How plants LINC the SUN to KASH

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Abbreviations: CCSD, canonical C-terminal SUN domain; ER, endoplasmic reticulum; INM, inner nuclear membrane; KASH, Klarsicht/ANC-1/Syne homology; LINC, linkers of the nucleoskeleton, NE, nuclear envelope; ONM, outer nuclear membrane; PM3, plant-prevalent mid-SUN 3 transmembrane; SUN, Sad1/UNC-84

The nuclear envelope (NE) is a double membrane system consisting of an inner nuclear membrane (INM) and an outer nuclear membrane (ONM). Studies in opisthokonts revealed that the two membranes are bridged by protein complexes formed by the INM Sad1/UNC-84 (SUN) proteins and the ONM Klarsicht/ANC-1/Syne homology (KASH) proteins.

The founding member of SUN proteins is Caenorhabditis elegans Sad1, a component of the spindle pole body. This domain was subsequently found to interact with a C-terminal domain conserved in a series of ONM proteins including Klarsicht, ANC-1, and Syne (also known as Nesprin). Therefore, the two interacting domains were named Sad1/UNC-84 (SUN) domain and Klarsicht/ANC-1/Syne homology (KASH) domain, respectively. SUN proteins typically locate at the INM with their C-terminal SUN domain positioned in the perilamin space (PNS) (Fig. 1 and see below). KASH proteins are tail-anchored proteins at the ONM harboring a C-terminal KASH domain, which contains a short PNS tail (~30 amino acids) typically ending with a PPPX (X represents any amino acid) motif that is essential for interacting with the SUN domain. The SUN-KASH complexes bridge their binding partners across the NE (Fig. 1). In the nucleoplasm, the N-termini of many SUN proteins interact directly or indirectly with nuclear lamins, which are intermediate filament proteins located underneath the INM and are considered components of the nucleoskeleton. At the cytoplasmic side, KASH proteins are linked to motor proteins, intermediate filaments, microtubules, or F-actin.

Mammalian SUN1 and SUN2 interact with lamin A, and the NE localization of SUN2 depends on lamin A. SUN1 and SUN2 interact with the mammalian KASH proteins nesprin-1 and nesprin-2 at the NE. These two KASH proteins consist of N-terminal F-actin-binding calponin homology domains; a long stalk domain composed of spectrin repeats, and a C-terminal KASH domain. The spectrin repeats each assemble into a three-helix bundle, which makes the protein flexible in length and may assist in buffering against mechanical stress. Nesprin-1 and -2 interact with F-actin and connect the INM lamins through SUN1 and SUN2 (Fig. 1A). These LINC complexes are responsible for anchoring the synaptic nuclei at the mouse neuromuscular junction. Nesprin-1 and -2 also link the centrosome to the nucleus through interactions with the dynamin-dynactin complex (Fig. 1A). This connection is essential for interkinetic nuclear migration and nucleuskinesis in mice.

In Drosophila, the localization of Klarsicht at the nuclear periphery depends on a type B lamin and a SUN protein, klaroid. Klaroid also forms nuclear aggregates in transgenic flies expressing a mutated lamin C that lacks the first A2 amino acids, suggesting that Klaroid might be associated with lamin C. Klarsicht is connected to microtubules and is responsible for the apical nuclear migration during photoreceptor formation.

Linkers of the nucleoskeleton to the cytoskeleton (LINC) complexes formed by SUN and KASH proteins are conserved eukaryotic protein complexes that bridge the nuclear envelope (NE) via protein-protein interactions in the NE lumen. Revealed by opisthokont studies, LINC complexes are key players in multiple cellular processes, such as nuclear and chromosomal positioning and nuclear shape determination, which in turn influence the generation of gametes and several aspects of development. Although comparable processes have long been known in plants, the first plant nuclear envelope bridging complexes were only recently identified. WPP domain-containing proteins at the outer NE have little homology to known opisthokont KASH proteins, but form complexes with SUN proteins at the inner NE that have plant-specific properties and functions. In this review, we will address the importance of LINC complex-regulated processes, describe the plant NE-bridging complexes and compare them to opisthokont LINC complexes.

