HHS Public Access

Author manuscript

Biol Chem. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

Biol Chem. 2015 April 1; 396(4): 295–310. doi:10.1515/hsz-2014-0267.

Making the LINC: SUN and KASH protein interactions

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Abstract

Cell nuclei are physically integrated with the cytoskeleton through the LINC complex (for LInker of Nucleoskeleton and Cytoskeleton), a structure that spans the nuclear envelope to link the nucleoskeleton and cytoskeleton. Outer nuclear membrane KASH domain proteins and inner nuclear membrane SUN domain proteins interact to form the core of the LINC complex. In this review we provide a comprehensive analysis of the reported protein-protein interactions for KASH and SUN domain proteins. This critical structure, directly connecting the genome with the rest of the cell, contributes to a myriad of cellular functions and, when perturbed, is associated with human disease.

Keywords

cytoskeleton; KASH; LINC complex; nuclear envelope; nucleus; SUN

Introduction

Mechanical integration of the nucleus and cytoskeleton was revealed by experiments that pulled on cell surface integrin receptors to induce concomitant deformation of the nucleus(Maniotis et al., 1997). Since those original observations, we have come to understand the primary structure by which the nucleus is physically integrated with the cytoskeleton and many of the various ways it influences cellular organization and function. Called the LINC-complex for LInker of the Nucleoskeleton and Cytoskeleton(Crisp et al., 2006), the structure spans the nuclear envelope (NE) to physically connect the cytoskeleton to the nucleoskeleton. The NE is a specialized extension of the endoplasmic reticulum (ER) that encases the genome during interphase, thus separating the nucleus from the cytoplasm. As a component of the ER, the NE has two membranes, an outer and inner nuclear membrane (ONM and INM, respectively) as well as a lumenal region called the perinuclear space (PNS). These two membranes are continuous with the rest of the ER and the PNS is continuous with the ER lumen. The INM and ONM are directly connected by numerous

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annulate junctions of high membrane curvature. Within these NE gaps reside large proteinaceous nuclear pore complexes (NPCs) that, among other functions, enable and regulate nucleo-cytoplasmic trafficking. Underlying the INM is a major constituent of the nucleoskeleton called the nuclear lamina, composed of type-V intermediate filaments called lamins. The LINC complex is fundamentally composed of ONM-resident KASH domain proteins that translumenally interact with INM-resident SUN domain proteins (hereafter simply called KASH and SUN proteins). In this way the KASH and SUN proteins create a structure that spans the NE. By association of KASH proteins with cytoskeletal proteins and SUN proteins with nucleoskeletal proteins, the LINC complex can integrate these mechanical networks. Although cytoskeletal and nucleoskeletal proteins contribute to the LINC complex, this review will focus on interacting partners of the core LINC complex, the KASH and SUN proteins.

Structure of the LINC complex

SUN proteins are type-II transmembrane proteins with nuclear-oriented N-termini and lumenal C-termini. Named for SUN domain family members (Sad1p, Unc-84) (Malone et al., 1999) they all share a conserved SUN domain at the C-terminus of the lumenal domain. This lumenal region of SUN proteins also includes an extended coiled-coil domain. The nucleoplasmic domain of SUN proteins is structurally less characterized, but at least in some mammalian family members, has been shown to be capable of nuclear localization independent of the rest of the protein(Haque et al., 2006; Hodzic et al., 2004; Liu et al., 2007). KASH domain proteins, named for Klarsicht, Anc-1, Syne-1 homology (Starr and Han, 2002), are also type-II transmembrane proteins that have a short luminal tail with a very loosely conserved PPPX motif at the C-terminus (this is most conserved in mammals where X is variable residue), and a far more diverse and sizeable cytoplasmic N-terminus.

Based on structural analysis of human Sun2 (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012b), the coiled-coil domain of SUN proteins homo-typically associate to create an extended trimeric stalk. Within this complex, each of the three globular SUN domains are positioned proximate to the ONM where they can each bind to an individual KASH domain in the binding pocket the SUN domains form, in part via its PPPX motif. Conserved cysteine residues, one each in the KASH and SUN domain, may stabilize this association by disulfide bond formation. In this way the KASH and SUN proteins interact to create a stable tether between the INM and ONM. Although their exact stoichiometry remains unclear, many KASH proteins have also been shown to homotypically oligomerize, potentially contributing to the mechanical stability of these complexes. In mammals there appears to be general promiscuity of KASH-SUN protein interactions. Consequently, there is no clear preference for a specific SUN protein to bind a particular KASH protein (Stewart-Hutchinson et al., 2008).

Functions of an ancient LINC

The LINC complex has been studied in yeast, worms, flies, fish, mammals and plants. The fundamental constituents of the structure are well conserved throughout eukaryotic cells, although there is a diverse repertoire of KASH and SUN proteins that varies between

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species. The number of KASH and SUN domain proteins generally increases with organism complexity. In mammals there is clear evidence for cell-type specific members of the LINC complex. Many of the functions of the LINC complex are also well conserved. These functions include nuclear retention, nuclear migration, cell polarity, cytoskeletal organization, meiotic chromosome movement to facilitate recombination, protein recruitment to the ONM and potentially mechanotransduction. Not surprisingly there is growing evidence of LINC complex involvement in human disease. In humans, autosomal dominant mutations in KASH protein genes, *SYNE1* and *SYNE2*, are reportedly associated with Emery-Dreifuss Muscular Dystrophy (EMD) (Wheeler et al., 2007; Zhang et al., 2007a). In addition, autosomal recessive mutations in *SYNE1* are associated with cerebellar ataxia (Gros-Louis et al., 2007) and arthrogyrposis (Attali et al., 2009). A frameshift mutation of Nesprin4 was recently identified in hereditary hearing loss (Horn et al., 2013a). Mutations in genes encoding SUN proteins have recently been associated with muscular dystrophy (Meinke et al., 2014). Interestingly, loss of Sun1 ameliorates the pathology resulting from altered expression of A-type lamins in mouse models of EMD and Hutchinson-Gilford Progeria Syndrome (HGPS) (Chen et al., 2012).

