Accessorizing and anchoring the LINC complex for multifunctionality

Wakam Chang,[1] Howard J. Worman,[1,2] and Gregg G. Gundersen[1]

[1]Department of Pathology and Cell Biology and [2]Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, NY 10032

The linker of nucleoskeleton and cytoskeleton (LINC) complex, composed of outer and inner nuclear membrane Klarsicht, ANC-1, and Syne homology (KASH) and Sad1 and UNC-84 (SUN) proteins, respectively, connects the nucleus to cytoskeletal filaments and performs diverse functions including nuclear positioning, mechanotransduction, and meiotic chromosome movements. Recent studies have shed light on the source of this diversity by identifying factors associated with the complex that endow specific functions as well as those that differentially anchor the complex within the nucleus. Additional diversity may be provided by accessory factors that reorganize the complex into higher-ordered arrays. As core components of the LINC complex are associated with several diseases, understanding the role of accessory and anchoring proteins could provide insights into pathogenic mechanisms.

Introduction

The linker of nucleoskeleton and cytoskeleton (LINC) complex is widely recognized as the major means by which the nucleus is mechanically linked to the cytoskeleton in eukaryotic cells. It is composed of Klarsicht, ANC-1, and Syne homology (KASH) domain proteins in the outer nuclear membrane and Sad1 and UNC-84 (SUN) domain proteins in the inner nuclear membrane (Fig. 1). The KASH domain projects into the perinuclear space between the inner and outer nuclear membranes, where it interacts with the SUN domain of SUN proteins. This interaction prevents the KASH protein from diffusing out of the outer nuclear membrane into the contiguous ER. KASH proteins extend into the cytoplasm and allow the LINC complex to bind to different cytoskeletal elements and signaling molecules. SUN proteins in turn are localized in the inner nuclear membrane, anchoring the LINC complex in the nucleus by interactions with A-type lamins, chromatin-binding proteins, and other proteins.

At its core, the LINC complex is a two-membrane adhesive assembly that is capable of transmitting mechanical force across the nuclear envelope. This capability is adapted for a diverse range of functions including moving the nucleus, maintaining the centrosome–nucleus connection, shaping the nucleus, signal transduction, DNA repair, and moving chromosomes within the nucleus (Burke and Roux, 2009; Starr and Fridolfsson, 2010). This functional diversity is achieved by assembling the LINC complex from distinct KASH proteins that interact with different cytoskeletal filaments and by associating with accessory factors. The LINC complex must be dynamic in order to switch between these functions, and to allow assembly of higher-ordered arrays that can transmit force to the nucleus as a whole or, alternatively, into the nucleus.

We review the core LINC complex and interacting partners that alter cytoskeletal functionality and reinforce the core complex to permit force transduction. We consider how the LINC complex is differentially anchored for transmitting force to or into the nucleus. Furthermore, we examine data revealing that LINC complex components interact with signaling molecules, which suggests a role in signal transduction. Finally, we examine higher-ordered assemblies of LINC complexes and the role that accessory and anchoring proteins play in their formation and function. We do not address the function of short isoforms of KASH proteins that are generated by alternative transcriptional start sites or splicing, as these forms either do not localize to the nuclear membrane (KASH-less isoforms) or are unlikely to form LINC complexes, given their localization in the inner nuclear membrane (see Rajgor et al., 2012 for further discussion). Additionally, we refer the reader to reviews that cover other aspects of the LINC complex such as the discovery of its components and functions (Starr and Fridolfsson, 2010), three-dimensional structure (Sosa et al., 2013), role in nuclear positioning (Gundersen and Worman, 2013) and meiosis (Hiraoka and Dernburg, 2009), and association with disease (Burke and Stewart, 2014).

Correspondence to Gregg G. Gundersen: ggg1@columbia.edu

Abbreviations used in this paper: ABD, actin-binding domain; CH, calponin homology; KASH, Klarsicht, ANC-1, and Syne homology; LINC, linker of nucleoskeleton and cytoskeleton; SR, spectrin repeat; SUN, Sad1 and UNC-84; TAN, transmembrane actin–associated nuclear.

The Rockefeller University Press
J. Cell Biol. Vol. 208 No. 1 11–22
www.jcb.org/cgi/doi/10.1083/jcb.201409047

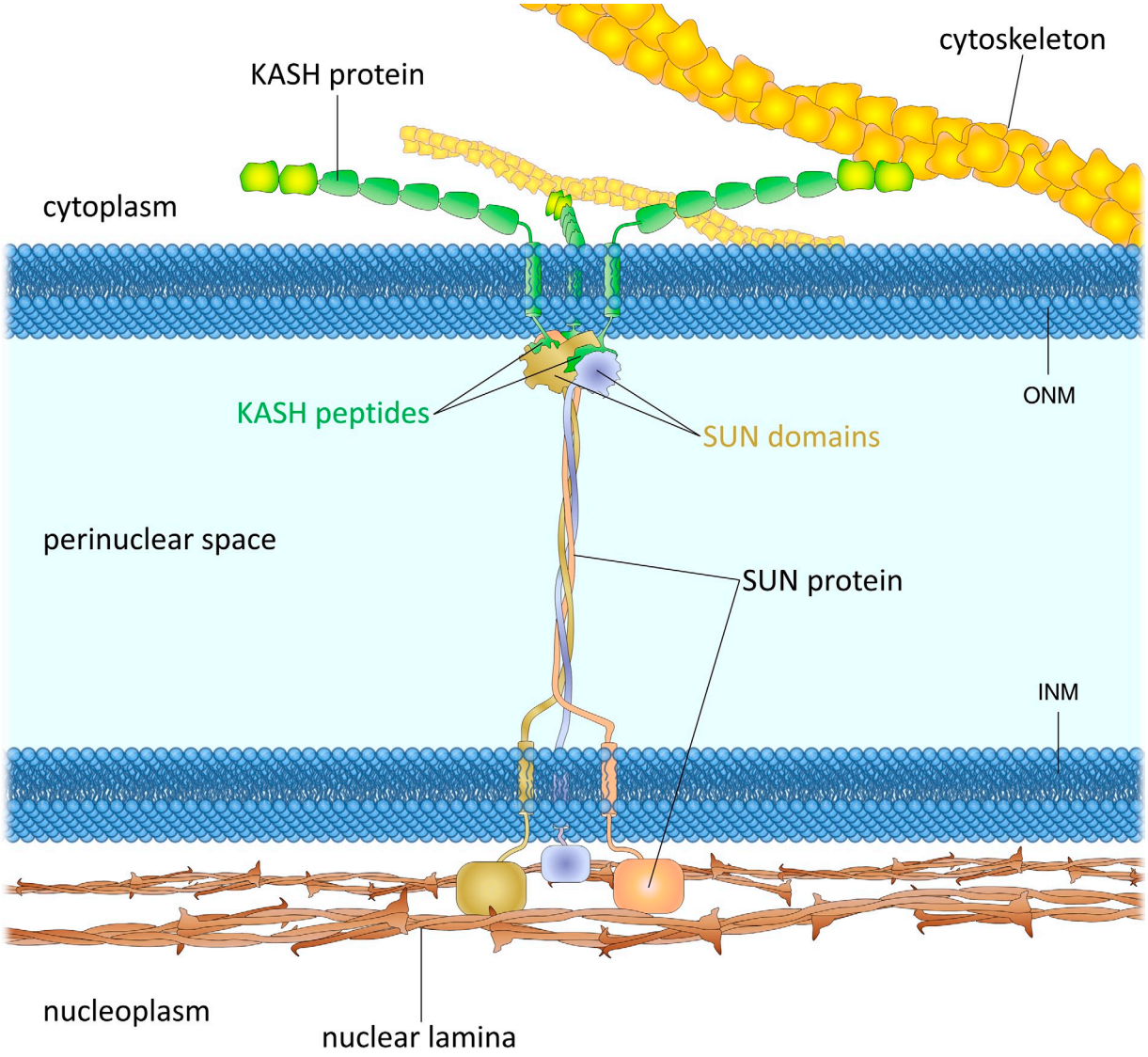

Figure 1. **The LINC complex bridges the cytoskeleton and nucleoskeleton.** The LINC complex is composed of KASH proteins in the outer nuclear membrane and SUN proteins in the inner nuclear membrane. The lumenal region of SUN proteins forms a triple helical coiled-coil, allowing trimerization of their SUN domains. The hydrophobic groove between neighboring SUN domains is required for the KASH peptide to bind, and this interaction is further strengthened by a KASH-lid of the SUN domain (see text). The cytoplasmic extensions of KASH proteins vary in size and interact with different cytoskeletal elements. Mammalian KASH proteins typically contain several SRs (see text). The nucleoplasmic domains of SUN proteins anchor the LINC complex to the nucleoskeleton, through its interaction with nuclear lamina, as well as chromosome-binding proteins and probably other anchoring proteins (see Fig. 3). INM, inner nuclear membrane; ONM, outer nuclear membrane.

