN3 protein structure) and maize (ZmSUN3, ZmSUN4, and ZmSUN5; Murphy et al., 2010; Murphy and Bass, 2012; Graumann, 2014), while one mid-SUN protein each has been characterized in mice (osteopetrotia; Sohaskey et al., 2010) and \textit{S. cerevisiae} (SUN-like protein 1; Friederichs et al., 2012). Mid-SUN proteins differ in both structure and localization from Cter-SUN proteins. In addition to the central localization of the SUN domain, mid-SUN proteins usually consist of three TMDs and plant mid-SUN proteins also contain a conserved PAD domain (Fig. 1A; Murphy et al., 2010; Graumann, 2014). While antibody labelling of ZmSUN3 indicates NE localization, fluorescent protein fusions of AtSUN3 and AtSUN4 were localized in both the ER and the NE at similar levels (Murphy et al., 2010; Graumann...
et al., 2014). However, protein mobility in the two membranes is different, with AtSUN3 appearing less mobile and AtSUN4 more mobile in the ER than in the NE. This suggests different binding properties and therefore possible different functions (Graumann, 2014). ER localization was also shown for the two opisthokont mid-SUN proteins (Sohaskey et al., 2010; Friederichs et al., 2012). While the additional ER localization may suggest functions specific to mid-SUN proteins, they also appear to be involved in processes similar to Cter-SUNs. Mid-SUN proteins interact with Cter-SUNs and, in plants, also interact with the KASH proteins AtWIP1 and AtTIK (see below), indicating that plant mid-SUN proteins are components of NE bridges (Friederichs et al., 2012; Graumann, 2014). Arabidopsis mid-SUN proteins play a role in nuclear morphology and plant development: the sun3-1 single null mutant has reduced nuclear polarity, the sun4-1 sun5-1 double null mutant has reduced nuclear size, and the sun3-1 sun4-1 sun5-1 triple null mutant is not viable (Graumann, 2014).

**Discovery of plant KASH proteins**

Although SUN proteins are conserved in plants, no plant homologues of known opisthokont KASH proteins have been identified using a BLASTP search. The first plant KASH proteins, WPP domain-interacting proteins (WIPs), were identified because they share a characteristic signature with the opisthokont KASH domain: a short amino acid tail, located C-terminal of a TMD and terminating in a conserved four amino acid motif (X. Zhou et al., 2012). Deleting the last four amino acids of AtWIP1 or mutating the C-terminal tail domain of AtWIP1 impaired the interaction with AtSUN1 and AtSUN2 (X. Zhou et al., 2012). This domain of AtWIP1 was also found to interact with AtSUN3 (Graumann, 2014). Deleting the SUN domain of AtSUN2 impaired binding of AtWIP1, and, as shown for animal KASH proteins, the NE localization of AtWIP1 depends on AtSUN1 and AtSUN2 (X. Zhou et al., 2012).

The success in identifying AtWIP1 as a plant KASH protein inspired the development of the DORY algorithm to identify novel plant KASH proteins (Zhou et al., 2014). DORY reads a protein database and collects protein sequences that contain only one TMD and a putative KASH domain fulfilling the following criterion: a short tail that is C-terminal to the TMD and terminates in a four amino acid motif. The collected protein sequences are then grouped into protein families based on amino acid sequence similarity. Within a true KASH protein family, the putative KASH domain should be evolutionarily conserved. Therefore, the outputs of DORY need a manual verification: (i) collect known family members using BLASTP to search the NCBI non-redundant protein database and (ii) verify the presence of the putative KASH domain in the collected family members.

