Protein interactions at the higher plant nuclear envelope: evidence for a linker of nucleoskeleton and cytoskeleton complex

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INTRODUCTION

The nuclear envelope (NE) fulfills many important functions: protecting and enclosing the genetic material, facilitating transport, involvement in cell signaling, and providing physical and structural bridges. These functions of support, transport, and communication are required to different extents in various organisms from single cells to complex metazoans and require a high level of complexity. This is achieved by differentiation of the proteins of the inner and outer nuclear membranes (INM and ONM, respectively), each having a unique protein composition providing specific cytoplasm-facing and nucleoplasm-facing functions.

Communication across the NE occurs through protein bridges that link across the periplasmic space between the INM and ONM. These protein bridges have multiple functions, providing support and anchorage for the genetic material and nucleoskeleton, positioning, and moving the nucleus and acting as a pathway of signaling. While these functions and structures are conserved in eukaryotes, there are marked differences in the proteins that are involved (see below and Zhou and Meier, 2013). This mini review will consider advances in the identification of the proteins of the higher plant NE with a focus on proteins that bridge the membrane.

BRIDGING THE NUCLEAR PERIPLASM; THE LINC COMPLEX

Micrographs of the nucleus reveal many connections between structural proteins of the cytoplasm and nucleoplasm, the nuclear membrane and the nuclear pores (Fiserova et al., 2009). The membranes are separated by a space of about 50 nm and it is in this space that interactions between ONM and INM proteins occur. The major bridge in this space is the linker of nucleoskeleton and cytoskeleton (LINC) complex (Crisp et al., 2006). It is conserved across eukaryotes and has remarkable diversity of function through modification of two families of constituent proteins. It provides anchorage for NE-associated proteins of the nucleoskeleton and cytoskeleton, which support, move and shape the NE and chromatin and position the nuclear pores (Figure 1).

The LINC complex has two key constituents, the SAD1/UNC84 (SUN) domain proteins of the INM and the Klarsicht/Anc/Syne-1 homology (KASH) domain proteins of the ONM (Crisp et al., 2006). They attach by the interaction of the SUN and KASH domains located at the extreme C-termini of the respective proteins. In most opisthokonts, there are two ubiquitously expressed SUN domain proteins (as well as other family members with more specialized expression patterns); while KASH domain proteins are far more diverse in structure and function.

SUN DOMAIN PROTEINS

The name SUN domain derives from UNC84, described by Malone et al. (1999) in Caenorhabditis elegans embryos and Sad1, a spindle pole body component in Schizosaccharomyces pombe (Hagan and Yanagida, 1995). Both contain a C-terminal SUN-domain. Initial studies revealed two ubiquitously expressed homologs in opisthokonts which are type II integral membrane proteins. The C-terminal SUN domain is located in the nuclear periplasm, with the N-terminus in the nucleoplasm where it interacts with B-type lamins (Crisp et al., 2006). A coiled coil domain is located between the transmembrane domain and the SUN domain in the nuclear
It is now clear that C-SUN domain proteins (SUNs 1–2) in the inner nuclear membrane interact with proteins of the outer nuclear membrane and the nucleoskeleton. The role of mid-SUNs (SUNs 3–5) remains to be elucidated, but they also interact with C-SUNs and may also constitute a key component of anchorages for the cytoskeleton, nucleoskeleton, and nuclear pores. Anchorage of SINE, Wit, Wip, and RanGAP all depend on SUN interaction. The plant LINC complexes are involved in two actin-NE linkages – the direct SINE1-actin connection and the WIP-Wit-myosin XI-1 – actin linkage.