Structure of the LINC complex: implications for force transmission

Two groups have described the crystal structure of the SUN2 protein in complex with the KASH domain of Syne-2/nesprin-2 (Sosa et al., 2012; Wang et al., 2012). (Note: the original KASH proteins in mice were named Syne-1 and Syne-2 [Apel et al., 2000], but as the family expanded, most KASH proteins in vertebrates became known as nesprins, for nuclear envelope spectrin repeat [SR] protein [Zhang et al., 2001], a term we use here.) SUN2 is a trimer with a globular head composed of SUN domains and a stalk composed of a triple helical coiled-coil (Fig. 1). The KASH peptide binds along a hydrophobic groove between adjacent SUN domains, with additional interaction provided by a "KASH-lid" that covers part of the peptide. Consistent with the binding interface lying between adjacent SUN domains, multimerization of SUN monomers through the triple helical coiled-coil is required for KASH peptide binding (Sosa et al., 2012; Zhou et al., 2012b). In addition to multiple noncovalent interactions, the KASH peptide can make a disulfide bond to the SUN domain. The extensive interactions between the KASH and SUN domains provide an explanation for how the LINC complex resists mechanical forces applied on KASH proteins by the cytoskeleton.

Based upon the projected length of the coiled-coil of the SUN trimer, it has been proposed that the LINC complex maintains the spacing of the inner and outer nuclear membranes (Sosa et al., 2012). Data are mixed on this issue. In HeLa cells, disrupting the SUN–KASH interaction with dominant-negative versions or knockdowns alters spacing of the two membranes

(Crisp et al., 2006). However, disruption of the SUN protein UNC-84 in *Caenorhabditis elegans* only affects spacing when nuclei are actively under force, as in body wall muscle cells, and deleting a large portion of the luminal domain does not change the spacing (Cain et al., 2014). As nuclei in HeLa cells and other adherent cells are under constant tension, these studies suggest that the LINC complex only contributes to spacing when the nucleus is under stress.

A striking feature of SUN protein structure is its trimeric nature. It is clear from the crystal structure that SUN domain interfaces are required for KASH peptide binding, and individual SUN2 domains fused with an unrelated trimeric coiled-coil restore their KASH binding (Sosa et al., 2012). Yet, the trimeric nature of the SUN protein suggests additional features of LINC complex function. The triple helical nature of the SUN stalk may be required for efficient force transmission across the nuclear membranes and to withstand the high loads required for bulk movement of the nucleus or movements of meiotic chromosomes. Another possibility, which we consider later, is that the trimer contributes to the formation of higher-ordered arrays of LINC complexes.

Accessorizing the LINC complex through KASH protein interactions

KASH protein cytoplasmic extensions. Specificity of the LINC complex for attachment to cytoskeletal elements is determined by specific KASH proteins. These proteins have cytoplasmic extensions with distinct domains that bind directly or indirectly to cytoskeletal filaments. The repertoire of KASH proteins expressed in vertebrates and invertebrates allows for binding to actin and microtubules and, in vertebrates, intermediate filaments (Gundersen and Worman, 2013). Yeast and plants have divergent KASH-like proteins that engage microtubules in *Schizosaccharomyces pombe* (Chikashige et al., 2006; King et al., 2008) and actin filaments in *Saccharomyces cerevisiae* (Conrad et al., 2008; Koszul et al., 2008) and plants (Tamura et al., 2013; Zhou et al., 2014). KASH protein cytoplasmic extensions vary greatly in size from <30 kD to >1 MDa in mammals, *C. elegans*, and *Drosophila melanogaster*. For most KASH proteins, and the large ones in particular, the most prominent structural feature in their cytoplasmic extensions is the presence of extended regions containing predicted SRs or coiled-coil domains (Fig. 2). In the "giant" KASH proteins in mammals, the vast majority of the cytoplasmic extension is predicted to be composed of SRs, with 74 in nesprin-1G and 56 in nesprin-2G (Simpson and Roberts, 2008; Autore et al., 2013). Smaller KASH proteins in mammals (nesprin-3, nesprin-4, and small isoforms of nesprin-1 and nesprin-2 arising from alternative splicing and transcriptional initiation) also contain SRs. However, except for the *Drosophila* Msp-300, SRs are not found in other KASH or KASH-like proteins, including the giant *C. elegans* ANC-1, which instead is predicted to contain coiled-coil segments within tandem repeats (Fig. 2).

The significance of the dramatic size variation among KASH proteins is unclear. It has been proposed that the large size and presumed extended length of the giant KASH proteins may enhance their interaction with the cytoskeleton (Starr and Han, 2002). However, small KASH proteins such as nesprin-3α and nesprin-4 in mammals and UNC-83 in *C. elegans* also interact with cytoskeletal elements. Additionally, in at least one case, a small chimeric variant of nesprin-2G is capable of functionally rescuing actin-dependent nuclear movement defects when expressed in cells depleted of nesprin-2G (Luxton et al., 2010). Alternatively, large KASH proteins may provide scaffolding functions that enhance resistance to mechanical force, influence signaling, or contribute to higher-ordered assemblies of LINC complexes.

Actin filaments. The giant KASH proteins nesprin-1G, nesprin-2G, ANC-1, and Msp-300 bind directly to actin filaments through paired calponin homology (CH) domains that strongly resemble those in other actin-binding proteins such as α-actinin. Aside from SRs or coiled-coils, these CH domains are one of the few recognizable structural domains in cytoplasmic extensions of KASH proteins (nesprin-4 contains a leucine zipper that may contribute to dimerization; Roux et al., 2009). In each case, the CH domains are at the amino terminus of the protein separated by a long stretch of SRs or coiled-coils from the C-terminal membrane-spanning KASH domain (Fig. 2).

The CH domains of giant KASH proteins are sufficient for recruiting actin filaments to the nuclear surface and are required for actin-dependent nuclear movement and positioning (Zhang et al., 2001; Starr and Han, 2002; Luxton et al., 2010). Yet, a recent study indicates that these domains alone are not sufficient to resist the mechanical load when actin moves the nucleus (Kutscheidt et al., 2014). Fibroblasts polarizing for migration move their nucleus rearward, resulting in reorientation of the centrosome (Gomes et al., 2005). This movement results from coupling of retrogradely moving dorsal actin cables to the nucleus by SUN2-nesprin-2G LINC complexes that assemble into linear arrays known as transmembrane actin–associated nuclear (TAN) lines (Luxton et al., 2010, 2011). The CH domains of nesprin-2G are necessary for TAN line formation and nuclear movement, yet nesprin-2G requires interaction with another actin-binding protein, the formin FHOD1, to assemble TAN lines and move the nucleus (Kutscheidt et al., 2014). FHOD1 has a typical formin domain structure, but has a unique second actin-binding site in its amino terminus that, in conjunction with an adjacent site that binds to SR11-13 of nesprin-2G (see Fig. 2), is sufficient to cross-link nesprin-2G and actin filaments (Kutscheidt et al., 2014). The FHOD1-interacting domain of nesprin-2G is within one of two clusters of SRs that are highly evolutionarily conserved and not contained in nesprin-1G (Kutscheidt et al., 2014). This region is predicted to be a site for protein–protein interaction (Autore et al., 2013), and also binds to the membrane protein meckelin, which participates in ciliogenesis (Fig. 2; Dawe et al., 2009).

Plant KASH-like proteins have been identified based upon their interaction with SUN proteins, localization to the nuclear envelope, and conserved carboxyl-terminal domains (Tamura et al., 2013; Zhou et al., 2014). Two of the five newly identified plant KASH-like proteins, termed SUN-interacting nuclear envelope (SINE) proteins, bind actin filaments through their amino-terminal armadillo repeats and are required for actin-dependent anchorage of the nucleus in the center of guard cells (Zhou et al., 2014). Other plant KASH-like proteins called WPP