Functional interactions of the LINC complex

Yeast

In yeast, the LINC complex has two primary functions: providing chromosome movement and embedding the spindle pole body (SPB) within the NE. The ONM proteins that bind to the SUN proteins function like KASH proteins but lack the conserved proline residue adjacent to the C-terminus, found in many other eukaryotes.

S. cerevisiae

- SUN proteins—Mps3 interacts with Mps2, a KASH protein coupled with the SPB (Conrad et al., 2008; Friederichs et al., 2011; Koszul et al., 2008). In this way, the interaction between Mps3 and Mps2 leads to recruitment of the SPB into the NE.

Mps3 also functions during meiosis. In *S. cerevisiae*, meiotic telomeres are tethered to the INM and transiently clustered at one pole of the NE, where the SPB locates. In meiotic telomere clustering, often called bouquet, Mps3 interacts with a telomere adaptor protein Ndj1 (Conrad et al., 2008; Conrad et al., 2007; Kosaka et al., 2008; Wanat et al., 2008). The interaction of Ndj1 with Mps3 is crucial for telomere tethering. Loss of Ndj1 leads to meiotic chromosome segregation defects (Conrad et al., 2007). The force translocating telomeres is delivered by Csm4, a meiotic specific KASH protein that appears to interact with the actin cytoskeleton (Conrad et al., 2008; Kosaka et al., 2008; Koszul et al., 2008; Wanat et al., 2008).

Functions of Mps3 extend beyond retention of KASH proteins on the ONM. In interphase, telomeres are tethered at the NE largely by two different mechanisms. In both cases, the interaction between telomere binding proteins and Mps3 mediates telomere tethering (Bupp et al., 2007; Schober et al., 2009). The association between telomeres and the NE during interphase is thought to protect telomeres from transcription and inappropriate recombination. One mechanism of telomere tethering is mediated by the interaction between

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telomere-bound ribosome biogenesis factors and Mps3, which also recruits Sir4, a gene silencing protein (Bupp et al., 2007; Horigome et al., 2011). The other mechanism requires the interaction between Mps3 and DNA double-strand break ends binding proteins Ku70/Ku80 hetero-dimer protein (Antoniacci et al., 2007; Schober et al., 2009). Interestingly, chromosome regions with DNA double stand breaks (DSB) are also tethered by Msp3, which is assisted by Ku70/Ku80 (Oza et al., 2009).

In addition to the roles of Mps3 in chromosome organization, Mps3 has been shown to bind directly to Replication Factor C (RFC) complex subunits and histone variant Htz1, which are linked with DNA replication and repair mechanism(Haas et al., 2012). Mps3 also directly binds to acetyltransferase Eco1/Ctf7 resulting in post-translational modification of Mps3. Acetylation of Mps3 is required for accurate sister chromatid cohesion and for chromosome recruitment to the nuclear membrane (Antoniacci et al., 2004; Ghosh et al., 2012). Although Csm4, a KASH protein of *S. cerevisiae*, has been implicated to participate in the Mps3-mediated interphase chromosome tethering (Chan et al., 2011), the role of KASH proteins still remains elusive.

- KASH proteins—Mps2 is required to embed SPB in the NE, much like a NPC. The N-terminus of Mps2 interacts with Bbp1, which interacts with the core SPB protein Spc29 (Schramm et al., 2000). In turn, the C-terminus of Mps2 interacts with Mps3. Together, these three proteins, Mps3, Mps2, and Bbp1, form the half-bridge, a protein complex that anchors the SPB within the NE (Schramm et al., 2000). Another binding partner of Mps2 is Spc24, a peripheral kinetochore component involved in chromosome segregation (Le Masson et al., 2002). However, the role of Mps2 in chromosome segregation remains unclear.

Csm4 is retained on the ONM via association with Mps3 (Conrad et al., 2008; Kosaka et al., 2008; Koszul et al., 2008; Wanat et al., 2008). There is no direct evidence regarding the cytoplasmic binding partners of Csm4. Perturbation of the actin cytoskeleton leads to failure of telomere movement during meiosis, which resembles the phenotype of Csm4 deletion mutants (Koszul et al., 2008; Scherthan et al., 2007; Trelles-Sticken et al., 2005; Wanat et al., 2008; Yamamoto et al., 1999). Given these observations, it has been proposed that Csm4 interacts with the actin cytoskeleton to mediate the movement of Mps3-tethered telomeres in meiosis.

S. pombe

- SUN proteins—Sad1, the only known SUN protein in *S. pombe*, is required for the SPB insertion to the NE via its KASH partner Kms2. On the nucleoplasmic side, Sad1 works cooperatively to connect chromatins to the SPB during interphase. In this complex, integral membrane proteins Ima1 and Ndc80 stabilize the complex of Sad1 and centromeres (King et al., 2008). Interaction of Sad1 with centromeres, assisted by Ima1 and Ndc80, suggests the role of these proteins in maintenance of nuclear organization. However, recent studies suggest a dispensable role of Ima1 in linking centromeres to the SPB (Hiraoka et al., 2011).

During interphase, centromeres of *S. pombe* are concentrated at the NE where the SPB is inserted. This centromere anchoring is mediated by Sad1 and Csi1 (Hou et al., 2012). Csi1

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interacts both with Sad1 and kinetochore at the same time, thus bridging centromeres and Sad1. The current model suggests that the interaction between Sad1 and Csi1, which closely locates centromeres to the SPB, aids kinetochore capture microtubules as cells enter early mitosis. Csi1 mutants demonstrated defects in chromosome segregation and mitosis progression (Hou et al., 2012).

Sad1 also mediates a repetitive chromosome movement that stretches the NE during meiosis. This characteristic movement of chromosomes, often called "horse tail" movement, is led by the association between NE-tethered telomeres and dynein bound KASH protein Kms1 (Niwa et al., 2000; Shimanuki et al., 1997). Sad1 tethers telomeres to the NE via meiotic specific proteins, Bqt1 and Bqt2. The direct interaction between Sad1 and Bqt1 recruits Bqt2, which then allows interaction with a telomere binding protein Rap1 (Chikashige et al., 2006).