The opisthokont SUN and KASH Proteins

The founding member of SUN proteins in Caenorhabditis elegans UNC-84. The C-terminus of UNC-84 has a domain sharing homology with two human proteins and Schizosaccharomyces pombe Sad1, a component of the spindle pole body. This domain was subsequently found to interact with a C-terminal domain conserved in a series of ONM proteins including Klarsicht, ANC-1, and Syne (also known as Nesprin). Therefore, the two interacting domains were named Sad1/UNC-84 (SUN) domain and Klarsicht/ANC-1/Syne homology (KASH) domain, respectively. SUN proteins typically locate at the INM with their C-terminal SUN domain positioned in the perilamin space (PNS) (Fig. 1 and see below). KASH proteins are tail-anchored proteins at the ONM harboring a C-terminal KASH domain, which contains a short PNS tail (~30 amino acids) typically ending with a PPPX (X represents any amino acid) motif that is essential for interacting with the SUN domain. The SUN-KASH complexes bridge their binding partners across the NE (Fig. 1). In the nucleoplasm, the N-termini of many SUN proteins interact directly or indirectly with nuclear lamins, which are intermediate filament proteins located underneath the INM and are considered components of the nucleoskeleton. At the cytoplasmic side, KASH proteins are linked to motor proteins, intermediate filaments, microtubules, or F-actin.

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similar chromosomal bouquet mediated by a SUN1-KASH5 complex.7 Table 1 provides an overview of known SUN-KASH pairs from different organisms and their known or proposed functions.

The structure of the mammalian SUN-KASH complex was recently resolved.39,40 In this complex, three KASH domains are anchored in one cloverleaf-like trimer of SUN domains. The SUN domain protomer has several functional domains: an α-helical stalk, a compact β-sandwich core, a cation-loop, and a protruding anti-parallel β-sheet named the KASH-lid (Fig. 2). The α-helix serves as an extension of the trimeric coiled-coil domain of SUN proteins, which facilitates the trimerization. The trimer is further stabilized by a large inter-acting surface on the β-sandwich core and the hydrogen bonds between the α-helix of one protomer to the β-sandwich of the adjacent protomer. The KASH domain is clamped between the KASH-lid of one protomer and the β-sandwich core of the adjacent protomer. Without binding the KASH domain, the KASH-lid conformation is rather random.40 The very C-terminal “PPPX” motif is positioned in a KASH pocket formed by S641, Y703, Y707, H628 and the cation-loop (Q593-C601), which explains why these four amino acids are critical for SUN-KASH interactions.40

Nuclear Positioning in Plants

Nuclear positioning events are involved in considerable aspects of the plant life cycle. The most obvious example of nuclear movement is pollen tube growth, during which sperm cells and the vegetative nucleus migrate over long distances toward the tip of the growing pollen tube (for a recent review, see ref. 41). In

A similar nuclear migration process in mouse cone photoreceptor development is regulated by SUN1, but the responsible KASH proteins and the involved elements of the cytoskeleton are unknown.7 The localization at the nuclear periphery of another KASH protein, MSP-300 also depends on klaroid.23 MSP-300 is an ortholog of mammalian neosprit-1 and -2. Although its role in nuclear anchorage was previously unclear,24-26 a recent study revealed that MSP-300 interacts with D-Titin/Sallimus and anchors mitochondria and endoplasmic reticulum (ER) to the striated muscle Z-discs.27 It also cooperatively functions with Klaroid to promote even nuclear spacing in striated muscle.27 In C. elegans, UNC-84 co-localizes with Ce-lamin at the NE and its localization depends on Ce-lamin.28 UNC-84 recruits the KASH proteins UNC-83 and ANC-1 to the NE (Fig. 1A).29,30 UNC-83 in turn targets kinesin-1 and/or dynein to the nuclear periphery, and the force provided by the motor proteins drives the nuclear migration in C. elegans hypodermal P cells and embryonic hypodermal cells.30-32 ANC-1 interacts with F-actin and is required for nuclear anchorage in the adult C. elegans syncytial hypodermis.30