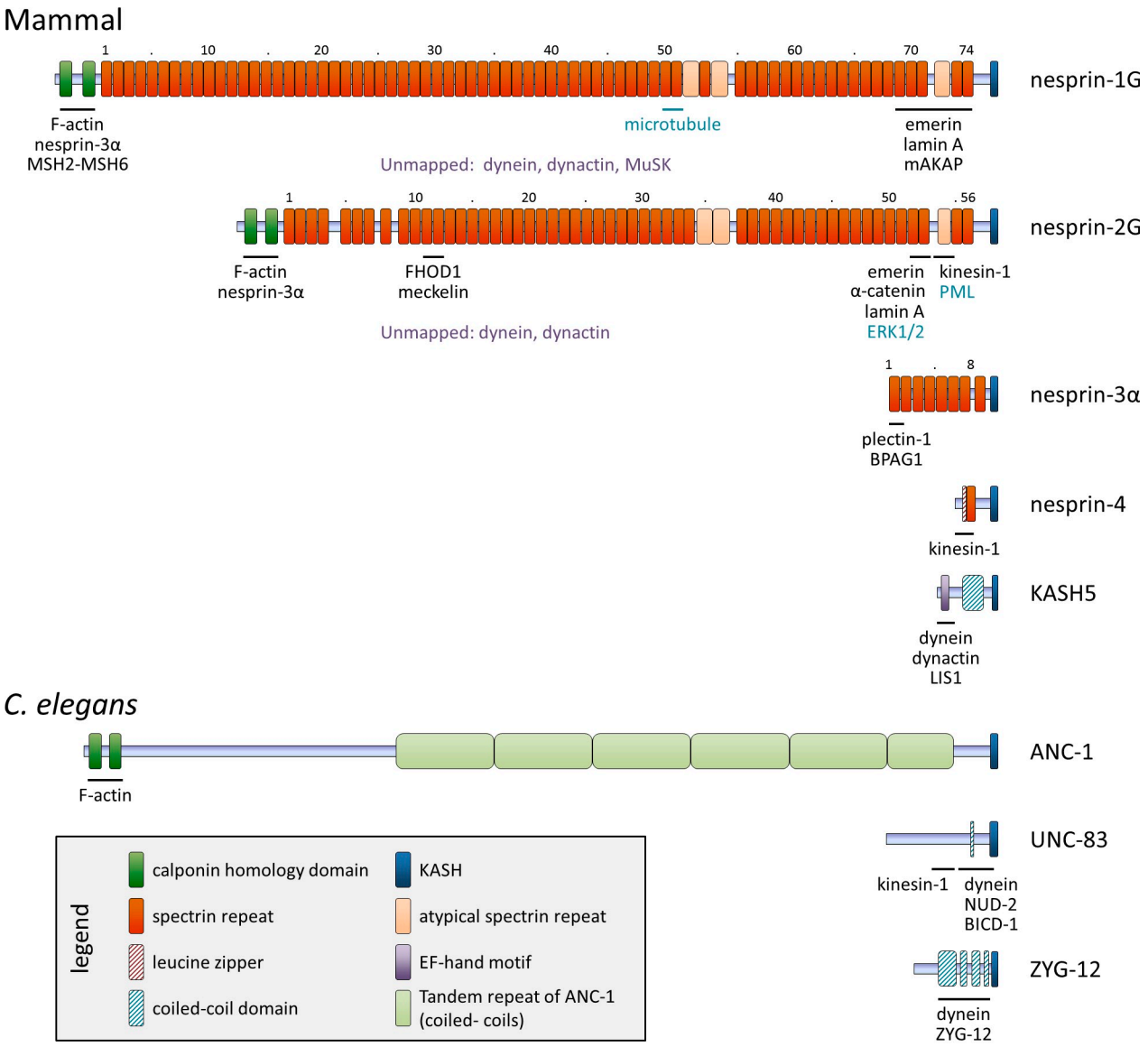

Figure 2. **Domain structures of KASH proteins and their interacting proteins.** Schematics are shown summarizing findings in mammals and *C. elegans* where the most information is available. Lines under KASH proteins indicate binding regions. "Unmapped" refers to proteins whose sites of interaction have not yet been identified. Giant KASH proteins (e.g., nesprin-1G, nesprin-2G, and ANC-1) contain CH domains that bind to F-actin, microtubule motors, and signaling proteins. The small isoforms typically interact with microtubule motors and/or their regulators. Interacting proteins in blue are characterized in isoforms lacking the KASH domain. Note that two KASH proteins, mammalian LRMP and *C. elegans* KDP-1, were omitted because of the lack of known interacting proteins.

domain–interacting protein (WIP) interact with myosin XI-i through another integral membrane protein called WPP domain–interacting tail-anchored protein (WIT). Myosin XI-i is recruited to the nuclear membrane by WIP-WIT proteins to regulate nuclear shape and dark-induced nuclear movement in plant cells (Zhou et al., 2012a; Tamura et al., 2013).

Microtubules. KASH proteins that interact with microtubules include nesprin-1, nesprin-2, nesprin-4, and probably KASH5 in mammals, *fue* in zebrafish, UNC-83 and ZYG-12 in *C. elegans*, Klar in *Drosophila*, Kif9 in *Dictyostelium discoideum*, and Kms1 and Kms2 in fission yeast (Starr and Fridolfsson, 2010; Gundersen and Worman, 2013). In almost all cases, the interaction with microtubules is mediated through association of the KASH protein with the motor proteins kinesin, dynein, or both. The *D. discoideum* KASH protein Kif9 itself contains a kinesin motor domain that appears to act as a microtubule depolymerase (Tikhonenko et al., 2013). An exception is the KASH-less $p50^{Nesp1}$ isoform that contains SRs 48–51 of nesprin-1 and interacts directly with microtubules in cosedimentation assays and colocalizes to P granules in cells (Rajgor et al., 2014). Presumably, all nesprin-1 isoforms containing SR 48–51 have the potential to directly interact with microtubules.

In many cases, association of KASH proteins with microtubule motor proteins is direct and occurs through discrete regions in their cytoplasmic extensions and specific subunits of motor proteins (Fig. 2). Nesprin-1, nesprin-2, KASH5, UNC-83, and Zyg-12 interact either directly or indirectly with cytoplasmic dynein (Fig. 2). Detailed mapping has shown that a site near the KASH domain of UNC-83 binds dynein light chain DLC-1 and the dynein regulators NUD-2 (a homologue of mammalian

NudE) and BICD-1 (Fridolfsson et al., 2010). Mammalian KASH5 also binds dynein and dynein regulators near its KASH domain (Morimoto et al., 2012; Horn et al., 2013b). Mammalian NudE/EL may indirectly interact with the LINC complex, as SUN1/2 and NudE/EL are required for dynein-dependent removal of nuclear membranes from chromatin during nuclear envelope breakdown (Turgay et al., 2014). Sites of dynein interaction with nesprin-1 and nesprin-2 have not yet been identified. Several KASH proteins also bind kinesin-1 motors, including nesprin-2 (Zhang et al., 2009; Schneider et al., 2011; Yu et al., 2011), nesprin-4 (Roux et al., 2009), and UNC-83 (Meyerzon et al., 2009; Fridolfsson et al., 2010). The interaction is usually mediated by direct binding of kinesin-1 light chains, through their tetratricopeptide repeats, to sites near the KASH domains (Fig. 2).

An emerging theme is that KASH proteins do not simply interact with a single motor or cytoskeletal filament but rather functionalize the surface of the nucleus by providing binding sites for multiple cytoskeletal elements. For example, nesprin-2G binds actin filaments at one end through its amino-terminal CH domains and FHOD1 interaction site, whereas its other end binds kinesin-1 (Fig. 2). This calls into question how KASH protein-bound motors and other elements are coordinated to yield the largely unidirectional and single cytoskeletal track movements of nuclei that have been observed. For example, nesprin-2G is involved in actin-dependent nuclear movement in polarizing fibroblasts (Luxton et al., 2010) as well as microtubule- and dynein-dependent movement of nuclei in migrating neurons and developing photoreceptor cells (Zhang et al., 2009; Yu et al., 2011). Clearly, KASH proteins and/or their associated motors must be regulated to select one activity over another.

Interaction with multiple microtubule motors may allow nuclei to be moved predominantly in one direction but with the capability of "back-tracking" to negotiate obstacles. In a detailed analysis of nuclear movement in hypodermal precursors in *C. elegans*, which involves the KASH protein UNC-83 and its binding partners kinesin and dynein, microtubule plus end–directed movements were interspersed with minus end–directed movements and rolling movements (Fridolfsson and Starr, 2010). In the absence of dynein, these latter movements were lost and nuclei failed to move efficiently toward the plus ends.

KASH protein engagement of microtubule motors also facilitates centrosome association with the nucleus, one of the first functions attributed to the LINC complex. In *C. elegans*, the KASH protein ZYG-12 interacts with dynein through its light intermediate chain and a centrosome-localized splice variant that lacks the KASH domain to maintain the centrosome near the nucleus (Malone et al., 2003). Several KASH proteins, including nesprin-1, nesprin-2, nesprin-3, and KASH5 in mammals and Kif9 in *D. discoideum*, have been implicated in maintaining the centrosome in close juxtaposition to the nucleus (Zhang et al., 2009; Schneider et al., 2011; Yu et al., 2011; Horn et al., 2013b; Tikhonenko et al., 2013). In contrast, overexpression of nesprin-4 increases the nucleus–centrosome distance (Roux et al., 2009). In yeast, KASH proteins are integral components of the spindle pole body and maintain the close association between the nucleus and microtubule organizing centers (Niwa et al., 2000; King et al., 2008).

Intermediate filaments. Nesprin-3α is the only KASH protein known to interact with cytoplasmic intermediate filaments. It is one of two known isoforms and contains a unique region at its amino terminus that interacts with the actin-binding domain (ABD) of plectin, which binds intermediate filaments through its plakin domain (Fig. 2; Wilhelmsen et al., 2005). The same region of nesprin-3α also interacts with the ABD of BPAG1n/dystonin-2a (Wilhelmsen et al., 2005; Young and Kothary, 2008). It is unknown whether the binding of these proteins' ABDs to nesprin-3α prevents simultaneous binding to actin. Interestingly, nesprin-3α also binds to the ABD (i.e., CH domains) of nesprin-1G and nesprin-2 and may control nuclear size through formation of a nesprin meshwork (Lu et al., 2012). Nesprin-3 also appears to function in the cellular response to shear stress and force transmission (Lombardi et al., 2011; Morgan et al., 2011; Chambliss et al., 2013) and in fibroblast migration in 3D matrices, where the cells use a distinct form of migration in which the front and rear of the cell are compartmentalized by the nucleus and associated ER so that actomyosin-dependent forward movement of the nucleus creates pressure in the front of the cell to generate lobopodial protrusions (Petrie et al., 2014).