SAD1/UNC84 domain proteins are involved in multiple cellular functions. The yeast SUN domain proteins Sad1 (S. pombe) and Mps3 (Saccharomyces cerevisiae) are located at spindle pole bodies (SPBs) and the INM (Tran et al., 2001; Bupp et al., 2007). Specifically, SpSad1 and ScMps3 are localized to the half bridge, part of the central plaque of the SPB associated with the NE (Hagan and Yanagida, 1995; Jaspersen et al., 2006). As well as the ubiquitously expressed SUN1 and 2, mammals contain three SUN domain proteins (SUN3, 4, and 5) which are tissue specific in distribution (Razafsky and Hodzic, 2009). Some SUN domain proteins are expressed differentially during development; for instance, C. elegans SUN1 is expressed in the germ line and early embryo, while UNC84 is found in adult somatic cells and embryos after the 24-cell stage (Lee et al., 2002; Fridkin et al., 2004).

PLANT SUN DOMAIN PROTEINS – THE EVIDENCE

First descriptions for homologs of plant SUN domain proteins were of SpSad1 in Arabidopsis thaliana by van Damme et al. (2004), and Oryza sativa by Moriguchi et al. (2005) and indicated location at the phragmoplast and mitotic spindle. The significance of the higher plant SUN domain proteins was overlooked until the first detailed characterisation by Graumann et al. (2010). This study revealed AtSUN1 and AtSUN2 to be localized to the NE in interphase and provided the first evidence of components of a putative LINC complex in plants.

Genomic sequencing reveals that proteins with a classical C-terminal SUN domain are present throughout the kingdom plantae, including the club moss Selaginella moellendorffii, the moss Physcomitrella patens, algae, and monocot and dicot species. They have been characterized in detail in the dicot A. thaliana (Graumann et al., 2010; Graumann and Evans, 2011; Oda and Fukuda, 2011) and the monocot Zea mays (Murphy et al., 2010). Each species has two proteins with a C-terminal SUN domain (AtSUN1 and AtSUN2; Graumann et al., 2010, and ZmSUN1 and ZmSUN2; Murphy et al., 2010). AtSUN1 and 2 show a higher degree of homology (68% identity, 1.00 E−178) with each other than with either ZmSUN1 or 2 (41%, 4.0 E−79 and 2.0 E−70, respectively) suggesting separate gene duplication events. Some difference in function and binding as well as location is suggested for the different SUN proteins. The two plant C-terminal SUN domain proteins are significantly smaller than mammalian and closest in size to yeast Sad1. There is a strong structural resemblance with a single coiled coil domain located between the N-terminal transmembrane domain and the C-terminal SUN domain (Graumann and Evans, 2010; Graumann et al., 2010).

Recently, in addition to interaction with putative plant KASH domain proteins in the outer NE (see below), Graumann (2014) has demonstrated that plant SUN domain proteins interact with putative nucleoskeletal proteins of the NMCP/LINC family (Giska and Moreno, 2013, 2014). Specifically, the N-terminus of AtSUN1 and 2 can interact with LINC1 and immobilize it at the nuclear periphery (Graumann, 2014). In the absence of lamins in plants, these SUN–LINC interactions are a first indicator of SUN-nucleoskeletal anchorage in plants.

Studies of higher plants reveal an additional family of proteins containing a central SUN domain in addition to the C-terminal SUNs (Murphy et al., 2010). Genome analysis of
Carugo et al., 2002; Mislow et al., 2002). Therefore, a SUN–KASH
Like SUN, KASH-domain proteins form homomers (Djinovic-
ing domain separated from a single transmembrane domain by
KASH domain proteins have an N-terminal cytoskeletal bind-
size; the largest being the 1300 kDa protein Msp-300/nesprin.
and Fridolfsson, 2010). KASH domain proteins vary widely in
(Razafsky and Hodzic, 2009).
appears important for this binding and is widely conserved
the SUN domain trimer. Specifically, the penultimate proline
the KASH domain fits into a hydrophobic pocket formed by
proteins (Starr and Fridolfsson, 2010). They are generally
them: location at the ONM; KASH domain mediated
localisation at the NE. Friederichs et al. (2012) therefore hypothe-
size a role for the mid-SUN protein in the maintenance of the
Mps3 pool at the NE. Whether the SUN proteins with a cen-
tral SUN domain are involved in nucleo-cytoplasmic bridging, in
plants or opisthokonts, remains unclear but is beginning to be
investigated.