SUN-KASH complexes can also link chromosomes to the cytoskeleton. In Schizosaccharomyces pombe, centromeres are tethered to the SUN protein Sad1 through Csl1 (Fig. 1B).33 Loss of Sad1 or Csl1 leads to high-frequency centromere clustering defects.33 At meiotic prophase, telomeres are tethered to Sad1 through Bqt1, Bqt2 and telomere-associated proteins Taz1 and Rap1.7 Telomeres are further linked to dynein motors by the KASH protein Kms1 and Kms2 (Fig. 1B).34-37 This results in telomere clustering and later in nuclear oscillation between the cell poles, which facilitates homologous pairing and recombination.30 In mammals, a recent study revealed the formation of a similar chromosomal bouquet mediated by a SUNI-KASH5 complex.7 Table 1 provides an overview of known SUN-KASH pairs from different organisms and their known or proposed functions. The structure of the mammalian SUN-KASH complex was recently resolved.7 Table 1 provides an overview of known SUN-KASH pairs from different organisms and their known or proposed functions.
| SUN | KASH | Cytoplasmic partner: function |
|-----|------|------------------------------|
| Mammalian | | |
| SUN1/2 | Nesprin-1 | F-actin: anchoring the synaptic nuclei under the mouse neuromuscular junction. |
| SUN1/2 | Nesprin-2 | Dynein/dynactin complex: anchoring the nucleus to centrosome for interkinetic nuclear migration and nucleokinesis. |
| SUN1/2 | Nesprin-3 | Dynein/dynactin complex and kinesin: connecting the nucleus to centrosome for interkinetic nuclear migration and nucleokinesis. |
| Maybe SUN1/2 | Nesprin-4 | Kinesin-1: predicted to promote nuclear migration toward the base of the secretory epithelial cells. |
| SUN1/2 | KASH5 | Dynein/dynactin complex: telomere movement during meiosis. |
| SUN3 | Nesprin-1 | Proposed to be kinesin II, dynein/dynactin, or F-actin: probably links the nucleus to posterior manchette during sperm head formation. |
| SUN1η | Nesprin-3 | Proposed to be plectin: proposed to be a non-NE complex anchoring acrosome to the anterior actin filaments during sperm head formation. |
| SPAG4 | Unknown | Anchoring microtubules to the NE in striated muscle. |
| SPAG4L4L-2 | Unknown | Unknown: restricted to the apical nuclear region of round spermatids facing the acrosomal vesicle, and probably involved in linkage of the acrosomal vesicle to the spermatid nucleus and in acrosome biogenesis. |
| Drosophila | | |
| Klaroid | MSP-300 | F-actin: nuclear anchoring during Drosophila oogenesis. |
| MSP-300 | D-Titin/Sallimus: anchoring mitochondria and endoplasmic reticulum to the striated muscle Z-discs. |
| MSP-300 | Unknown | Anchoring microtubules to the NE in striated muscle. |
| MSP-300 | Unknown | Anchoring the nuclei to myofibril compartment in striated muscle. |
| Klaroid | Klarsicht | Proposed to be microtubule motors: nuclear migration during eye development. |
| KLAR | Unknown | Anchoring microtubules to the NE in striated muscle. |
| SPAG4/GLA | Unknown | Unknown: promoting even myonuclear spacing in both striated muscle and nonstriated myotubes. |
| C. elegans | | |
| UNC-84 | ANC-1 | F-actin: nuclear anchorage in the adult C. elegans syncytial hypodermis. |
| UNC-84 | UNC-83 | Kinesin-1 and dynein: nuclear migration in embryonic C. elegans hypodermal cells. |
| SUN-1/matefin | ZYG-12 | Dynein and ZYG-12A: linkage between the centrosome and nucleus; meiotic chromosome paring and synapsis, and nuclear positioning within the syncytial gonad. |
| SUN-1/matefin | KDP-1 | Unknown: cell-cycle progression. |
| S. pombe | | |
| Sad1 | Kms1 and Kms2 | Dynein and centrosomes: meiotic chromosome pairing and synapsis. |
| S. cerevisiae | | |
| Mps3 | Unknown | Unknown: Mps3 is involved in spindle pole body insertion into the NE and NE homeostasis; it interacts with Mps2 to connect the spindle pole body to the NE and functions in spindle pole body duplication. |
| Mps3 | Csm4 | Probably F-actin: meiotic telomeres are tethered to Mps3 at the NE by Ndc1 and further connected to the cytoskeleton (perhaps actins) by Csm4. |
| Mps3 | Unknown | Unknown: Mitotic telomeres are tethered to Mps3 at the NE by Sir4 and the telomere clustering is mediated by two Mps3 associated proteins, Ebp2 and Lpl1. |
| Dictyostelium | | |
| SUN-1 | Unknown | Unknown: SUN-1 connects the centrosome to chromatin and ensures genome stability. |
| Unknown | Interaptin | F-actin: Function unknown. |
| Arabidopsis | | |
| AISUN1/2 | AtWIP1/2/3 | RanGAP: anchoring RanGAP to the NE. |
| AISUN1/2 | AtWIP1/2/3 | Unknown: nuclear shape determination. |
Plant SUN Proteins