An initial search using the C-terminal four amino acid motif ‘XXPT’ (X represents any amino acid) and the Arabidopsis protein database identified four new putative KASH proteins—SUN-interacting nuclear envelope protein 1 (SINE1), SINE2, SINE3, and SINE4 (see Fig. 1B for domain structure; Zhou et al., 2014). SINE1 and SINE2 belong to the same protein family, which is conserved throughout land plants. Homologues of the SINE3 family can only be found in eudicots, and homologues of the SINE4 family can only be found in *A. thaliana* and *A. lyrata* and the closely related

![Fig. 1. Domain structure of plant SUN and KASH proteins. (A) Domain structure of plant SUN proteins. (B) Domain structure of plant KASH proteins.](https://academic.oup.com/jxb/article-abstract/66/6/1649/2889896)
species *Capsella rubella*. A new terminal four amino acid motif pattern [DTVAMFLFY][VAPIL][PT] was then summarized from the homologues of AtWIP1, SINE1, SINE2, SINE3, and SINE4. This pattern was used to search for putative KASH proteins in the NCBI non-redundant protein database. The SINE5 protein family (see Fig. 1B for domain structure of SINE5) and six other protein families were identified (Zhou et al., 2014). The SINE5 family is specific to *Medicago truncatula* and *Medicago sativa*, while the other six protein families are also confined to a limited number of plant species. SINE1, SINE2, SINE3, SINE4, and SINE5 have been confirmed to be plant KASH proteins by the following experiments: (i) N-terminal green fluorescent protein (GFP)-tagged proteins were localized to the *Arabidopsis* NE; (ii) the NE localization of the tested proteins depended on their last four amino acids and on SUN proteins; and (iii) co-immunoprecipitation assays showed their interaction with the SUN domain of AtSUN1 and AtSUN2 through their C-terminal tails.

In addition, a new *Arabidopsis* KASH protein, AtTIK, has been identified by a split-ubiquitin-based membrane yeast two-hybrid screen (Fig. 1B; Graumann, 2014). AtTIK interacts with AtSUN1, AtSUN2, and AtSUN3, but its interaction with AtSUN2 is weak (Graumann, 2014). The KASH domain of AtTIK, terminating in ‘PPPS’, is required for its interaction with SUN proteins (Graumann, 2014). Homologues of AtTIK can be found outside the plant lineage, due to its N-terminal putative Toll-Interleukin-Resistance (TIR) domain that is present in a large family of proteins widely conserved in eukaryotes. Nonetheless, AtTIK homologues that contain a KASH domain have only been found in *A. thaliana*, *A. lyrata*, and *Thellungiella halophila* (Graumann, 2014). Together, six plant KASH protein families have been experimentally confirmed, and an additional six predicted KASH protein families await experimental verification.

**Function of plant SUN and KASH proteins**

The SUN–WIP complex as the NE anchor of RanGAP and WPP proteins

WPPs were first identified as plant NE anchors of Ran GTPase-activating proteins (RanGAPs; Xu et al., 2007). Unlike opisthokont RanGAPs, plant RanGAPs have an additional N-terminal WPP domain (Rose and Meier, 2001; Jeong et al., 2005). Aside from plant RanGAPs, the WPP domain is only present in a group of plant-specific small proteins similar to the N-termini of RanGAPs, and in *Arabidopsis* they are named AtWPP1, AtWPP2, and AtWPP3 (Patel et al., 2004; Meier et al., 2010). Except for AtWPP3, which is cytoplasmic, AtWPP1 and AtWPP2 are probably anchored at the NE through the same mechanism as RanGAP (Patel et al., 2004). The WPP domain interacts with the cytoplasmic CCD of AtWIP1, AtWIP2, and AtWIP3 (Xu et al., 2007). The concentration of AtRanGAP1 at the NE requires AtWIP and AtSUN, and an AtSUN2–AtWIP1–AtRanGAP1 triple complex was shown by co-immunoprecipitation (X. Zhou et al., 2012). Another ONM protein family, WPP domain-interacting tail-anchored protein (WIT; Zhao et al., 2008) is involved in RanGAP1–NE association. WIT family proteins also contain an N-terminal cytoplasmic CCD and a C-terminal TMD but lack a PNS tail domain (Zhao et al., 2008). The *Arabidopsis* genome encodes two WIT genes—AtWIT1 and AtWIT2 (Zhao et al., 2008). AtWIT1 interacts with all three AtWIPs, and its NE localization does not depend on its TMD (Zhao et al., 2008). AtRanGAP1 is anchored to the NE by AtWIT1, AtWIT2, AtWIT1p, AtWIT2p, and AtWIP3 (Fig. 2A; Xu et al., 2007; Zhao et al., 2008). Although this mechanism has only been examined in *Arabidopsis* roots, this AtRanGAP NE-targeting mechanism probably also applies to other plant tissues. Interestingly, the protein level, but not the RNA level of AtWIT1 depends on AtWIP1, AtWIP2, and AtWIP3 (Zhao et al., 2008). These findings suggest that WITs probably function as binding adaptors or enhancers for WIPs, and that WITs that are not in a complex with WIPs are unstable.