KASH DOMAIN PROTEINS: MULTI-FUNCTIONAL
COMPONENTS OF THE LINC COMPLEX

The term KASH domain derives from members of a family of pro-
teins, described in D. melanogaster (Klarsicht), C elegans (ANC-1;
Starr and Han, 2002) and mammals (Syne-1 and 2, also known as
Nesprin 1 and 2; Apel et al., 2000) which interact with SUN
domain proteins (Starr and Fridolfsson, 2010). They are generally
generated by plants located at the ONM, with a conserved
C-terminal KASH domain in the perinuclear space close to a
transmembrane domain. Starr (2009) proposed four criteria to
characterize them: location at the ONM; KASH domain mediated
interaction with the SUN domain; ONM localisation dependent
on the SUN–KASH domain interaction (Crisp et al., 2006); and
a non-conserved, cytoplasmic N-terminal domain that interacts
with cytoskeletal proteins like actin and dynein.

The KASH domain includes a transmembrane domain and a
short stretch of amino acids (typically between 9 and 35) in the
periplasm ending in a conserved sequence which in most ani-
mal KASH proteins is PPPX (Razaﬁsky and Hodzic, 2009; Starr
and Fridolfsson, 2010). KASH domain proteins vary widely in
size; the largest being the 1300 kDa protein Msp-300/nesprin.
KASH domain proteins have an N-terminal cytoskeletal bind-
ing domain separated from a single transmembrane domain by
a series of spectrin repeats or coiled coils (Lenne et al., 2000).
Like SUN, KASH-domain proteins form homomers (Djinovic-
Carugo et al., 2002; Mislow et al., 2002). Therefore, a SUN–KASH
complex comprises three SUN domain proteins (as a homo-
or hetero-trimer) each associated with a KASH domain pro-
tein which may be associated with other adjacent KASH domain
proteins (Figure 1). Binding occurs when the PPPT motif of the
KASH domain fits into a hydrophobic pocket formed by
the SUN domain trimer. Speciﬁcally, the penultimate proline
appears important for this binding and is widely conserved
(Razaﬁsky and Hodzic, 2009).

Metazoan nesprins vary widely in size and interactions. Largest
are the proteins formed by the Syn1/Nesp1 gene that encodes a
range of splice isoforms, the biggest being the 1000 kDa Nesprin 1
Giant (Nesp1G). Nesp1G has an N-terminal actin binding domain,
made up of two calponin homology domains. Nesprin 2 (also
called Syne2/NUANCE) encodes Nesprin2G of 800 kDa also with an
N-terminal cytoplasmic actin binding domain (Apel et al., 2000;
Zhang et al., 2002; Zhen et al., 2002). Nesprin 3, 100 kDa, lacks
an actin binding domain, but binds intermediate ﬁlaments via an
interaction with plectin; while Nesprin 4 is smaller (42 kDa) and
interacts with the cytoskeleton via kinesin. Some smaller nesprins
co-localize and interact with the INM protein, emerin (Mislow
et al., 2002; Zhang et al., 2005; Wheeler et al., 2007).

Klarsicht/Anc/Syne-1 homology domain proteins also have a
role in controlling nuclear size in non-plant systems (Lu et al.,
2012). Nesprin 1 and 2 interact with Nesprin 3 so that both.ends
contact the nuclear surface, ﬁrst via their C-terminal KASH-
domains and second by interaction between their N-termini and
Nesprin 3. Nesprin interaction with the cytoskeleton also forms
a lattice-like ﬁlamentous network covering the ONM (Lu et al.,
2012).