Two types of SUN protein have been identified in plant genomes: the canonical C-terminal SUN domain (CCSD) type and the plant-prevalent mid-SUN 3 transmembrane (PM3) type. The CCSD type has a SUN domain at the C-terminus, while the signature of the PM3 type is a centrally positioned SUN domain followed by a highly conserved domain of unknown function. The PM3 type was first discovered in maize, but is also present in other plant species, as well as in opisthokonts and fungi.

Despite the importance of nuclear positioning and chromosome movement during the plant life cycle, little is known about the molecular players involved. The recent identification of NE-bridging complexes in plants has now provided tools to begin investigating the underlying molecular mechanisms.
A combination of co-immunoprecipitation and fluorescence recovery after photobleaching experiments demonstrated that AtWIP1, AtWIP2, and AWIP3 interact with AtSUN1 and AtSUN2 at the plant NE. The PNS tail of AtWIP1, especially the “VPT” motif, is required for the interaction, similar to the interactions between mammalian Nesprins and SUNs. Surprisingly, the PNS tail of WIP1 is only 9 amino acids long. The PNS tail of Homo sapiens Nesprin-2 is 30 amino acids long, and its C-terminal fragment of 14 amino acids is the shortest one able to bind the SUN domain. The ability of Arabidopsis SUN proteins to bind a very short PNS tail might be connected to the presence of a stretch of additional conserved residues in plant SUN domains; however, further work is required to understand the exact biochemical nature of the unusual plant NE-bridging complex.

**Function of the AtSUN-AtWIP Complex in RanGAP NE Anchoring**

The NE localization of AtWIP1 is reduced in a sun1-knockout sun2-knockdown (sun1-ko sun2-ko) mutant, suggesting that the localization of WIPs depends on AtSUNs, analogous to the animal KASH proteins. Consistent with these findings, the NE localization of Arabidopsis RanGAP1 is currently the only confirmed cytoplasmic part of the RanGAP-NE-bridging complex containing the SUMO E3-ligase nucleoporin RanBP2, SUMOylated RanGAP1 and the E2 SUMO-conjugating enzyme UBC9. The finding that plants have recruited an NE-bridging complex to anchor RanGAP to the NE suggests that there may be additional functions for such complexes beyond linking the nucleus and the cytoskeleton. Thus, novel, cytoskeleton-unrelated binding partners might also exist for opisthokont KASH proteins. One such KASH protein candidate might be C. elegans KDP-1, which functions in cell cycle progression from late S to M phase, and for which a cytoskeleton partner is currently unknown.

**Function of the AtSUN-AtWIP Complex in Nuclear Shape Determination**

It is possible that WIPs additionally interact with motor proteins or the cytoskeleton. However, neither the wip1-1 wip2-1 mutant, indicating that plant SUN proteins play a role in RanGAP-NE association by forming a RanGAP-WIP-SUN complex. The existence of this complex is supported by co-immunoprecipitation data.