Nuclear shape and nuclear movement mediated by the SUN–WIP complex

The SUN–WIP complex plays a role in maintaining the elongated shape of the nucleus in *Arabidopsis* root cells, trichomes, and leaf epidermal cells (Fig. 2C; Oda and Fukuda, 2011; X. Zhou et al., 2012). In *Arabidopsis* root hairs in particular, the nucleus is highly elongated and appears like a spindle with two thin tails attached to its poles (Chytilova et al., 2000; X. Zhou et al., 2012). In the *Arabidopsis* *wip1-1 wip2-1* triple null mutant and a *sun1 knockdown* mutant, the nuclei in these cell types are spherical (Oda and Fukuda, 2011; X. Zhou et al., 2012). Spherical nuclei have also been observed in an *Arabidopsis* *wit1-1 wit2-1* double null mutant and in *kaku1* mutants (Tamura et al., 2013). *Arabidopsis KAKU1* encodes AtMyosin XI-i and was identified in a forward genetic screen for nuclear shape (Tamura et al., 2013). AtMyosin XI-i and its C-terminal domain lacking the motor domain (XI-iMmotor) have been localized at the NE, and the NE localization of XI-iMmotor depends on AtWIT1 and AtWIT2 (Tamura et al., 2013). AtWIT2 was identified in the immunoprecipitate of yellow fluorescent protein (YFP)–XI-iMmotor by mass spectrometry (Tamura et al., 2013). A pull-down assay also showed a direct interaction between XI-iMmotor and AtWIT1 (Tamura et al., 2013). These data suggest that AtWIT1 and AtWIT2 recruit AtMyosin XI-i to the NE and transfer the motor forces to the NE, resulting in the observed elongated nuclear shape (Fig. 2C; Tamura et al., 2013).

AtMyosin XI-i is also involved in nuclear movement in *Arabidopsis* (Tamura et al., 2013). Nuclei undergo bidirectional movement in mature *Arabidopsis* root hair cells (Chytilova et al., 2000) and this movement is slowed down in *wit1-1 wit2-1* and *kaku1* mutants (Tamura et al., 2013). In *Arabidopsis* leaf mesophyll cells, blue light irradiation leads to nuclear migration to the anticlinal wall while dark treatment leads to movement to the periclinal wall (Iwabuchi et al., 2007, 2010). Although blue light-induced nuclear migration was not affected in *wit1-1 wit2-1* and *kaku1* mutants,
dark-induced nuclear migration was impaired (Tamura et al., 2013). Therefore, in Arabidopsis, an AtSUN–AtWIP–AtWIT–AtMyosin XI-i complex probably exists and mediates nuclear shape and nuclear migration (Fig. 2C), because (i) AtWIT1 interacts with AtWIPs at the ONM; (ii) the protein level of AtWIT1 depends on AtWIPs; (iii) the NE localization of AtWIP1 depends on its interaction with AtSUNs; and (iv) the spherical nuclear shape phenotype is shared among sun1-knockout sun2-knockdown, wip1-1 wip2-1 wip3-1, wit1-1 wit2-1, and kaku1 mutants. So far, no developmental defects have been associated with either the nuclear shape change or nuclear migration defects in roots and leaves.