- KASH proteins—Kms1 mediates "horsetail" movement during meiosis via dynein. Although identified genetically (Niwa et al., 2000; Shimanuki et al., 1997), the physical interaction between dynein and Kms1 is not fully characterized.

Kms2 accompanies the SPB during interphase. Recently, core SPB components Cut12 and Pcp1 were identified as direct binding partners of Kms2 (Walde and King, 2014). Loss of Kms2 led to late mitotic entry, disruption of stable bipolar spindle formation, and abnormal insertion of the SPB. Interestingly, defects in recruitment of polo kinase, Plo1, to the SPB at mitotic entry were also observed in Kms2 deletion mutant cells (Walde and King, 2014), suggesting a role of Kms2-Sad1 in regulation of the SPB integration and mitotic entry.

Insects

The LINC complex in *Drosophila melanogaster* is involved in nuclear migration and positioning. Although highly conserved with the mammalian LINC complex, the mechanisms of these functions of the LINC complex in *D. melanogaster* remain unclear.

- SUN proteins—Spag4, also known as Giacomo, is a homolog of the mammalian SUN protein Spag4, which is specifically expressed in the male reproductive organ (Kennedy et al., 2004; Shao et al., 1999). Spag4 plays a role in nuclear and centriolar attachment during spermatogenesis. Knockout of Spag4 leads to sterility in males suggesting that it has a crucial role in spermatogenesis. A coiled-coil cytoplasmic protein Yuri Gagarin and dynein-dynactin are also involved during this process and have been found to colocalize with Spag4. Intriguingly, other known drosophila KASH proteins, Klarsicht and MSP-300, are dispensable for this process (Kracklauer et al., 2010).

Klaroid is essential to localize its KASH proteins Klarsicht and Msp-300 (Kracklauer et al., 2007; Malone et al., 2003; Technau and Roth, 2008). With these KASH protein partners, Klaroid is necessary for nuclear migration in differentiation of the eye disc and muscle cells. The B-type lamin, Lam Dm0, is required for proper targeting of Klaroid (Patterson et al., 2004).

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- KASH proteins—Klarsicht (Klar) is an essential KASH-domain protein for proper migration of nuclei during the eye development (Fischer-Vize and Mosley, 1994; Kracklauer et al., 2007; Mosley-Bishop et al., 1999; Patterson et al., 2004). In either Klar or dynein mutants, the nuclei in the eye disc fail to migrate (Kracklauer et al., 2007; Patterson et al., 2004; Swan et al., 1999). Since Klar co-localizes with microtubules at the NE, it is speculated that microtubule-dependent motor protein complexes associated with Klar may mediate nuclear migration (Fischer et al., 2004; Mosley-Bishop et al., 1999; Patterson et al., 2004; Welte, 2004). However, a Klar-associated motor protein has not yet been defined.

Msp-300 interacts with the actin cytoskeleton via its N-terminal actin binding domain. The interaction between Msp-300 and the actin cytoskeleton was originally proposed as an essential KASH-domain protein for the positioning of nuclei during muscle and ovarian nurse cell development (Rosenberg-Hasson et al., 1996; Volk, 1992; Yu et al., 2006). More recently, however, several other groups contest that Msp-300 is dispensable in the previously mentioned tissues (Technau and Roth, 2008; Xie and Fischer, 2008). Msp-300 has been found to form a specialized ring structure at the periphery of larval myogenic NE. In this ring structure, Msp-300 associates with Klar, suggesting that the interactions between Mps-300 and Klar link microtubules to the NE (Elhanany-Tamir et al., 2012; Volk, 2013).

Worms

The LINC complex of *Caenorhabditis elegans* plays an important role in nuclear migration, positioning, and meiotic chromosome movement.

- SUN proteins—SUN1/MTF-1 and UNC-84 are the only identified SUN domain proteins in *C. elegans*. SUN1/MTF1 is required for localization of ZYG-12 and Kdp-1 on the ONM (Malone et al., 2003; McGee et al., 2009). SUN1/MTF1 is involved in chromosome movement during meiosis. *C. elegans* chromosomes harbor specific regions called pairing centers where meiosis specific zinc-finger proteins Him-8, Zim1, Zim2, and Zim3 mediate the attachment of meiotic chromosomes to the NE. In the pairing center, SUN1/MTF1 is also found co-localized with Him-8 (Sato et al., 2009). Interestingly, Sun1/MTF1 is involved in apoptosis. It binds to pro-apoptotic factor CED4 (Tzur et al., 2006a) and subsequently shifts the location of CED4 from mitochondria to the NE. The interaction between Sun1/MTF1 and CED4 is thought be required for apoptosis.

Unc-84 is essential to localize Unc-83 and Anc-1 on the ONM (Starr and Han, 2002; Starr et al., 2001). The interaction with Ce-lamin is required for targeting of Unc-84 (Lee et al., 2002).

- KASH proteins—Anc-1 bridges the NE to the actin cytoskeleton via its N-terminal actin binding domain (Starr and Han, 2002). Anc-1 is known to contribute in tethering clustered nuclei to the actin cytoskeleton in hypodermal cells. Loss of function mutants of Anc-1 lead to unanchored nuclei that aggregate in the cytoplasm (Starr and Han, 2002).

A recent study revealed a novel interaction between Anc-1 and the PHR protein Rpm-1, a large signaling protein involved in numerous developmental events in neurons. This interaction positively regulates a β-catenin protein, Bar-1, which functions in development

of axon termination in the mechanosensory neurons and synapse formation in the GABAergic motor neurons (Tulgren et al., 2014).