Signaling scaffolds. Growing evidence indicates that KASH proteins act to tether signaling molecules. Early studies showed that nesprin-1 interacts with muscle-specific tyrosine kinase (Apel et al., 2000) and muscle A kinase anchoring protein (mAKAP; Pare et al., 2005). A KASH-less form of nesprin-2 interacts with active mitogen-activated protein kinases and promyelocytic leukemia protein (Warren et al., 2010). More recently, nesprin-2 has been shown to interact with α-catenin (Neumann et al., 2010). Through this interaction and its interaction with the nuclear envelope protein emerin, nesprin-2 positively regulates the nuclear localization of active β-catenin and Wnt signaling (Neumann et al., 2010). Curiously, emerin, which interacts with both nesprin-2 and β-catenin, negatively regulates Wnt signaling by restricting nuclear accumulation of β-catenin (Zhang et al., 2005; Markiewicz et al., 2006). Given that emerin and α-catenin interact with the same region of nesprin-2 (see Fig. 2), it is possible that competition between emerin and catenins for nesprin-2 binding may explain the opposing roles of nesprin-2 and emerin in Wnt signaling.

KASH protein regulation of β-catenin and Wnt signaling may be phylogenetically conserved. *C. elegans* ANC-1 interacts with Regulator of Presynaptic Morphology 1 (RPM-1), a regulator of neuronal development and regeneration (Tulgren et al., 2014). Genetic analysis suggests that RPM-1, ANC-1, and β-catenin function together to regulate synapse formation in motor neurons and axon termination in the mechanosensory neurons. This function of ANC-1 requires its nuclear localization and is negatively regulated by emerin as in mammalian cells (Tulgren et al., 2014). Although additional research is required to understand how KASH proteins contribute to Wnt signaling, an attractive hypothesis is that they enhance the perinuclear concentration of active β-catenin.

The LINC complex has also been implicated in very rapid mechanochemical signaling to the nucleus (Isermann and Lammerding, 2013). It is clear that the nucleus responds to force and that LINC complex components are necessary for this force

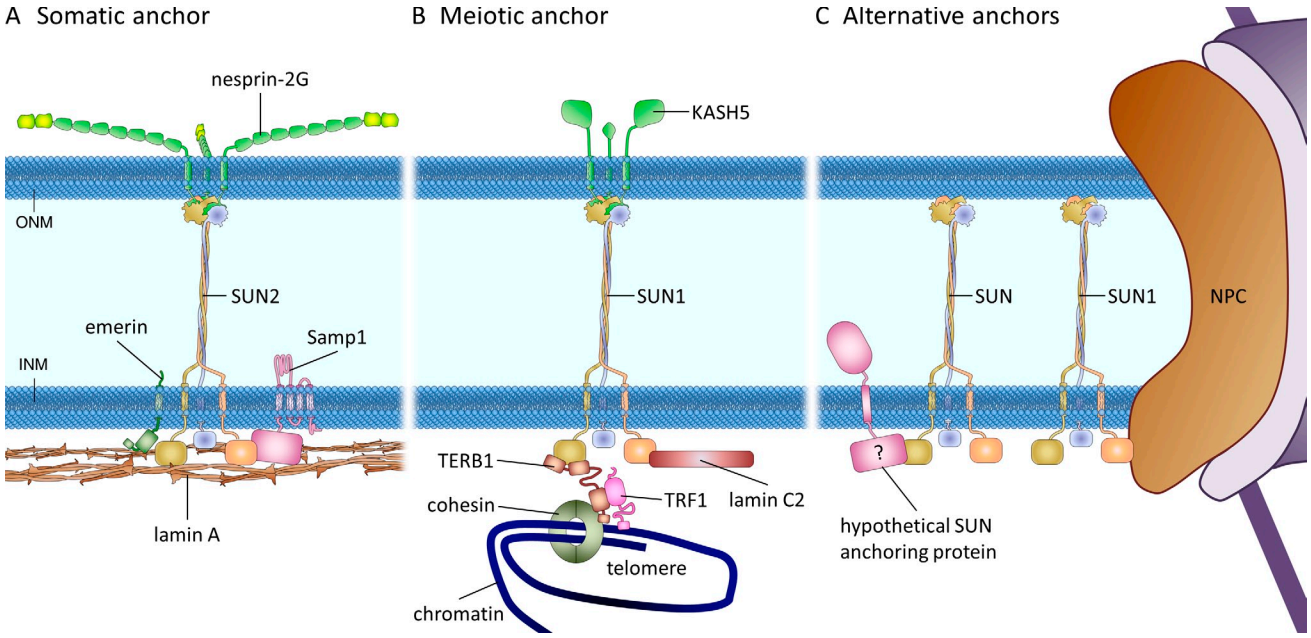

Figure 3. **Anchoring the LINC complex.** Mammalian SUN proteins are anchored to the inner nuclear membrane through at least three different mechanisms. (A) The nucleoplasmic tail of SUN2 binds to lamin A and anchors the LINC complex to the nuclear lamina in somatic cells. Samp1 and emerin are required to strengthen this anchorage during nuclear movement, presumably to resist the high mechanical force. For clarity, nesprin-2G is shown as a shorter protein without all of its 56 SRs. INM, inner nuclear membrane; ONM, outer nuclear membrane. (B) The nucleoplasmic tail of SUN1 binds TERB1 and anchors the LINC complex to chromosomes through telomere binding proteins (TRF1 and cohesion) in meiotic cells. Lamin C2 also associates with this complex, probably through SUN1 binding. (C) Nucleoplasmic tails of SUN proteins shown binding to nuclear pores (SUN1) and a hypothetical protein as possible alternative anchors for the LINC complex in somatic cells. As described in the text, the localization of SUN1 and SUN2 in the nuclear membrane is only slightly affected in somatic cells lacking all lamins, which indicates the presence of additional anchoring factors.

transmission (Maniotis et al., 1997; Lombardi et al., 2011; Chambliss et al., 2013). Nevertheless, it has been difficult to determine whether the LINC complex directly transmits mechanical force into chemical signals within the nucleus. The first evidence for direct mechanosensing by the LINC complex comes from a recent ground-breaking study in which magnetic tweezers were used to pull on nesprin-1 antibody-coated beads attached to isolated nuclei (Guilluy et al., 2014). Pulling on nesprin-1 resulted in a stiffening response in which greater force was required to displace the bead. Stiffening was accompanied by and required the recruitment of A-type lamins to the LINC complex, activation of Src, and tyrosine phosphorylation of emerin. This study identifies the first mechanotransduction pathway into the nucleus and raises several provocative questions including how tension on nesprin-1 activates Src and whether other KASH proteins also mediate mechanotransduction.

Anchoring the LINC complex

Nuclear lamins. To position and move the nucleus, the LINC complex must be anchored so that it can transmit force to the nucleus. Several studies clearly show that lamins contribute to nucleoplasmic anchoring of the LINC complex (Fig. 3 A). In mammals, the three lamin genes encode lamin B1, lamin B2 (and an alternatively spliced lamin B3), and the A-type lamins, which include the alternatively spliced isoforms lamin A, lamin C, and lamin C2 (Worman, 2012). In support of a LINC complex anchoring function, the carboxyl terminus of lamin A binds to SUN proteins, whereas binding to lamin B1 and lamin C appears to be very weak (Crisp et al., 2006; Haque et al., 2006). Additionally, both SUN1 and SUN2 have increased diffusional mobility in nuclei of mouse fibroblasts lacking A-type lamins compared with cells from wild-type mice (Östlund et al., 2009). Finally, experiments on actin-dependent nuclear movement in migrating fibroblasts that lack A-type lamins show that nesprin-2G-SUN TAN lines are relatively unstable and slip over the nucleus rather than move with it, indicating a defect in anchoring (Folker et al., 2011). Similarly, nuclear migration in *C. elegans* is impaired when the interaction between UNC-84 and the lamin LMN-1 is weakened (Bone et al., 2014).

Nevertheless, studies on the intracellular localization of SUN proteins show that factors other than lamin A binding must contribute to anchoring the LINC complex, particularly in mammalian cells. Expression of the single *C. elegans* lamin is apparently required for proper localization of UNC-84 (Lee et al., 2002), yet its closest mammalian orthologue SUN1 is properly localized in cells lacking A-type lamins or both A-type and B-type lamins (Padmakumar et al., 2005; Crisp et al., 2006; Haque et al., 2006; Hasan et al., 2006). Similarly, SUN2 is only minimally displaced to the ER when lamins A and C are lacking, which suggests that additional factors are also involved in its localization (Crisp et al., 2006). It should be noted that the *Lmna*$^{-/-}$ fibroblasts used in some of these studies actually expresses a truncated lamin A (Jahn et al., 2012), which may have dominant-negative effects because some of the phenotypes in the *Lmna*$^{-/-}$ cells cannot be rescued by reexpression of wild-type lamin. However, most of the results were confirmed with siRNA knockdown. These studies indicate that at least in mammalian cells, other factors contribute to SUN protein

localization in the nucleus and hence potentially in anchoring the LINC complex.

A-type lamins also interact directly with nesprin-1 and nesprin-2 through SRs near their KASH domains (Fig. 2). However, this interaction is not likely to contribute to LINC complex anchoring, given that nesprins in the outer nuclear membrane do not contact the lamina. Indeed, nesprin-2G localization in the outer nuclear membrane is not strongly affected by the absence of A-type lamins (Folker et al., 2011). Instead, A-type lamin's interactions with nesprins are likely to reflect interactions with smaller nesprin isoforms that enter the inner nuclear membrane.