During fertilization, AtWIPs and AtWITs are essential for pollen vegetative nuclear migration and successful pollen tube reception (Fig. 2F; Zhou and Meier, 2014). A mature Arabidopsis pollen grain is a vegetative cell engulfing two sperm cells (McCue et al., 2011). The vegetative nucleus and the two sperm cells are linked by a narrow bridge of sperm cell plasma membrane and migrate together as the male germ unit (McCue et al., 2011). Both AtWIP1 and AtWIT1 were localized to the NE of the vegetative nucleus, and reduced seed production was observed in wip1-1 wip2-1 wip3-1, and wit1-1 wit2-1 mutants (Zhou and Meier, 2014). During wild-type pollen tube growth, the vegetative nucleus enters the pollen tube first, followed by the sperm cells, and the vegetative nucleus leads the migration of the male germ unit. However, in wit1-1 wit2-1 pollen tubes, the vegetative nucleus loses its locomotion, and the migration of the male germ unit is guided by the sperm cells (Zhou and Meier, 2014). This leads to an increased distance between the vegetative nucleus and the sperm cells or a complete dissociation of the vegetative nucleus from the sperm cells. The reception of wit1-1 wit2-1 pollen tubes is also impaired, evident by the stalled or overgrown pollen tubes inside ovules and by multiple pollen tubes.
that target one ovule (Zhou and Meier, 2014). A similar phenotype was also observed in wip1-1 wip2-1 wip3-1 (Zhou and Meier, 2014). The defect of pollen tube reception is probably due to the loss of the vegetative nucleus during pollen tube growth, because only sperm cell nuclei were found around or inside the ovules that failed to be fertilized (Zhou and Meier, 2014). The presence of the vegetative nucleus at the pollen tube tip is thus probably essential for sensing signals upon pollen tube arrival at ovules (Zhou and Meier, 2014).

**AtTIK in root development and nuclear size**

AtTIK is predominantly expressed in roots and is involved in root development (Fig. 2B; Graumann, 2014). In attik-knockout seedlings, roots are significantly smaller and grow more slowly (Graumann et al., 2014). In addition, the size of root nuclei is smaller than in wild type. However, the functions of AtTIK and the molecular link between small nuclei and small roots remain to be investigated (Graumann, 2014).

**SINE1 and SINE2 in guard cell nuclear anchorage and plant immunity**

*Arabidopsis* SINE1 and SINE2 have similar expression patterns in roots; however, in leaves, SINE1 is specifically expressed in guard cells and guard cell mother cells, while SINE2 is predominantly expressed in epidermal and mesophyll cells (Zhou et al., 2014). Both SINE1 and SINE2 contain N-terminal armadillo repeats, but only the armadillo repeats of SINE1 co-localize with actin filaments (Zhou et al., 2014). SINE1 forms F-actin-dependent interwoven filaments across the NE in guard cells (Zhou et al., 2014). In wild-type *Arabidopsis* guard cells, the nuclei are usually positioned in the centre. Loss of SINE1 impaired the central nuclear position of guard cells (Zhou et al., 2014). A similar defect was also observed in sun1-knockout sun2-knockdown leaves or wild-type leaves when F-actin was depolymerized by latrunculin B. This suggests that the SUN–SINE1 complex is essential for guard cell central nuclear anchorage in an F-actin-dependent manner (Fig. 2D; Zhou et al., 2014). No guard cell phenotype was observed in sine2 mutants, but, instead, SINE2 plays a role in innate immunity against *Hyaloperonospora arabidopsis* (*Hpa*), an oomycete pathogen (Fig. 2E; Zhou et al., 2014). *Hpa* invades *Arabidopsis* through the junction of epidermal cells, grows through mesophyll cells, and inserts haustoria into these cells for nutrition absorption from the hosts (Coates and Beynon, 2010). The expression pattern of SINE2 in leaves correlated with the invasion track of *Hpa*, but the detailed function of SINE2 in anti-*Hpa* defence needs further investigation.