Unc-83 plays a role in nuclear migration, which is developmentally important in a wide variety of tissues. It was originally found to associate both with kinesin and dynein (Fridolfsson et al., 2010; Meyerzon et al., 2009). Mutations in kinesin-1 demonstrated disorganized nuclei in hypodermal cells, which lead to developmental defects in neurons and vulva cells (Meyerzon et al., 2009). The loss of dynein, however, caused minimal defects (Fridolfsson et al., 2010). A recent study revealed dynein induces a short backward movement of hypodermal nuclei migration, which contributes to efficient nuclear migration (Fridolfsson et al., 2010). These observations suggest that the association between Unc-83 and kinesin has a major role during nuclear migration of hypodermal cells, as assisted by dynein.

Zyg-12 plays an essential role in pronuclei fusion that requires the association of the NE and centrosome. During pronuclear fusion, Zyg-12 is retained by Sun1 at the NE where it interacts with dynein. In contrast, the KASH-less isoform of Zyg-12 (Zyg-12A) associates with the centrosome (Malone et al., 2003; Meyerzon et al., 2009). Centrosome attachment is completed by the dimerization of both membrane and centrosome bound Zyg-12 (Malone et al., 2003). Zyg-12 also functions in early meiosis when it accumulates at the pairing center (MacQueen et al., 2005; Phillips and Dernburg, 2006) and associates with dynein. The interaction between dynein and Zyg-12 drives the tethered chromosomes to form a meiotic bouquet and facilitates proper pairing of chromosomes (Sato et al., 2009).

Kdp-1 is a recently identified KASH-domain protein. The cytoplasmic binding partner of Kdp-1 has not yet been identified. Kdp-1 is essential for viability and development of *C. elegans*, suggesting its role in cell cycle progression (McGee et al., 2009).

Fish

LRMP is the only known KASH protein in *Danio rerio*. It is involved in pronuclear fusion and attachment of the centrosome to the NE (Lindeman and Pelegri, 2012). The cytoplasmic binding partners of LRMP, as well as the identity of cognate SUN proteins, are currently unknown.

Mammals

The mammalian LINC complex is structurally conserved with other organisms' LINC complex. There are several KASH and SUN proteins, some with tissue-specific expression and functions. SUN proteins appear to share their functions to retain KASH proteins at the ONM. Evidence of unique roles for SUN protein variants remains limited.

- SUN proteins—There are five conventional SUN proteins in mammals. Sun1 and 2 are widely expressed whereas Sun3-5 are restricted to the testis(Crisp et al., 2006; Haque et al., 2006; Hodzic et al., 2004; Shao et al., 1999; Tzur et al., 2006b). Despite the interaction with A-type lamin, targeting of SUN1 and 2 is not primarily dependent on A-type lamins (Crisp et al., 2006; Haque et al., 2006). A sixth mammalian Sun-like protein is osteopotentia, which

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harbors an internal SUN domain. Whether osteopotentia has the capacity to bind KASH-domain proteins remains unclear (Sohaskey et al., 2010).

In mammalian meiosis, Sun1is required for tethering meiotic telomeres to the NE (Ding et al., 2007; Horn et al., 2013b; Morimoto et al., 2012). In this way, Sun1 and KASH5 mediate telomere movement and proper pairing of chromosomes during meiosis (Horn et al., 2013b). However, the nuclear proteins that mediate telomeres and Sun1 are currently unknown.

Sun1η is a newly identified splicing variant of Sun1 which interacts with Nesprin3 at the anterior of the outer acrosomal membrane of sperm (Gob et al., 2010). In addition, it was proposed that Sun3 and Nesprin-1contribute to formation of polarized sperm heads at the posterior of acrosomal membrane of sperm (Gob et al., 2010).

As for SUN proteins' role within the NE, recent studies have revealed the involvement of Sun1 and Sun2 in the DNA damage response (DDR). Both were found to interact with DNA-dependent kinase (DNAPK), Ku70, and Ku80 to elicit DNA damage response. Sun1/Sun2 double knockout fibroblasts exhibit DNA damage and compromised DDR activation (Lei et al., 2012). In addition to lamin-A, Sun1 has been shown to associate with hALP (NAT10) and be involved in chromosome de-condensation following mitosis (Chi et al., 2007). Both Sun1 and Sun2 have been shown to bind to SAMP1 (TMEM201) (Borrego-Pinto et al., 2012; Jafferali et al., 2014), an INM protein that appears to regulate the role of the LINC complex in cell migration (Borrego-Pinto et al., 2012).

- KASH Proteins—Nesprin-1 and Nesprin-2 encompass many splice isoforms, the largest of which, called giant isoforms, are ~800–1,000 kDa (Zhang et al., 2002; Zhang et al., 2001). The largest isoforms feature an N-terminal actin binding domain followed by numerous spectrin repeats within their cytoplasmic domain. Given their size, functional domains, and splicing isoforms, Nesprin-1 and -2 can interact with diverse proteins. Indeed, they are involved in many important cellular processes as described below.

As expected, the ONM targeting ability of Nesprin-1 and -2 requires SUN proteins, but several smaller KASH-domain containing isoforms of Nepsrin-1 and -2 were found to reside within the nucleus (Rajgor et al., 2012; Zhang et al., 2005). There are also KASH-less isoforms of Nesprins-1 and-2 found in a variety of cellular locations which are reported to associate with diverse proteins. The role of KASH-less isoforms needs to be clarified (Gough et al., 2003; Mislow et al., 2002; Warren et al., 2010; Zhang et al., 2002; Zhang et al., 2005).

Likely via interactions with their binding partners, Nesprin-1 and -2 are also involved in mechanotransduction, a process that monitor physical forces and trigger biological responses (Anno et al., 2012; Lombardi and Lammerding, 2010; Stewart-Hutchinson et al., 2008). Recently, forces transmitted via Nesprin-1 in isolated nuclei led to changes in nuclear stiffness, resulting in phosphorylation of emerin (Guilluy et al., 2014). Interestingly, this study further illustrates that changes in nuclear stiffness affect stress fiber formation and SRF (serum response factor)-dependent gene expression (Guilluy et al., 2014). In cardiomyocytes, compared to wild type or single deletion of Nesprin-1 and-2, loss of both

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Nesprin-1 and -2 induced altered nuclei positioning, shape, and gene expression of biomechanical response genes upon mechanical stimulation (Banerjee et al., 2014). These observations suggest that Nesprin-1 and -2 play important roles in sensing mechanical force and regulating gene expression.