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**Figure 2.** Computed three-dimensional model of the SUN domain of AtSUN1. (A) The S251-D453 fragment of AtSUN2 was modeled using MODELER. The SUN domain of HsSUN2 was used as a template (PDB: 4F19). Three models were computed and the one with the lowest DOPE score is shown. Magenta, model of the AtSUN1 SUN domain. Cyan, SUN domain of HsSUN2. Gray, Nesprin-2 KASH domain in the HsSUN2-KASH complex. (B) Computed surface of the binding pocket for the KASH C-terminus in AtSUN1. Red, V301-N318 fragment of AtSUN1 corresponding to the KASH lid of HsSUN2 (Y1567-S1581). Orange, S324-C333 fragment of AtSUN1, corresponding to the cation-loop of HsSUN2 (S593-C601). Purple, residue H360, S371, H439, and Y463 of AtSUN1, corresponding to H624, S641, Y703, Y707 of HsSUN2, respectively. Images were generated using UCSF Chimera package and POVRay (http://www.povray.org/).

**Figure 3.** (See opposite page.) Amino acid sequence alignment of plant SUN domains with the SUN domain of HsSUN2. Alignment was performed using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) with default settings, except that the output sequences were kept as input order. Image was generated using JalView and ClustalX color. GI, NCBI GenInfo identifier. Black frames in the alignment are numbered at the top. Frame 1 indicates Cys63 in HsSUN2, which forms a disulfide bond with Homo sapiens Nesprin-2 Cys682. This disulfide bond is dispensable for SUN-KASH interaction, and in plant SUN domains this position is instead a conserved D or E. Frame 2 indicates the KASH lid in HsSUN2, however, the sequences have low similarity between HsSUN2 and plant SUN proteins. Frame 3 represents the cation loop in HsSUN2. This cation loop and residues indicated by frame 4, 6, 7, 8 (correspond to H624, S641, Y703, and Y707 of HsSUN2, respectively) form the pocket holding the KASH C-terminus and are well conserved in plant SUN domains. Frame 5 represents N636 of HsSUN2, the N-glycosylation site. N-glycosylation of HsSUN2 is dispensable for KASH binding, and this position is conserved D in plant SUN domains.
Arabidopsis root hair cells, trichomes, and some of the leaf epidermal cells, the nucleus is spindle-shaped. Especially in root hair cells, the super-elongated nucleus resembles a "pod" with two thin tails attached to its poles. The only phenotype observed in these mutants was a reduced nuclear polarity in root hairs, leaf epidermal cells, and trichomes. In wild-type Arabidopsis root hair cells, trichomes, and some of the leaf epidermal cells, the nucleus is spindle-shaped. Especially in root hair cells, the super-elongated nucleus resembles a "pod" with two thin tails attached to its poles. In the wip3-1 wip3-1 wip3-1 nor the sun1-KO sun2-KD mutant has obvious defects in nuclear positioning or plant development. The only phenotype observed in these mutants was a reduced nuclear polarity in root hairs, leaf epidermal cells, and trichomes.
mutant or the sun1-KO sun2-KD mutant, this spindle shape is lost, indicating that both WIP and SUN are required for maintaining an elongated nuclear structure in these cell types. It is possible that an unknown factor anchored by the SUN-WIP complex regulates the nuclear shape in the Arabidopsis epidermis (Fig. 1C, indicated by the oval with a question mark).

Intriguingly, loss-of-function mutants of two Arabidopsis nuclear long, coiled-coil proteins, LITTLE NUCLEI 1 and 2 (LINC1 and LINC2), recently renamed to CRWN1 and CRWN2, respectively, to avoid confusion with the LINC complex, also lead to the loss of nuclear elongation, in addition to a reduced nuclear size. Plant genomes do not encode homologs of animal lamin genes. The Daucus carota L. (carrot) nuclear matrix constituent protein 1 (NMCP1) and its Arabidopsis homo-
lologs—CRWN1, CRWN2, CRWN3 and CRWN4—have been proposed as putative plant counterparts of animal lamins. They are conserved in many plant species and contain long coiled-coil domains similar to lamins, though at almost twice the size, and NMCP1 and CRWN1 are located at the nuclear periphery. In addition to the nuclear shape change, the crwn1-1 crwn2-1 double mutant has a significantly reduced nuclear volume, fewer chromocenters, and a dwarf plant phenotype.