**SUN and WIP in meiosis**

The NE serves as a platform for chromosome organization during meiotic prophase I and, in mammals, *C. elegans*, and yeast, the meiotic telomeres are linked to the cytoplasmic motors through the SUN–KASH NE bridges (Kracklauer et al., 2013; Yamamoto, 2014). The cytoplasmic forces transferred to the telomeres cause telomere clustering at the NE, which pulls the chromosomes into a ‘bouquet’. Telomere movement at the NE and the formation of the chromosomal bouquet are important for homologous chromosome pairing and recombination. The first piece of evidence that plant SUN proteins are also involved in meiosis came from the *Zea mays desynaptic mutant* (Murphy and Bass, 2012). The desynaptic mutant is defective in telomere clustering, homologous chromosome synopsis, recombination, and chromosome segregation (Murphy and Bass, 2012). The expression of a truncated mid-SUN protein, ZmSUN3, in the desynaptic mutant may be responsible for these defects (Murphy and Bass, 2012). Recent evidence from various groups indicates that in plants, Cter–SUN proteins and WIP proteins also play important meiotic roles.

During maize meiotic prophase I, ZmSUN2 was found to form a unique belt-like structure at the NE (Murphy et al., 2014). Immunolabelling experiments showed that ZmSUN2 clustered to an NE belt at the leptotene stage, changed to a half belt at the zygotene stage, and returned to the full belt afterwards (Murphy et al., 2014). Telomeres were localized inside this SUN2 NE belt, but neither F-actin nor microtubules was observed to form structures related to this belt (Murphy et al., 2014). Although the developmental function of the ZmSUN2 belt is unclear, the belt was disrupted in meiosis-specific mutants (Murphy et al., 2014). Similar to the SUN belt in maize, AtSUN1 and AtSUN2 also have meiotic prophase I-specific localization (Varas et al., 2015). Expressed as GFP fusions driven by their own promoters, AtSUN1 and AtSUN2 appear to localize evenly at the NE in interphase of pollen mother cells. In meiotic prophase I, however, both proteins accumulate in punctate structures—usually one per nucleus. Further, instead of being evenly distributed at the NE, the two proteins become polarized to one side of the nucleus (Varas et al., 2015). Similar to maize, this polarization is hypothesized to mirror telomere clustering at the NE (Varas et al., 2015).

In *Arabidopsis*, deficiency of AtSUN1 and AtSUN2 caused defects in the distribution of meiotic crossovers sites (Duroc et al., 2014; Varas et al., 2015) A similar phenotype was also observed in an *Arabidopsis atpss1* null mutant and an *atsun1 atpss1* triple null mutant (Duroc et al., 2014). *AtPSSI* (*Arabidopsis Pollen Semi-Sterility1*), also known as AtKin-1, encodes a kinesin I-like protein homologous to rice OsPSSI (Zhou et al., 2011; Duroc et al., 2014; Wang et al., 2014). The synopsis and distribution of meiotic crossovers are impaired in *atpss1*. AtPSSI was found to interact with AtWIP1 and AtWIP2 in a yeast two-hybrid assay (Duroc et al., 2014). It is therefore possible that SUN, WIP, and AtPSSI form a complex which mediates telomere movement and chromosome synopsis during meiotic prophase I (Fig. 2G; Duroc et al., 2014). This is also supported by Varas et al. (2015), who found severe meiotic defects in the absence of full-length AtSUN1 and AtSUN2. These include delayed meiotic progression, incomplete synopsis, and formation of unresolved interlocks, which all lead to reduced seed count and severely affect plant fertility (Varas et al., 2015). Interestingly, AtSUN1...
and AtSUN2 appear to be able to substitute for each other, because single null mutants do not have any of these defects (Varas et al., 2015). These studies provided the first direct genetic evidence that SUN proteins function in plant meiosis.