1. Actin: The actin binding ability of Nesprins-1 and -2 is the primary mechanism anchoring the clustered nuclei at the neuromuscular junction (NMJ) (Padmakumar et al., 2004; Zhang et al., 2002; Zhen et al., 2002). In Nesprin-1-deficient mice, the clustered nuclei at the NMJ are displaced; however, loss of Nesprin-2 has no similar effect (Zhang et al., 2007b), which suggests Nesprin-2 is functionally redundant. Yet the loss of both Nesprins leads to perinatal mortality due to malfunction of the diaphragm (Zhang et al., 2007b). Nesprin2 giant and Sun2 are involved in polarized nuclear movement, which mediated by the LINC complex perpendicularly aligned with the actin cytoskeleton at the nuclear periphery. (Gomes et al., 2005; Luxton et al., 2010). The specialized actin cytoskeleton and its aligned LINC-complex were subsequently termed transmembrane actin-associated nuclear (TAN) line. The role of TAN lines *in vivo* remains unclear.

2. Microtubule: Nesprin-1 and -2 are essential for nuclear migration during neuronal development. The neuronal nuclei move between the apical and basal surface according to the cell cycles. In the mouse embryonic brain and retinal cells, Nesprin-1 and -2 interact with dynein complex (Yu et al., 2011; Zhang et al., 2009). Nesprin-2, in turn, interacts with kinesin (Yu et al., 2011; Zhang et al., 2009). Given this observation, Nesprin-1 and -2 are speculated to participate in movement of neuronal nuclei toward the apical or basal surface in the developing brain or retina. Mice deficient in both Nesprin-1 and -2 suffer severe developmental defects in the brain and retina, likely due to the failure of neuronal nuclear migration (Yu et al., 2011; Zhang et al., 2009). This abnormal neuronal development may also contribute to the neonatal lethality of the mice deficient in Nesprin-1 and -2.

3. Nesprin3: Giant isoforms of Nesprin-1 and -2 were shown to interact with Nesprin3 via a direct interaction (Taranum et al., 2012). Based on this observation, a new model for the architecture of nuclear-cytoskeletal interactions has been proposed. In this model, Giant Nepsrin-1, and -2 are drawn down to the periphery of the NE and form interchains with Nepsrin-3. In this manner, the interchain potentially serves as a “filamentous cage” that, among other things, can regulate nuclear size (Taranum et al., 2012).

4. Nuclear proteins: In mammals, Nesprin-1 and -2 were shown to interact with various proteins at the inner nuclear membrane. Small isoforms of Nesprin-1 and -2 bind to the nucleoplasmic domain of Sun proteins (Haque et al., 2010), emerin (Mislow et al., 2002; Wheeler et al., 2007; Zhang et al., 2005), and lamin A/C (Libotte et al., 2005; Mislow et al., 2002; Zhang et al., 2005). However, the role beyond the association between Nesprins and inner nuclear membrane proteins remains unclear.

5. TorsinA: The lumenal AAA+ protein torsinA was shown to associate with the KASH domains of Nesprins 1 and -2 (Nery et al., 2008). Interestingly, a dystonia-

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associated mutant of torsinA requires Sun1 for its aberrant localization to the NE (Jungwirth et al., 2011).

Nesprin3 localizes to the NE in a Sun1 and Sun2 dependent manner and is rather ubiquitously expressed. Considerably smaller than Nesprin-1 and Nesprin-2, Nesprin3can form a large protein complex by which it binds to cyto-linker protein plectin (Ketema et al., 2007; Wilhelmsen et al., 2005). Plectin is a multi-functional protein that binds the intermediate filament system, actin cytoskeleton, and the cytoplasmic domain of integrin β4 (Geerts et al., 1999; Ketema et al., 2007; Postel et al., 2011; Wilhelmsen et al., 2005). Given that plectin can form extended oligomers, the interaction between Nesprin3 and plectin has been proposed to potentially connect the nucleus to the plasma membrane (Ketema et al., 2013). The loss of Nesprin3 in zebra fish and mice demonstrates a reduced association between the intermediate filament system and the NE; however, this loss revealed at most a minimal effect on embryonic development, viability, and fertility (Ketema et al., 2013; Postel et al., 2011). Nesprin3 and Sun1η, a spermiogenesis specific Sun1 isoform, have been proposed to function in the formation of sperm heads (Gob et al., 2010).

Nesprin4 was originally detected in secretory epithelial cells (Roux et al., 2009). By interacting with kinesin-1, Nesprin4 was proposed to have a role in positioning the nucleus at the basal membrane of secretory epithelial cells (Roux et al., 2009). Subsequently, patients with hereditary hearing loss were shown to have predicted loss-of-function mutations in the gene that encodes Nesprin4. Furthermore, Nesprin4-deficient mice also exhibit hearing loss with defective basal nuclear positioning and maintenance of outer hair cells (Horn et al., 2013a). Interestingly, Sun1-deficient mice have similar deficits in hearing and outer hair cell polarity (Horn et al., 2013a). It appears that outer hair cells lack Nesprin-2 (another kinesin-binding KASH protein), suggesting functional redundancy of Nesprin-2 and -4 in secretory epithelial cells.

KASH5, unlike Nesprins, does not contain cytoplasmic spectrin repeats and its expression appears restricted to germ cells during and after meiosis (Horn et al., 2013b; Morimoto et al., 2012). During mammalian meiotic prophase I, dynein-bound KASH-5 colocalizes with NE-tethered telomeres and Sun1 (Horn et al., 2013b; Morimoto et al., 2012). Mice deficient in KASH5 are infertile and display an arrested telomere movement (Horn et al., 2013b), indicating that KASH5 is a mechanism conveying telomeres during meiosis prophase I. Recently, Link et al. reports that KASH5 can target to the NE in a Sun1 independent manner, presumably via interaction with Sun2 (Link et al., 2014).