Phenotypes of genetically modified mice imply that lamins other than A-type lamins may participate in anchoring the LINC complex in certain cell types. Although they develop growth retardation, muscular dystrophy, and cardiomyopathy after birth, mice with germline deletion of A-type lamins develop to term, suggesting that critical LINC complex–mediated events occur in these mice during embryonic development (Sullivan et al., 1999). B-type lamins, despite their weak interaction with SUN proteins, may play a role. In fact, lamin B1–, lamin B2–, nesprin1/2-, and SUN1/2-deficient mice all show similar defects in neuronal migration, which suggests that B-type lamins may contribute to anchoring the LINC complex in migrating neurons during development (Zhang et al., 2009; Coffinier et al., 2010, 2011). Perhaps the weak interaction between B-type lamins and SUN proteins is strengthened by other factors. Alternatively, B-type lamins may play an indirect role in anchoring the LINC complex, for example by overall stiffening of the nucleus.

Lamin-associated proteins. Two A-type lamin–associated proteins, Samp1 (also known as NET5) and emerin, have also been implicated in LINC complex anchoring. Both of these proteins depend on A-type lamins for their localization to the inner nuclear membrane (Sullivan et al., 1999; Borrego-Pinto et al., 2012). Samp1 was initially reported to interact with SUN1 and emerin and to be required for proper localization of emerin in the inner nuclear envelope (Gudise et al., 2011). Subsequently, Samp1 was found to be necessary for actin-dependent nuclear movement in fibroblasts and to interact with SUN2, lamin A, and lamin C, although localization of these proteins was not dependent on Samp1 (Borrego-Pinto et al., 2012). Samp1 also colocalized with nesprin-2G and SUN2 in TAN lines in fibroblasts polarizing for migration, although the effect of Samp1 depletion on TAN lines was not addressed. SAMP1 colocalization in TAN lines and its requirement for nuclear movement suggest that it enhances anchoring of TAN lines by providing a second interacting site for SUN2 in addition to that provided by A-type lamins. Such a model is supported by a recent report on *C. elegans* SAMP-1 (Bone et al., 2014). Interestingly, the LINC complex anchoring function of Samp1 was originally reported for its yeast orthologue Ima1 (see the following sections).

Emerin may also contribute to LINC complex anchoring. Emerin associates with SUN1 and SUN2, and the interaction between SUN1 and emerin has been mapped to their nucleoplasmic domains (Haque et al., 2010). Consistent with a possible role in anchoring the LINC complex, depletion of emerin from polarizing fibroblasts leads to abnormal nuclear migration and slipping of TAN lines on the nucleus (Chang et al., 2013). Thus, emerin may function together with A-type lamins and Samp1 in the nucleoplasmic anchoring of the LINC complex.

Anchors not clearly associated with lamins. Mouse embryonic stem cells harboring deletions of all lamin genes exhibit normal proliferation and can differentiate into fibroblast-like cells, beating cardiomyocytes and neural progenitor cells in vitro (Kim et al., 2011), which suggests that at least some LINC-complex–mediated functions can occur. Combined with data showing that lack of lamins does not completely disrupt the nuclear location of SUN proteins (discussed earlier), this suggests that proteins other than lamins or those bound to lamins can function in anchoring the LINC complex in the nucleoplasm (Fig. 3 C). In mammals, one possible candidate is the nuclear pore. SUN1 associates with the nuclear pore complex, and disruption of SUN1 interferes with nuclear pore assembly and distribution (Liu et al., 2007; Talamas and Hetzer, 2011). Yet it is unclear whether this association reflects SUN1 in a LINC complex, and there are no reports of KASH proteins contributing to nuclear pore distribution. In yeast, which lack nuclear lamins, it has been proposed that the yeast SUN protein, Sad1, interacts with Ima1 to anchor it within the nuclear membrane (King et al., 2008). The binding of Ima1 to centromeric DNA may provide the resistive force to anchor Sad1. Indeed, in the absence of Ima1, the Sad1–Kms2 LINC complex is partially disrupted, causing microtubule-dependent forces to distort the nucleus and depleting spindle pole body components from the nucleus. However, this finding has been questioned recently because it was found that some of the Ima1 deletion strains did not disrupt Ima1 (Hiraoka et al., 2011). Nonetheless, the authors report that Ima1 and two LEM domain proteins, Man1 and Lem2, interact with Sad1, and that when all three are disrupted similar nuclear phenotypes result, as originally reported for Ima1.

Meiotic chromosome anchorage. The LINC complex functions in chromosome movements and pairing in meiosis, and for this function, the anchoring of the LINC complex is distinct from that in somatic cells. In meiosis the LINC complex is mobile within the plane of the nuclear membrane and is tethered to defined regions of chromosomes (telomeres in mice and yeast or pairing centers in *C. elegans*; Fig. 3 B). Tethering is required for movements of chromosomes and is mediated by specific meiotic proteins that link factors at the chromosomal sites with SUN proteins in LINC complexes, which in turn engage the cytoskeleton. Reflecting their distinct chromosomal binding sites, these tethering proteins are not conserved among mice, *C. elegans*, *Drosophila*, and yeast. For example, in mice SUN1 interacts with telomeres through TERB1, a meiosis-specific protein that binds telomere protein TRF1 and telomere repeat sequences, and recruits cohesin that encircles and holds sister telomeres together (Fig. 3 B; Daniel et al., 2014; Shibuya and Watanabe, 2014). Through its interaction with a meiotic-specific KASH protein KASH5, telomere-associated SUN1 engages dynactin and dynein to mediate meiotic chromosome movement (Morimoto et al., 2012; Horn et al., 2013b). SUN2 is also associated with sites of telomere tethering at the nuclear envelope, but may not be required for meiosis (Schmitt et al., 2007). In fission yeast, the telomeric Rap1/Taz1 complex recruits the SUN protein Sad1 to telomeres (Chikashige et al., 2006). This interaction

is indirect and is bridged by meiotic prophase-specific Bqt1/2 (Chikashige et al., 2006). On the outer nuclear membrane, the KASH protein Kms1 binds to dynein to facilitate the movement of telomeres, which is essential for telomere clustering and the formation of the "telocentrosome" (Shimanuki et al., 1997; Yoshida et al., 2013). In *C. elegans*, specific pairing center proteins (HIM-8 and ZIM-1-3) attach chromosomes to LINC complexes composed of SUN1/Matefin and the KASH protein ZYG-12, which in turn binds dynein (Phillips and Dernburg, 2006; Sato et al., 2009). Budding yeast also use a telomere-specific binding protein (Ndj1) to attach to LINC components Csm4 and Mps3 for actin-dependent chromosome movements during meiosis (Conrad et al., 2007, 2008; Koszul et al., 2008; Wanat et al., 2008).

How the LINC complex in meiotic cells is modified to allow force transmission to chromosomes rather than, for example, the lamina, is still unclear. One possibility is that LINC complex components are posttranslationally regulated. In *C. elegans*, specific phosphorylation of Ser/Thr residues in the nucleoplasmic tail of SUN-1/Matefin occurs during meiosis and is required for meiotic chromosome movements (Penkner et al., 2009). These modifications may contribute to the reduced constraints on LINC complex mobility that have been observed at the onset of meiosis in *C. elegans* (Wynne et al., 2012).

Another possibility is that the lamina itself is modified. In mammalian germ cells, a meiotic-specific A-type lamin, lamin C2, is expressed and localizes to sites of LINC complex–mediated telomere tethering (Jahn et al., 2010; Link et al., 2013). Lamin C2 lacks the amino-terminal head and part of the central α-helical rod domain necessary for assembly into filaments, and shows higher diffusional mobility than lamin C when expressed in somatic cells (Jahn et al., 2010). Lamin C2 overexpression in somatic cells alters the distribution of lamin B1 and SUN proteins, which suggests that it may modify their normal anchoring mechanisms. Despite these considerations, tethering of telomeres to the nuclear periphery and their rearrangement into the characteristic bouquet conformation occurs normally in meiocytes lacking lamin C2, indicating that the formation of LINC complexes at telomeres and their initial movements during meiosis do not require this protein. Release of chromosomes from the bouquet stage was affected, so perhaps lamin C2 is only required for these later movements.

Chromosome anchorage during DNA repair. Evidence has accumulated that the LINC complex also functions in DNA repair. Initial work in budding yeast showed that the SUN protein Mps3 was required for localization of DNA double-strand breaks to the cell periphery, delaying homologous repair and enhancing repair through an alternative pathway (Oza et al., 2009). More recently, in fission yeast both LINC complex components Sad1 and Kms1 were shown to be localized at sites of DNA double-strand breaks and participate in repair (Swartz et al., 2014). Interestingly, microtubules are also colocalized to these sites, presumably through interaction with Kms1, and promote movements of the complexes and DNA repair. The LINC complex also appears to participate in DNA repair in mammalian cells: SUN1 and SUN2 interact with DNA-dependent protein kinase that functions in DNA repair, and early events in the repair process are defective in cells lacking SUN1 and SUN2 (Lei et al., 2012). Nesprin-1 interacts with the DNA damage response proteins MSH2 and MSH6 through its CH domains (Sur et al., 2014). It is not yet clear whether DNA breaks associate with the LINC complex in mammalian cells as appears to be the case in yeasts.