SUN proteins in mitotic NE breakdown and regeneration

Animal SUN proteins play a role in NE breakdown and NE–chromosome separation during mitosis (Chi et al., 2007; Patel et al., 2014; Turgay et al., 2014). In plants, localization studies of AtSUN1 and AtSUN2 in synchronized tobacco cell cultures and Arabidopsis root tips have revealed the first insights into SUN protein functions in mitosis (Graumann and Evans, 2011; Oda and Fukuda, 2011). In higher plants, like in metazoans, the NE breaks down at the onset of mitosis and reforms around the separating chromatin in the final phases of division (Graumann and Evans, 2013). NE proteins are known to disperse throughout the mitotic membranes after NE breakdown, and AtSUN1 and AtSUN2 are also found in these mitotic membranes (Graumann and Evans, 2013). Unlike metazoans, where the mitotic chromosomes are free of membranes in metaphase and anaphase, in tobacco cell culture, mitotic membranes are known to traverse the spindle and both AtSUN1 and AtSUN2 are present in these spindle membranes, which can be in close proximity to the chromosomes (Graumann and Evans, 2011). Interestingly, once the aligned chromosomes began to separate in anaphase, both SUN proteins started to accumulate on the side of the chromosomes that face the spindle poles. This protein accumulation then extended down the ‘sides’ of the decondensing chromosomes and eventually reached the other side facing the division plane (Graumann and Evans, 2011; Oda and Fukuda, 2011). This spatiotemporal localization of AtSUN1 and AtSUN2 in the reforming NE is thought to be linked to microtubule dynamics (Oda and Fukuda, 2011) and is very likely to be functional, since a non-functional NE marker, lamin B receptor (LBR)–GFP, did not display this pattern, but rather appeared evenly around the telophase chromosomes (Irons et al., 2003; Graumann and Evans, 2011). Moreover, the SUN proteins are more immobilized in the reforming NE and during NE breakdown than they are in the mitotic membranes, indicating that they are functional during NE breakdown and NE reformation (Graumann and Evans, 2011). The spatiotemporal pattern observed for AtSUN1 and AtSUN2 during NE reformation has also been observed for their interaction partners AtWIP1, AtWIP2, and AtWIP3 (Xu et al., 2007), and Apium graveolens CRWN1 (see below; Kimura et al., 2010), indicating that NE bridging complexes may have functional significance during NE reformation.

Association of plant lamins with SUN proteins

The animal INM is associated with an electron-dense laminar material named nuclear lamina (Aaronson and Blobel, 1975; Fawcett, 1966) which is composed of type V intermediate filaments—lamins (Aebi et al., 1986; Gerace et al., 1978; Burke and Stewart, 2013). Animal lamins bind SUN proteins and are linked to the cytoskeletons through LINC complexes, essential for nuclear shape, nuclear positioning, and mecha-notransduction (ISermann and Lammerding, 2013; Razafsky et al., 2014; Stroud et al., 2014).

Although no homologues of lamins are encoded in plant genomes, electron microscopy has confirmed an INM-associated meshwork, similar to the animal lamina (for a recent review, see Cisca and Moreno Diaz de la Espina, 2014). Currently, the best protein candidates for plant lamins are members of the nuclear matrix constituent protein (NMCP) family (Cisca and Moreno Diaz de la Espina, 2014). Daucus carota NMCP1 (DcNMCP1) was the first one isolated and characterized (Masuda et al., 1997), and later its homologues were reported in other species (Dittmer et al., 2007; Kimura et al., 2010; Cisca et al., 2013; Sakamoto and Takagi, 2013; Wang et al., 2013). NMCPs share some characteristics with lamins, especially the middle long CCD (Cisca and Moreno Diaz de la Espina, 2014). The Arabidopsis genome encodes four NMCPs which are also known as CROWDED NUCLEI I (CRWN1, also known as LITTLE NUCLEI I), CRWN2, CRWN2, and CRWN4. Like DcNMCP1, CRWN1 and CRWN4 were localized to the nuclear periphery and were also isolated from the crude nuclear lamina fraction (Dittmer et al., 2007; Masuda et al., 1997; Sakamoto and Takagi, 2013; Kimura et al., 2014). In contrast, CRWN2 was localized to the nucleoplasm and CRWN3 was mainly localized at the nuclear periphery and was partially present in the nucleoplasm (Sakamoto and Takagi, 2013). Deficiencies of animal lamins cause NE deformation (Butin-Israeli et al., 2012). Similarly, mutants of crwn1 and crwn4, but not mutants of crwn2 or crwn3, bear nearly spherical nuclei with reduced size (Sakamoto and Takagi, 2013; Wang et al., 2013). Combining a crwn1 mutation with a crwn2, crwn3, or crwn4 mutation led to a synergistic effect on nuclear size reduction (Wang et al., 2013).