LRMP, also called Jaw1, is an atypical mammalian KASH protein. The KASH domain of LRMP is sufficient to target the NE in a SUN-dependent manner (Horn et al., 2013b). However, posttranslational proteolytic processing can remove the luminal region, resulting in a potentially variable targeting of LRMP to the NE (Behrens et al., 1996; Horn et al., 2013b). Unlike other Nesprins, LRMP does not appear to associate with the cytoskeleton but instead binds inositol triphosphate receptor (IP3R), a Ca^{2+} channel located at the ER (Shindo et al., 2010). LRMP is highly expressed in immune cells and certain taste receptor cells though its function remains unclear.

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6. Plant

The LINC complex in plants, like other organisms, plays a role in many cellular events including nuclear positioning and shape, yet little is known about the mechanism involved.

- SUN proteins—In *Arabidopsis thaliana*, two SUN proteins AtSun1 and AtSun2 have been identified (Zhou et al., 2012a). AtSun1 and AtSun2 are shown to associate with Little Nuclei Proteins (LINC) that contain structural homology with lamins (Graumann et al., 2010). In addition to C-terminal SUN proteins ZmSun1, ZmSun2, *Zea mays* has three mid-SUN domain containing proteins (ZmSun3-5) (Murphy et al., 2010). However, the role of these mid-SUN domain containing proteins remains uncertain.

-KASH proteins—AtWIPs (AtWIP1, AtWIP2, and AtWIP3) are KASH domain proteins in *A. thaliana*, which harbor relatively short KASH domains. Originally identified as proteins anchoring AtRanGAP1 to the NE (Meier et al., 2010; Xu et al., 2007), AtWIPs are indeed KASH-domain proteins contingent on AtSun1 and AtSun2 (Zhou et al., 2012a). AtWIPs also interact with WIT1 and 2 that seem to aid in recruitment of AtRanGAP1 to the NE (Zhao et al., 2008). Interestingly, WIT1 and 2 physically interact with a plant specific myosin XI, thus bridging the actin cytoskeleton to the NE (Tamura et al., 2013). Disruptions of the interaction between AtWIPs and AtSuns have no obvious defects except less elongated nuclear shapes in epidermal cells (Zhou et al., 2012a). The consequence of the less elongated nuclear shape is currently unknown.

Recently, a group of proteins named Sun interacting NE (SINE) proteins were reported as KASH proteins. SINE1 is involved in actin-dependent nuclear positioning in guard cells, suggesting that it associates with the actin cytoskeleton (Zhou et al., 2014). SINE2 contributes to innate immunity to oomycete pathogens (Zhou et al., 2014).

Conclusions

The LINC complex bridges the nucleoskeleton to the cytoskeleton. However, there is growing evidence demonstrating that the LINC complex also functions as more than a simple physical tether. In yeast and *C. elegans*, the LINC complex participates in transcription, DNA repair, and signaling pathways. These observations suggest that the mammalian LINC complex has the potential to regulate gene expression and signaling pathways. It is not clear if KASH proteins retained by SUN proteins at the NE also interact with the previously mentioned signaling proteins. In addition to studying the functional consequences of the LINC complex-mediated signal transduction, elucidating the interaction between the LINC complex and non-cytoskeleton proteins will shed light on our understanding about the role of the LINC complex.

Many LINC complexes have been characterized by their respective cytoplasmic binding partners. Nevertheless, the function of SUN proteins in the nucleoplasm remains unclear. Although yeast do not have nuclear lamina, yeast SUN proteins play important roles in chromosome organization, gene expression, and DNA repair. Given the findings that Sun1 and Sun2 interact with DNAPK, a protein complex responding to DNA damage in mammals (Lei et al., 2012), there is an implication about the role of the SUN proteins in the

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nucleoplasm. Studies to clarify the function of SUN proteins will provide more clues about the role of the LINC complex in the nucleus. It also remains to be shown whether Sun proteins have unique or conserved functions within organisms. In mammals, the SUN proteins demonstrate a considerable degree of structural redundancy. Mouse models lacking either Sun protein have defects in nuclear anchorage in muscle cells, while double knockout mice were perinatally lethal (Lei et al., 2009). However, Sun1 was found closely associated with the nuclear pore complex (NPC) while Sun2 is more clustered (Liu et al., 2007). These observations suggest that Sun1 and 2 may have discrete roles. Thus, dissecting the role of SUN proteins will greatly enhance our understanding about the LINC complex.

Acknowledgments

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award numbers RO1GM102203, RO1GM102486 and RO1EB014869. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We apologize to the researchers whose works could not be cited due to practical space limits.

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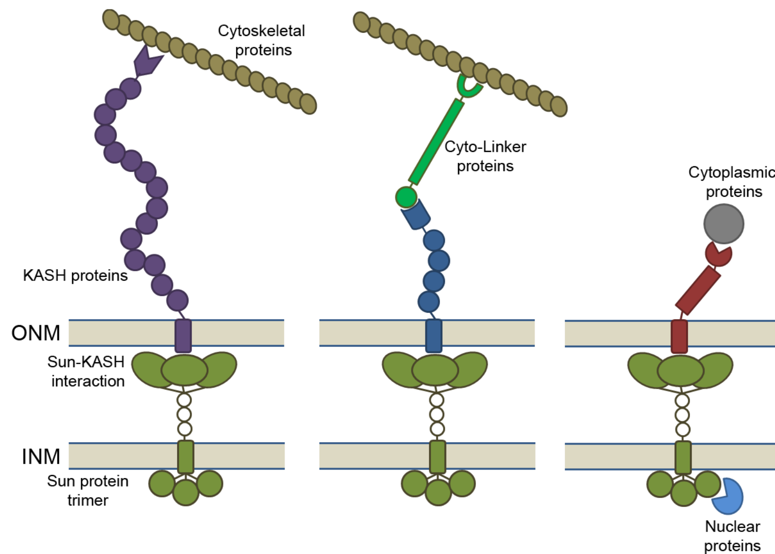

Figure 1. Schematic overview of SUN-KASH interactions

SUN domain protein trimers form a binding pocket for KASH domain proteins in the perinuclear space. Given that KASH proteins homo-typically oligomerize in an unknown stoichiometry upon SUN domain trimer, KASH proteins are simplified as monomers in this model. Through SUN-KASH luminal coupling, KASH proteins can directly interact with cytoskeletal proteins such as actin (Left). The interactions of KASH proteins and cytoskeletal proteins can be mediated by cyto-linker proteins such as plectin (Middle) or microtubule motor proteins. In addition to the cytoskeletal proteins, KASH proteins are known to bind to diverse cytoplasmic proteins while SUN proteins interact with nuclear proteins likely via the nuclear domain of SUN proteins (Right). The structure of nuclear domain of SUN proteins is remains poorly characterized.