Assembling higher-ordered arrays of LINC complexes

A fascinating aspect of the LINC complex is its formation of higher-ordered assemblies. These assemblies function to move nuclei in fibroblasts polarizing for migration (Luxton et al., 2010, 2011), to position nuclei in adherent smooth muscle cells (Nagayama et al., 2014), and to move meiotic chromosomes in numerous organisms. In polarizing fibroblasts, the TAN lines are higher-ordered linear alignments of SUN2-nesprin-2G LINC complexes along dorsal actin cables. Similar linear arrays of nesprin-1 also align with dorsal actin fibers in smooth muscle cells (Nagayama et al., 2014). In contrast, the higher-ordered arrays observed during meiosis in *S. pombe*, *C. elegans*, and mice are spot-weld clusters of LINC complexes that tether chromosomes to the nuclear envelope and allow for chromosome movements, which are usually powered by microtubules and dynein (Chikashige et al., 2006; Ding et al., 2007; Schmitt et al., 2007; Penkner et al., 2009; Sato et al., 2009; Morimoto et al., 2012).

The formation of TAN lines and meiotic clusters appears to involve different topological mechanisms. TAN lines only form when dorsal actin cables contact the nucleus, and disruption of actin cables by actin or myosin inhibitors or myosin II knockdown completely prevents their formation (Luxton et al., 2010; Chang et al., 2013). This "outside-in" initiation of TAN line formation is further emphasized by their absence in cells depleted of the formin FHOD1, which is primarily cytoplasmic (Kutscheidt et al., 2014), and by the observation that "nesprin-2G–only" TAN lines form in cells lacking SUN2 (Folker et al., 2011). In contrast, meiotic patches form by an "inside-out" mechanism triggered by the accumulation of meiotic-specific proteins at telomeres (or pairing center-associated proteins in *C. elegans*). Evidence for this includes: (1) the temporal correlation between appearance (and disappearance) of telomere/pairing center–associated proteins and the LINC complex patches in meiotic prophase, (2) the failure of LINC complexes to redistribute into patches in cells deficient in telomere/pairing center–associated proteins, (3) the formation of clusters of SUN proteins in somatic cells ectopically expressing the telomere-associated proteins, and (4) the absence of the effects of SUN or KASH protein depletion on the accumulation of the telomere/pairing center–associated proteins (Chikashige et al., 2006; Ding et al., 2007; Schmitt et al., 2007; Penkner et al., 2009; Sato et al., 2009; Morimoto et al., 2012). In contrast to TAN line formation, disruption of the associated cytoskeleton in meiocytes does not prevent the formation of meiotic patches of LINC complexes, although it reduces their size and increases their number, presumably reflecting the inability to cluster patches in the absence of cytoskeletal-derived forces (Sato et al., 2009). A similar inside-out mechanism may function during homology-directed DNA repair in yeast as shown by the accumulation of Sad1-Kms1 LINC complexes after initiation of double-strand DNA breaks (Swartz et al., 2014).

In order for higher-ordered assemblies of LINC complexes to form, SUN and KASH proteins must have sufficient mobility in the membrane to permit their clustering. In fact, fluorescence recovery after photobleaching experiments in fibroblasts show that both SUNs and nesprins are relatively mobile in the nuclear envelope (Östlund et al., 2009). In TAN lines, nesprin-2G becomes relatively immobilized (Luxton et al., 2011). In meiosis, both KASH proteins and SUNs redistribute from dispersed sites in mice and *C. elegans* and from the spindle pole body in fission yeast, which implies their mobility.

To understand these and perhaps other higher-ordered assemblies of LINC complexes still to be discovered, it is worth considering the trimeric nature of the SUN2 protein and the possibility that LINC complexes themselves may contribute to clustering. As noted by Sosa et al. (2012), clustering of LINC complexes could occur if oligomeric KASH proteins were attached through their KASH domains to separate SUN trimers. Although the oligomeric state of KASH proteins is unknown, evidence suggests that they may form homo-oligomers (Mislow et al., 2002; Ketema et al., 2007) or even hetero-oligomers, as shown by the binding of nesprin-1 and nesprin-2 CH domains to nesprin-3 (Lu et al., 2012). Zhou et al. (2012b) offered the alternative possibility that SUNs themselves cluster through formation of hybrids of their amino-terminal dimeric and carboxyl-terminal trimeric coiled-coils. Another possibility is that other proteins interacting with SUN or KASH proteins contribute to clustering, as seems to be the case for SUN interaction with telomere-binding proteins in meiosis and by the requirement for the dimeric formin FHOD1 for nesprin-2G TAN line formation (Kutscheidt et al., 2014). These possibilities are not mutually exclusive and more than one may contribute to the assembly of higher-ordered LINC complexes. The varied possibilities suggest that exploring the basis for higher-ordered assemblies of LINC complexes will be a rich area for further understanding of how the nucleus is attached to the cytoskeleton.

Future perspectives

The identification of LINC complex–associated proteins has begun to explain how the LINC complex is adapted to the growing list of functions attributed to it. We have considered how proteins associated with KASH proteins determine its specificity for the cytoskeleton, enhance its resistance to mechanochemical force, and contribute to both conventional and mechanosensitive signaling activities. In an analogous fashion, proteins associating with SUN proteins may alter or adapt the anchoring of the LINC complex for specific tasks. It is likely that more proteins will be identified that contribute to the known functions of the LINC complex and suggest new functions. Indeed, in the only comprehensive screen of KASH protein–interacting proteins, myriad new potential interactors of *C. elegans* UNC-83 were identified in a yeast two-hybrid screen (Fridolfsson et al., 2010).

Understanding how the LINC complex function is specified or modified by its associated proteins will also enhance our understanding of how alterations in its protein components contribute to disease and whether certain sets of proteins function in a LINC complex pathway. Several diseases and syndromes are caused by mutations in genes encoding core LINC complex components, including Emery-Dreifuss muscular dystrophy, cerebellar ataxia, arthrogryposis, and progressive high-frequency hearing loss (Gros-Louis et al., 2007; Zhang et al., 2007; Attali et al., 2009; Horn et al., 2013a; Meinke et al., 2014). Polymorphisms in genes encoding LINC complex components have also been putatively linked to autism, bipolar disorder, and several cancers (Sjöblom et al., 2006; Doherty et al., 2010; O'Roak et al., 2011; Green et al., 2013; Schoppmann et al., 2013; Yu et al., 2013). Emery-Dreifuss muscular dystrophy is a particularly provocative case given that disease-causing mutations occur in genes encoding SUNs and nesprins as well as in genes encoding their binding proteins emerin and A-type lamins (Bione et al., 1994; Bonne et al., 1999; Zhang et al., 2007; Puckelwartz et al., 2010; Taranum et al., 2012; Meinke et al., 2014). All of these proteins function in nuclear positioning, which suggests that mispositioning of nuclei may be a contributing factor to the disease. Future research should determine whether other proteins associated with the LINC complex can be implicated in specific pathways that reflect their contribution to disease pathogenesis.

The authors are supported by National Institutes of Health grants R01GM099481, R01/R56NS059352, R01HD070713, and R01AR048997, the Muscular Dystrophy Association (MDA294537), and the Los Angeles Thoracic and Cardiovascular Foundation.

The authors declare no competing financial interests.

Submitted: 9 September 2014
Accepted: 5 December 2014

References

Apel, E.D., R.M. Lewis, R.M. Grady, and J.R. Sanes. 2000. Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. *J. Biol. Chem.* 275:31986–31995. http://dx.doi.org/10.1074/jbc.M004775200

Attali, R., N. Warwar, A. Israel, I. Gurt, E. McNally, M. Puckelwartz, B. Glick, Y. Nevo, Z. Ben-Neriah, and J. Melki. 2009. Mutation of SYNE-1, encoding an essential component of the nuclear lamina, is responsible for autosomal recessive arthrogryposis. *Hum. Mol. Genet.* 18:3462–3469. http://dx.doi.org/10.1093/hmg/ddp290

Autore, F., M. Pfuhl, X. Quan, A. Williams, R.G. Roberts, C.M. Shanahan, and F. Fraternali. 2013. Large-scale modelling of the divergent spectrin repeats in nesprins: giant modular proteins. *PLoS ONE*. 8:e63633. http://dx.doi.org/10.1371/journal.pone.0063633

Bione, S., E. Maestrini, S. Rivella, M. Mancini, S. Regis, G. Romeo, and D. Toniolo. 1994. Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat. Genet.* 8:323–327. http://dx.doi.org/10.1038/ng1294-323

Bone, C.R., E.C. Tapley, M. Gorjánácz, and D.A. Starr. 2014. The *Caenorhabditis elegans* SUN protein UNC-84 interacts with lamin to transfer forces from the cytoplasm to the nucleoskeleton during nuclear migration. *Mol. Biol. Cell*. 25:2853–2865. http://dx.doi.org/10.1091/mbc.E14-05-0971

Bonne, G., M.R. Di Barletta, S. Varnous, H.M. Bécane, E.H. Hammouda, L. Merlini, F. Muntoni, C.R. Greenberg, F. Gary, J.A. Urtizberea, et al. 1999. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat. Genet.* 21:285–288. http://dx.doi.org/10.1038/6799