The interaction between CRWNs and SUN proteins has been shown indirectly. Using fluorescence resonance energy transfer experiments, the N-terminal nucleoplasmic domains of AtSUN1 and AtSUN2 was shown to interact with CRWN1 (Graumann, 2014). This N-terminal domain also affected the NE localization and the mobility of CRWN1 (Graumann, 2014). Recently, another INM protein, KAKU4, was identified in Arabidopsis. Mutants of KAKU4 also have spherical nuclei with reduced size, similar to crwn1 mutants (Goto et al., 2014). Although KAKU4 does not contain a TMD, overexpressing KAKU4 caused extra membrane growth at the NE (Goto et al., 2014). This phenotype was enhanced when CRWN1 was co-expressed with KAKU4 (Goto et al., 2014). Therefore, KAKU4 is another candidate for a plant lamina component, and it will be informative to study the relationship among KAKU4, CRWN1, AtSUN1, and AtSUN2.

An evolutionary view of SUN and KASH proteins

It is interesting to note that plant KASH proteins (except for AtTIK), WITS, NMCPs, and the KAKU4 family proteins are specific to plants. In addition, many known opisthokont
NE proteins are absent in plants, such as opisthokont KASH proteins, animal lamin, animal LEM (Lap2, Emerin, Man1)-domain proteins (Wagner and Krohne, 2007), and animal Samp1 (homologous to S. pombe Ima1; Borrego-Pinto et al., 2012). These proteins form an interaction network connected by laminins. In vertebrates, laminins also interact with the nuclear pore complex at the nucleoplasmic side by binding Nup153 (Smythe et al., 2000; Al-Haboubi et al., 2011). Nup153 is also not present in plants (Tamura et al., 2010). Instead, plant Nup136 is believed to be the functional homologue of Nup153, and interactions between NMCPs and Nup136 have been proposed (Tamura et al., 2010; Tamura and Hara-Nishimura, 2011). Although SUN proteins are well conserved in eukaryotes, the homology lies only in the SUN domain. Alignment of plant SUN proteins reveals a conserved N-terminus specific to angiosperms. In the eudicot lineage in particular, an N-terminal ‘SASTVSIT’ motif followed by another ‘RR[RT]-q2.5[DE]KK’ motif is conserved (‘q,2.5’ represents 2–5 amino acids with at least two hydrophobic amino acids in the middle; see Supplementary Fig. S2 at JXB online). Therefore, homology of SUN and KASH proteins in plants and opisthokonts is currently confined to the PNS interaction domains. Since a plethora of mammalian SUN proteins have been identified through proteomics (Schirmer et al., 2003; Korfali et al., 2012; de las Heras et al., 2013), further studies are needed to determine whether plant genomes also encode homologues of these mammalian NE proteins or if plants have evolved a different cohort of NE proteins.

**Future perspective**

Opisthokont SUN proteins have cellular functions that have not been described for, but probably apply to, plant SUN proteins; microtubule organization (Malone et al., 2003; Zhang et al., 2009), nuclear pore complex formation (Liu et al., 2007; Talamas and Hetzer, 2011), maintenance of NE architecture under mechanical strain (Cain et al., 2014), DNA damage response (Oza et al., 2009; Lei et al., 2012), and karyogamy (Jaspersen et al., 2006; Xiong et al., 2011; Vasnier et al., 2014). Plants have evolved other cellular mechanisms that involve nuclear movement or signalling, such as anti-pathogen defence, plant–microbe symbiosis, physical stress response, and thigmotropism (van Der Luit et al., 1999; Bonfante and Genre, 2010; Takagi et al., 2011; Ranty et al., 2012; Charpentier and Oldroyd, 2013; Griffis et al., 2014). Together, these studies have implied exciting unknown functions of the plant NE. Our current knowledge of the SUN–KASH network can serve as a starting point for future discoveries.

**Supplementary data**

Supplementary data are available at JXB online.

**Figure S1.** Phylogenetic tree of species that contain SUN proteins.

**Figure S2.** Protein sequence alignment of the N-termini of SUN proteins in angiosperms.

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