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Table 1

List of KASH and SUN protein associations

Species	Cytoplasmic Binding proteins	KASH proteins	SUN proteins	Nuclear Binding proteins	Functions	Methods
S. cerevisiae	Bbp1	Mps2	Mps3		SPB integrity	Mps2 & Bbp1: coIP, GST pull down (Schramm et al., 2000) Mps2 & Mps3: IF, Y2H (Friederichs et al., 2011)
	Spc24	Mps2			Chromosome segregation	Mps2 & Spc24: coIP (Le Masson et al., 2002)
	Actin	Csm4	Mps3	Ndj1	Meiotic telomere movement Meiotic telomere tethering	Actin & Csm4: mutant animal study (Conrad et al., 2008; Kosaka et al., 2008; Wanat et al., 2008) Csm4 & Mps3: coIP (Conrad et al., 2008) Mps3 & Ndj1: coIP (Conrad et al., 2008; Kosaka et al., 2008)
			Mps3	Sir4, ribosome biogenesis factors	Telomere tethering Protection of telomeres from transcription	Mps3 & Sir4: coIP (Bupp et al., 2007) Mps3 & Ribosome biogenesis factors: coIP (Horigome et al., 2011), Y2H (Horigome et al., 2011)
			Mps3	Telomerase, Ku70, Ku80	Telomere tethering Protection of telomeres from transcription	Mps3 & telomerase: IF of reporter (Schober et al., 2009), GST pull down (Antoniacci et al., 2007) Mps4 & K70/Ku80: IF of reporter (Antoniacci et al., 2004), mutant animal study (Oza et al., 2009)
			Mps3	Replication factor C, Htz1	DNA damage response	Mps3 & Replication factor C, Htz1: CoIP, in vitro coupling (Haas et al., 2012)
			Mps3	Eco1/Ctf7	Chromosome tethering Sister chromatin cohesion	Mps3 & Eco1/Ctf7: GST pull down, coIP (Antoniacci et al., 2004)
S. pombe	Dynein (Dlc1)	Kms1	Sad1	Bqt1, Bqt2	Meiotic telomere movement Meiotic telomere tethering	Dlc1 & Kms1: Y2H (Miki et al., 2004), IF (Yoshida et al., 2013) Kms1 & Sad1: Y2H (Miki et al., 2004) Sad1 & Bqt1/2: IF, coIP, mutant animal study (Chikashige et al., 2006)
	Cut12, Pcp1	Kms2	Sad1		SPB integrity and tethering	Cut12 & Kms2: IF, GST pull down (Walde and King, 2014) Kms2 & Sad1: IF (King et al., 2008)

Species	Cytoplasmic Binding proteins	KASH proteins	SUN proteins	Nuclear Binding proteins	Functions	Methods
			Sad1	Csi	Centromere tethering	Sad1 & Csi1: coIP (Hou et al., 2012)
D. melanogaster	Yuri Gagarin, dynein, dynactin		Spag4		Centriolar coupling during spermatogenesis	Yuri Gagarin, dynein, dynactin & Spag4: IF (Kracklauer et al., 2010)
	Microtubule	Klarsicht	Klaroid	Lam Dm0	Nuclear migration during eye development	Microtubule & Klarsich: IF (Fischer et al., 2004; Patterson et al., 2004) Klarisicht & Klaroid: mutant animal study (Kracklauer et al., 2007) Klaroid & Lam Dm0: IF (Patterson et al., 2004)
	Actin	MSP-300	Klaroid	Lam Dm0	Nuclear anchorage in muscle fiber	Actin & MSP-300: IF, in vitro coupling (Volk, 1992) MSP-300 & Klaroid: mutant animal study (Technau and Roth, 2008)
	Klar	MSP-300	Klaroid	Lam Dm0	Microtubule coupling	MSP-300 & Klar: coIP (Elhanany-Tamir et al., 2012)
C. elegans	Actin	Anc-1	Unc-84	Ce-Lamin	Nuclear anchorage	Actin & Anc-1: in vitro coupling (Starr and Han, 2002) Anc-1 & Unc-84: mutant animal study (Starr and Han, 2002) Unc-84 & Ce Lamin: mutant animal study (Lee et al., 2002)
	Rpm1	Anc-1			Positive regulation of Bar-1 during neuronal development	Rpm1 &Anc1: coIP (Tulgren et al., 2014)
	Dynein (DLC-1/BICD-1/Nud-2)	Unc-83	Unc-84	Ce-Lamin	Nuclear migration of hypodermal cells	Dynein & Unc-83: GST pull down, coIP (Fridolfsson et al., 2010) Unc-83 & Unc-84: mutant animal study (Starr et al., 2001)
	Kinesin (KLC-2)	Unc-83	Unc-84	Ce-Lamin	Nuclear migration of hypodermal cells	Kinesin & Unc83: GST pull down, coIP, IF (Meyerzon et al., 2009)
	Zyg-12A	Zyg-12	Sun1/MTF1		Centrosome nucleus coupling	Zyg-12A & Zyg12: coIP (Malone et al., 2003) Zyg12 & Sun1/MTF: mutant animal study (Malone et al., 2003)
	Dynein (DLI-1/Lis-1/Arp-1/DHC1)	Zyg-12	Sun1/MTF1	Him-8, Zim1, Zim2, Zim3	Meiotic chromosome movement Meiotic chromosome tethering	Dynein & Zyg12: IF, mutant animal study (Malone et al., 2003; Sato et al., 2009; Zhou et al., 2009) Sun1 & Him-8, Zim-1,-2,-3: IF (Sato et al., 2009)
		KDP-1	Sun1/MTF1		Cell cycle progression	Kdp1 & Sun1/MTF1: Y2H, mutant animal study (McGee et al., 2009)