Borrego-Pinto, J., T. Jegou, D.S. Osorio, F. Auradé, M. Gorjánácz, B. Koch, I.W. Mattaj, and E.R. Gomes. 2012. Samp1 is a component of TAN lines and is required for nuclear movement. *J. Cell Sci.* 125:1099–1105. http://dx.doi.org/10.1242/jcs.087049

Burke, B., and K.J. Roux. 2009. Nuclei take a position: managing nuclear location. *Dev. Cell*. 17:587–597. http://dx.doi.org/10.1016/j.devcel.2009.10.018

Burke, B., and C.L. Stewart. 2014. Functional architecture of the cell's nucleus in development, aging, and disease. *Curr. Top. Dev. Biol.* 109:1–52. http://dx.doi.org/10.1016/B978-0-12-397920-9.00006-8

Cain, N.E., E.C. Tapley, K.L. McDonald, B.M. Cain, and D.A. Starr. 2014. The SUN protein UNC-84 is required only in force-bearing cells to maintain nuclear envelope architecture. *J. Cell Biol.* 206:163–172. http://dx.doi.org/10.1083/jcb.201405081

Chambliss, A.B., S.B. Khatau, N. Erdenberger, D.K. Robinson, D. Hodzic, G.D. Longmore, and D. Wirtz. 2013. The LINC-anchored actin cap connects the extracellular milieu to the nucleus for ultrafast mechanotransduction. *Sci. Rep*. 3:1087. http://dx.doi.org/10.1038/srep01087

Chang, W., E.S. Folker, H.J. Worman, and G.G. Gundersen. 2013. Emerin organizes actin flow for nuclear movement and centrosome orientation in migrating fibroblasts. *Mol. Biol. Cell*. 24:3869–3880. http://dx.doi.org/10.1091/mbc.E13-06-0307

Chikashige, Y., C. Tsutsumi, M. Yamane, K. Okamasa, T. Haraguchi, and Y. Hiraoka. 2006. Meiotic proteins bqt1 and bqt2 tether telomeres to form the bouquet arrangement of chromosomes. *Cell*. 125:59–69. http://dx.doi.org/10.1016/j.cell.2006.01.048

Coffinier, C., S.Y. Chang, C. Nobumori, Y. Tu, E.A. Farber, J.I. Toth, L.G. Fong, and S.G. Young. 2010. Abnormal development of the cerebral cortex and cerebellum in the setting of lamin B2 deficiency. *Proc. Natl. Acad. Sci. USA*. 107:5076–5081. http://dx.doi.org/10.1073/pnas.0908790107

Coffinier, C., H.J. Jung, C. Nobumori, S. Chang, Y. Tu, R.H. Barnes II, Y. Yoshinaga, P.J. de Jong, L. Vergnes, K. Reue, et al. 2011. Deficiencies in lamin B1 and lamin B2 cause neurodevelopmental defects and distinct nuclear shape abnormalities in neurons. *Mol. Biol. Cell*. 22:4683–4693. http://dx.doi.org/10.1091/mbc.E11-06-0504

Conrad, M.N., C.Y. Lee, J.L. Wilkerson, and M.E. Dresser. 2007. MPS3 mediates meiotic bouquet formation in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA*. 104:8863–8868. http://dx.doi.org/10.1073/pnas.0606165104

Conrad, M.N., C.Y. Lee, G. Chao, M. Shinohara, H. Kosaka, A. Shinohara, J.A. Conchello, and M.E. Dresser. 2008. Rapid telomere movement in meiotic prophase is promoted by NDJ1, MPS3, and CSM4 and is modulated by recombination. *Cell*. 133:1175–1187. http://dx.doi.org/10.1016/j.cell.2008.04.047

Crisp, M., Q. Liu, K. Roux, J.B. Rattner, C. Shanahan, B. Burke, P.D. Stahl, and D. Hodzic. 2006. Coupling of the nucleus and cytoplasm: role of the LINC complex. *J. Cell Biol.* 172:41–53. http://dx.doi.org/10.1083/jcb.200509124

Daniel, K., D. Tränkner, L. Wojtasz, H. Shibuya, Y. Watanabe, M. Alsheimer, and A. Tóth. 2014. Mouse CCDC79 (TERB1) is a meiosis-specific telomere associated protein. *BMC Cell Biol.* 15:17. http://dx.doi.org/10.1186/1471-2121-15-17

Dawe, H.R., M. Adams, G. Wheway, K. Szymanska, C.V. Logan, A.A. Noegel, K. Gull, and C.A. Johnson. 2009. Nesprin-2 interacts with meckelin and mediates ciliogenesis via remodelling of the actin cytoskeleton. *J. Cell Sci.* 122:2716–2726. http://dx.doi.org/10.1242/jcs.043794

Ding, X., R. Xu, J. Yu, T. Xu, Y. Zhuang, and M. Han. 2007. SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. *Dev. Cell*. 12:863–872. http://dx.doi.org/10.1016/j.devcel.2007.03.018

Doherty, J.A., M.A. Rossing, K.L. Cushing-Haugen, C. Chen, D.J. Van Den Berg, A.H. Wu, M.C. Pike, R.B. Ness, K. Moysich, G. Chenevix-Trench, et al. 2010. ESR1/SYNE1 polymorphism and invasive epithelial ovarian cancer risk: an Ovarian Cancer Association Consortium study. *Cancer Epidemiol. Biomarkers Prev.* 19:245–250. http://dx.doi.org/10.1158/1055-9965.EPI-09-0729

Folker, E.S., C. Ostlund, G.W. Luxton, H.J. Worman, and G.G. Gundersen. 2011. Lamin A variants that cause striated muscle disease are defective in anchoring transmembrane actin-associated nuclear lines for nuclear movement. *Proc. Natl. Acad. Sci. USA*. 108:131–136. http://dx.doi.org/10.1073/pnas.1000824108

Fridolfsson, H.N., and D.A. Starr. 2010. Kinesin-1 and dynein at the nuclear envelope mediate the bidirectional migrations of nuclei. *J. Cell Biol.* 191:115–128. http://dx.doi.org/10.1083/jcb.201004118

Fridolfsson, H.N., N. Ly, M. Meyerzon, and D.A. Starr. 2010. UNC-83 coordinates kinesin-1 and dynein activities at the nuclear envelope during nuclear migration. *Dev. Biol.* 338:237–250. http://dx.doi.org/10.1016/j.ydbio.2009.12.004

Gomes, E.R., S. Jani, and G.G. Gundersen. 2005. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell*. 121:451–463. http://dx.doi.org/10.1016/j.cell.2005.02.022

Green, E.K., D. Grozeva, L. Forty, K. Gordon-Smith, E. Russell, A. Farmer, M. Hamshere, I.R. Jones, L. Jones, P. McGuffin, et al. 2013. Association at SYNE1 in both bipolar disorder and recurrent major depression. *Mol. Psychiatry*. 18:614–617. http://dx.doi.org/10.1038/mp.2012.48

Gros-Louis, F., N. Dupré, P. Dion, M.A. Fox, S. Laurent, S. Verreault, J.R. Sanes, J.P. Bouchard, and G.A. Rouleau. 2007. Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia. *Nat. Genet.* 39:80–85. http://dx.doi.org/10.1038/ng1927

Gudise, S., R.A. Figueroa, R. Lindberg, V. Larsson, and E. Hallberg. 2011. Samp1 is functionally associated with the LINC complex and A-type lamina networks. *J. Cell Sci.* 124:2077–2085. http://dx.doi.org/10.1242/jcs.078923

Guilluy, C., L.D. Osborne, L. Van Landeghem, L. Sharek, R. Superfine, R. Garcia-Mata, and K. Burridge. 2014. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nat. Cell Biol.* 16:376–381. http://dx.doi.org/10.1038/ncb2927

Gundersen, G.G., and H.J. Worman. 2013. Nuclear positioning. *Cell*. 152:1376–1389. http://dx.doi.org/10.1016/j.cell.2013.02.031

Haque, F., D.J. Lloyd, D.T. Smallwood, C.L. Dent, C.M. Shanahan, A.M. Fry, R.C. Trembath, and S. Shackleton. 2006. SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. *Mol. Cell. Biol.* 26:3738–3751. http://dx.doi.org/10.1128/MCB.26.10.3738-3751.2006

Haque, F., D. Mazzeo, J.T. Patel, D.T. Smallwood, J.A. Ellis, C.M. Shanahan, and S. Shackleton. 2010. Mammalian SUN protein interaction networks at the inner nuclear membrane and their role in laminopathy disease processes. *J. Biol. Chem.* 285:3487–3498. http://dx.doi.org/10.1074/jbc.M109.071910

Hasan, S., S. Güttinger, P. Mühlhäusser, F. Anderegg, S. Bürgler, and U. Kutay. 2006. Nuclear envelope localization of human UNC84A does not require nuclear lamins. *FEBS Lett.* 580:1263–1268. http://dx.doi.org/10.1016/j.febslet.2006.01.039

Hiraoka, Y., and A.F. Dernburg. 2009. The SUN rises on meiotic chromosome dynamics. *Dev. Cell*. 17:598–605. http://dx.doi.org/10.1016/j.devcel.2009.10.014

Hiraoka, Y., H. Maekawa, H. Asakawa, Y. Chikashige, T. Kojidani, H. Osakada, A. Matsuda, and T. Haraguchi. 2011. Inner nuclear membrane protein Ima1 is dispensable for intranuclear positioning of centromeres. *Genes Cells*. 16:1000–1011. http://dx.doi.org/10.1111/j.1365-2443.2011.01544.x

Horn, H.F., Z. Brownstein, D.R. Lenz, S. Shivatzki, A.A. Dror, O. Dagan-Rosenfeld, L.M. Friedman, K.J. Roux, S. Kozlov, K.T. Jeang, et al. 2013a. The LINC complex is essential for hearing. *J. Clin. Invest.* 123:740–750.