Nuclear shape change caused by mutations in lamin genes was observed in a series of human diseases called laminopathies. One such disease is Hutchinson-Gilford progeria syndrome (HGPS), which is linked to point mutations in the human lamin A gene (Lmna). Patients suffer from a series of premature-aging symptoms—among others, loss of hair, restrictive joint mobility, and cardiovascular disease. One of the hallmarks of HGPS cells is the occurrence of blebbled and lobulated nuclei. This defect of nuclear shape is reversely proportional to the amount of nesprin-2 at the NE, and mutation or silencing of nesprin-2 also causes blebbled nuclei in both mouse and human cells. Unexpectedly, the mutation of SUN1 in Lmna- mice did not accelerate the pathological phenotypes, but instead ameliorated them and corrected the nuclear aberrations in fibroblasts. In light of the SUN1 overaccumulation observed in Lmna- cells, this implies SUN1 overabundance as a pathogenic event in HGPS and a trigger of nuclear shape aberrations.

The involvement of both lamins and lamin-like proteins and SUN-KASH complexes in nuclear shape in both plants and mammals and their connection to human disease make it worthwhile to further investigate the biological relevance of nuclear morphology at the cellular and organismal level.

Possible Function of Plant SUN Proteins in Mitosis and Meiosis

Mammalian SUN1 is associated with NPCs and interacts with lamins. It is suggested that this kind of INM protein links mitotic ER to chromatin in telophase and might mediate NE reassembly during mitosis. A similar mechanism might also exist in plants. In Arabidopsis, AtSUN1 diffuses to the ER after NE breakdown and is mainly located at the distal side of the separated chromosomes throughout anaphase. At telophase, an enriched AtSUN1 signal starts to enclose the chromosomes from the distal surface to the proximal surface. At the same time, the signal at the ER becomes gradually reduced, indicating the translocation of AtSUN1 to the newly formed NE. Studies of AtSUN1 and AtSUN2 in BY-2 cells showed similar results.

Additional work using BY-2 cells also showed that AtSUN1 and AtSUN2 are associated with membranes around the spindle and close to the chromosomes. During plant meiotic prophase I, the telomere bouquet associated with the NE has roles in interhomolog pairing, synapsis, and homologous recombination. Evidence for a possible involvement of plant SUN proteins in this process comes from the maize desynaptic (dy) mutant. This mutant is defective in chromosome synapsis, recombination, telomere-NE tethering, and chromosome segregation. Linkage mapping combined with a candidate-gene approach make it likely that a splice variant of maize SUN3 is responsible for this phenotype.

Perspectives

Our understanding of the nature and role of plant NE proteins has just begun. Compared with the nuclear pore proteins, NE proteins appear to be even less conserved between plants and animals. This suggests that the hunt for plant NE proteins largely will be by de novo identification, rather than homology searches. It is exciting to learn that NE-bridging complexes are conserved in plants and that they perform plant-specific functions through plant-specific ONM partners. A cornucopia of new questions follows from these findings: How is the SUN-WIP complex involved in the developmental changes in nuclear morphology? Are there additional plant NE-bridging complexes and are they connected to the cytoskeleton? Are they involved in nuclear migration? Do plant SUN proteins interact with other IMN proteins and do they interact with a plant lamina? How are plant SUN proteins involved in chromosomal positioning? Perhaps the answers to these questions will help resolve a more general enigma: why is the complement of NE proteins so different in plants? And what does this tell us about the separate evolution of NE functions in plants and opisthokonts? A rapid resolution of these questions is unlikely, but their answers will certainly lead to a broader, more comparative understanding of the physical interaction of the nucleus with its cellular environment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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