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Species	Cytoplasmic Binding proteins	KASH proteins	SUN proteins	Nuclear Binding proteins	Functions	Methods
			Sun1/MTF1	CED	Recruit CED to nucleus	Sun1 & CED: in vitro coupling (Tzur et al., 2006a)
D. rerio		LRMP			Pronuclear fusion Centrosome nucleus coupling	Binding partners unknown (Lindeman and Pelegri, 2012)
Vertebrate	Actin	Nesprin-1	Sun1/Sun2	Lamin A/C	Nuclear anchorage at the NMJ	Actin & Nesprin-1: in vitro coupling(Padmakumar et al., 2004), IP (Nikolova-Krstevski et al., 2011) Nesprins & Sun1,2: coIP (Crisp et al., 2006; Haque et al., 2006) Sun1,2 & Atype lamins: GST pull down (Crisp et al., 2006), coIP (Haque et al., 2006)
	Dynein, dynactin	Nesprin-1	Sun1/Sun2	Lamin A/C	Nuclear migration during neuronal development	Dynein & Nesprin-1: IF, IP (Yu et al., 2011; Zhang et al., 2009)
	Nesprin3	Nesprin-1	Sun1/Sun2	Lamin A/C	Regulation of nuclear size	Nepsrin1 & Nesprin3: GST, His-tag pull down, Y2H (Taranum et al., 2012)
		Nesprin-1	Sun3		Formation of acrosome	Nesprin-1 & Sun3: IF (Gob et al., 2010)
	Actin	Nesprin-2	Sun1/Sun2	Lamin A/C	Nuclear migration during neuronal development	Actin & Nesprin-2: in vitro coupling (Zhen et al., 2002) Nesprin-2 & Sun1/2: coIP (Crisp et al., 2006; Haque et al., 2006)
	Dynein, dynactin	Nesprin-2	Sun1/Sun2	Lamin A/C	Nuclear migration during neuronal development	Dynein & Nesprin-2: IP, IF (Yu et al., 2011; Zhang et al., 2009)
	Kinesin	Nesprin-2	Sun1/Sun2	Lamin A/C	Nuclear migration during retinal development	Kinesin & Nesprin-2: GST pull down (Schneider et al., 2011), IP (Yu et al., 2011)
	Plectin	Nesprin-3	Sun1/Sun2	Lamin A/C	Intermediate filament coupling	Plectin & Nesprin3: coIP, IF (Ketema et al., 2007; Wilhelmsen et al., 2005) Nesprin3 & Sun1/2: coIP, IF(Ketema et al., 2007)
		Nesprin-3	Sun1η		Formation of sperm heard	Nesprin3 & Sun1η: IF (Gob et al., 2010)
	KLC-1/kif5b	Nepsrin-4	Sun1/Sun2	Lamin A/C	Nuclear positioning of secretory epithelia cells Nuclear positioning of inner ear cells	KLC-1 & Nesprin4: coIP, IF (Roux et al., 2009), GST pull down (Wang et al., 2013) Nesprin4 & Sun1/2: IF (Roux et al., 2009)
	Dynein, Dynactin	KASH5	Sun1		Meiotic telomere movement	Dynein & KASH5: IP (Morimoto et al., 2012), coIP, IF (Horn et al., 2013b)

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Species	Cytoplasmic Binding proteins	KASH proteins	SUN proteins	Nuclear Binding proteins	Functions	Methods
						KASH5 & Sun1: IF (Horn et al., 2013b Horn et al., 2013b
	IP3R	LRMP	Sun1/Sun2	Lamin A/C	IP3R recruitment to the NE	IP3R & LRMP: coIP, IF (Shindo et al., 2010) LRMP & Sun1: IF (Horn et al., 2013b)
		Nesprin-1/-2		LaminA/C Emerin		Nesprin-1/Nesprin-2 & LaminA/C: GST pull down (Mislow et al., 2002), coIP, IP (Zhang et al., 2005) Nesprin-1/Nesprin-2 & Emerin: in vitro coupling (Mislow et al., 2002), GST pull down (Wheeler et al., 2007), coIP, IP (Zhang et al., 2005)
	TorsinA	Nesprin-1/-2/-3				TorsinA & Nesprins: coIP (Nery et al., 2008)
			Sun1	DNAPK, Ku70, Ku80, NAT10	DNA damage response	Sun1 & DNPK: coIP (Lei et al., 2012) Sun1 & NAT10:coIP (Chi et al., 2007)
			Sun1/Sun2	SAMP1	Cell migration	Sun2 & SAMP1: IF, coIP (Borrego-Pinto et al., 2012) Sun1 & SAMP1: MCLIP(Jafferali et al., 2014)
	RanGAP	AtWips	AtSun1/2	LINC	Recruitment of RanGAP	RanGAP & AtWips: IP, IF (Meier et al., 2010; Xu et al., 2007) AtWips & AtSuns: coIP, FRAP (Zhou et al., 2012a) AtSun1/2 & LINC: FRET (Graumann, 2014)
A. thaliana	WIT1, 2	AtWips	AtSun1/2		Actin coupling	AtWit1,2 & AtWips: IP (Zhao et al., 2008)
	WIT1, 2	AtWips	AtSun1/2		Actin coupling	AtWit1,2 & AtWips: IP (Zhao et al., 2008)

coIP: co-immunoprecipitation, IF: immunofluorescence, Y2H: yeasttwo-hybrid, IP: immunoprecipitation, MCLIP: Membrane protein cross-link immunoprecipitation

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