PLANT KASH DOMAIN PROTEINS

The search for plant KASH domain proteins took longer than that
for the SUN domain proteins. The breakthrough came from the
realization that a well-studied plant NE protein showed charac-
teristics similar to the KASH domain proteins and the subsequent
demonstration of its interaction with SUN domain proteins in
a collaboration between the Graumann and Meier laboratories
(Zhou et al., 2012).

Previous work (Xu et al., 2007) had identiﬁed an NE-associated
Arabidopsis family of the WPP (tryptophan-proline-proline)
domain proteins. These include WPP1, WPP2, and RanGTPase-
activating protein 1 (RanGAP1) and are characterized by the
presence of various repeats of the WPP motif in the protein
sequence (Meier, 2000; Rose and Meier, 2001). These had been
demonstrated to be localized to the NE by interactions with two plant-
speciﬁc protein families – the WIPs (WPP domain interacting
proteins) and the WITs (WPP interacting [all anchored proteins]).
WIPs and WITs oligomerise to provide the anchorage of the
WPP proteins. For instance, by anchoring RanGAP1 to the NE
(Xu et al., 2007), they are involved in generating the RanGTP
gradient necessary for transport through the nuclear pores and
due to nucleo-cytoplasmic transport. Both WIPs and WITs are
C-terminally anchored membrane proteins with the C-terminus
located in the periplasm. However, only the AtWIPs were found to
contain a highly conserved X-VPT motif (X, hydrophobic amino
acid; Zhou et al., 2012). This motif contains a penultimate pro-
line, similar to the opisthokont PPPX, and is conserved in other
plant species. Furthermore, Zhou et al. (2012) had also shown that
deletion of this extreme C-terminal VVPT of AtWIP1 reduced its
NE localization. Thus, the overall domain structure and locali-
sation of AtWIPs made them good candidates for plant KASH
proteins.

Confirmation of the WIPs as KASH domain proteins depended
on demonstration of their SUN interaction and that the SUN
binding was indispensable to their NE localization. Interaction
of the VVPT motif with SUN domain proteins was shown by a
number of means. Deletion of all, or parts of, the SUN domain

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abolished interaction of AtWIP1, 2, and 3 in a pull down assay with AtSUN2 (Zhou et al., 2012). Also, fluorescence recovery after photobleaching (FRAP) assays revealed significant increase in mobility of AtWIPs after deletion of the VVPT domain. Zhou et al. (2012) further demonstrated that in a sun1-KO/ sun2-KD mutant transformed to express GFP-AtWIP1, the fluorescent signal from the WIP protein was predominantly cytoplasmic, resembling the distribution shown for a WIP1 truncation mutant in which the C-terminal VVPT had been deleted, confirming the requirement for the SUN domain protein for nuclear localisation. Comparison of the AtWIP1 C-terminus shows a low degree of similarity to opisthokont KASH domains. It is small in size, though similar to C. elegans ZYG-12B and KDP-1 and D. discoideum Interapartin (Xiong et al., 2008; McGee et al., 2009; Minn et al., 2009). The penultimate proline is highly conserved, as is the terminal serine/threonine.

The location of RanGAP at the pore complex was the first bona fide function identified for a plant LINC complex; it differs from mammalian systems, where RanGAP anchorage occurs by direct attachment to the nuclear pores by RanBP2. Further functions of the plant LINC complex are being elucidated. Tamura et al. (2013) used a myosin XI-I mutant of Arabidopsis, kaku1, to explore attachment of the cytoskeleton to the NE. They were able to show that myosin XI-I localizes at the NE and attaches to W1T1, which interact with WIP proteins attached to the ONM. Plant nuclei move in a number of circumstances, including in response to blue light and fungal infection, and are known to involve an actin-rather than a microtubule-based system (Nagai, 1993; Skalamera and Apel, E. D., Lewis, R. M., Grady, R. M., and Sanes, J. R. (2000). Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. J. Biol. Chem. 275, 31986–31995. doi: 10.1074/jbc.M004775200

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