Horn, H.F., D.I. Kim, G.D. Wright, E.S. Wong, C.L. Stewart, B. Burke, and K.J. Roux. 2013b. A mammalian KASH domain protein coupling meiotic chromosomes to the cytoskeleton. *J. Cell Biol.* 202:1023–1039. http://dx.doi.org/10.1083/jcb.201304004

Isermann, P., and J. Lammerding. 2013. Nuclear mechanics and mechanotransduction in health and disease. *Curr. Biol.* 23:R1113–R1121. http://dx.doi.org/10.1016/j.cub.2013.11.009

Jahn, D., S. Schramm, R. Benavente, and M. Alsheimer. 2010. Dynamic properties of meiosis-specific lamin C2 and its impact on nuclear envelope integrity. *Nucleus*. 1:273–283. http://dx.doi.org/10.4161/nucl.11800

Jahn, D., S. Schramm, M. Schnölzer, C.J. Heilmann, C.G. de Koster, W. Schütz, R. Benavente, and M. Alsheimer. 2012. A truncated lamin A in the $Lmna^{-/-}$ mouse line: implications for the understanding of laminopathies. *Nucleus*. 3:463–474. http://dx.doi.org/10.4161/nucl.21676

Ketema, M., K. Wilhelmsen, I. Kuikman, H. Janssen, D. Hodzic, and A. Sonnenberg. 2007. Requirements for the localization of nesprin-3 at the nuclear envelope and its interaction with plectin. *J. Cell Sci.* 120:3384–3394. http://dx.doi.org/10.1242/jcs.014191

Kim, Y., A.A. Sharov, K. McDole, M. Cheng, H. Hao, C.M. Fan, N. Gaiano, M.S. Ko, and Y. Zheng. 2011. Mouse B-type lamins are required for proper organogenesis but not by embryonic stem cells. *Science*. 334:1706–1710. http://dx.doi.org/10.1126/science.1211222

King, M.C., T.G. Drivas, and G. Blobel. 2008. A network of nuclear envelope membrane proteins linking centromeres to microtubules. *Cell*. 134:427–438. http://dx.doi.org/10.1016/j.cell.2008.06.022

Koszul, R., K.P. Kim, M. Prentiss, N. Kleckner, and S. Kameoka. 2008. Meiotic chromosomes move by linkage to dynamic actin cables with transduction of force through the nuclear envelope. *Cell*. 133:1188–1201. http://dx.doi.org/10.1016/j.cell.2008.04.050

Kutscheidt, S., R. Zhu, S. Antoku, G.W. Luxton, I. Stagljar, O.T. Fackler, and G.G. Gundersen. 2014. FHOD1 interaction with nesprin-2G mediates TAN line formation and nuclear movement. *Nat. Cell Biol.* 16:708–715. http://dx.doi.org/10.1038/ncb2981

Lee, K.K., D. Starr, M. Cohen, J. Liu, M. Han, K.L. Wilson, and Y. Gruenbaum. 2002. Lamin-dependent localization of UNC-84, a protein required for nuclear migration in *Caenorhabditis elegans*. *Mol. Biol. Cell*. 13:892–901. http://dx.doi.org/10.1091/mbc.01-06-0294

Lei, K., X. Zhu, R. Xu, C. Shao, T. Xu, Y. Zhuang, and M. Han. 2012. Inner nuclear envelope proteins SUN1 and SUN2 play a prominent role in the DNA damage response. *Curr. Biol.* 22:1609–1615. http://dx.doi.org/10.1016/j.cub.2012.06.043

Link, J., D. Jahn, J. Schmitt, E. Göb, J. Baar, S. Ortega, R. Benavente, and M. Alsheimer. 2013. The meiotic nuclear lamina regulates chromosome dynamics and promotes efficient homologous recombination in the mouse. *PLoS Genet.* 9:e1003261. http://dx.doi.org/10.1371/journal.pgen.1003261

Liu, Q., N. Pante, T. Misteli, M. Elsagga, M. Crisp, D. Hodzic, B. Burke, and K.J. Roux. 2007. Functional association of Sun1 with nuclear pore complexes. *J. Cell Biol.* 178:785–798. http://dx.doi.org/10.1083/jcb.200704108

Lombardi, M.L., D.E. Jaalouk, C.M. Shanahan, B. Burke, K.J. Roux, and J. Lammerding. 2011. The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton. *J. Biol. Chem.* 286:26743–26753. http://dx.doi.org/10.1074/jbc.M111.233700

Lu, W., M. Schneider, S. Neumann, V.M. Jaeger, S. Taranum, M. Munck, S. Cartwright, C. Richardson, J. Carthew, K. Noh, et al. 2012. Nesprin interchain associations control nuclear size. *Cell. Mol. Life Sci.* 69:3493–3509. http://dx.doi.org/10.1007/s00018-012-1034-1

Luxton, G.W., E.R. Gomes, E.S. Folker, E. Vintinner, and G.G. Gundersen. 2010. Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. *Science*. 329:956–959. http://dx.doi.org/10.1126/science.1189072

Luxton, G.W., E.R. Gomes, E.S. Folker, H.J. Worman, and G.G. Gundersen. 2011. TAN lines: a novel nuclear envelope structure involved in nuclear positioning. *Nucleus*. 2:173–181. http://dx.doi.org/10.4161/nucl.2.3.16243

Malone, C.J., L. Misner, N. Le Bot, M.C. Tsai, J.M. Campbell, J. Ahringer, and J.G. White. 2003. The *C. elegans* hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. *Cell*. 115:825–836. http://dx.doi.org/10.1016/S0092-8674(03)00985-1

Maniotis, A.J., C.S. Chen, and D.E. Ingber. 1997. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA*. 94:849–854. http://dx.doi.org/10.1073/pnas.94.3.849

Markiewicz, E., K. Tilgner, N. Barker, M. van de Wetering, H. Clevers, M. Dorobek, I. Hausmanowa-Petrusewicz, F.C. Ramaekers, J.L. Broers, W.M. Blankesteijn, et al. 2006. The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. *EMBO J.* 25:3275–3285. http://dx.doi.org/10.1038/sj.emboj.7601230

Meinke, P., E. Mattioli, F. Haque, S. Antoku, M. Columbaro, K.R. Straatman, H.J. Worman, G.G. Gundersen, G. Lattanzi, M. Wehnert, and S. Shackleton. 2014. Muscular dystrophy-associated SUN1 and SUN2 variants disrupt nuclear-cytoskeletal connections and myonuclear organization. *PLoS Genet.* 10:e1004605. http://dx.doi.org/10.1371/journal.pgen.1004605

Meyerzon, M., H.N. Fridolfsson, N. Ly, F.J. McNally, and D.A. Starr. 2009. UNC-83 is a nuclear-specific cargo adaptor for kinesin-1-mediated nuclear migration. *Development*. 136:2725–2733. http://dx.doi.org/10.1242/dev.038596

Mislow, J.M., J.M. Holaska, M.S. Kim, K.K. Lee, M. Segura-Totten, K.L. Wilson, and E.M. McNally. 2002. Nesprin-1α self-associates and binds directly to emerin and lamin A in vitro. *FEBS Lett.* 525:135–140. http://dx.doi.org/10.1016/S0014-5793(02)03105-8

Morgan, J.T., E.R. Pfeiffer, T.L. Thirkill, P. Kumar, G. Peng, H.N. Fridolfsson, G.C. Douglas, D.A. Starr, and A.I. Barakat. 2011. Nesprin-3 regulates endothelial cell morphology, perinuclear cytoskeletal architecture, and flow-induced polarization. *Mol. Biol. Cell*. 22:4324–4334. http://dx.doi.org/10.1091/mbc.E11-04-0287

Morimoto, A., H. Shibuya, X. Zhu, J. Kim, K. Ishiguro, M. Han, and Y. Watanabe. 2012. A conserved KASH domain protein associates with telomeres, SUN1, and dynactin during mammalian meiosis. *J. Cell Biol.* 198:165–172. http://dx.doi.org/10.1083/jcb.201204085

Nagayama, K., S. Yamazaki, Y. Yahiro, and T. Matsumoto. 2014. Estimation of the mechanical connection between apical stress fibers and the nucleus in vascular smooth muscle cells cultured on a substrate. *J. Biomech.* 47:1422–1429. http://dx.doi.org/10.1016/j.jbiomech.2014.01.042

Neumann, S., M. Schneider, R.L. Daugherty, C.J. Gottardi, S.A. Eming, A. Beijer, A.A. Noegel, and I. Karakesisoglou. 2010. Nesprin-2 interacts with α-catenin and regulates Wnt signaling at the nuclear envelope. *J. Biol. Chem.* 285:34932–34938. http://dx.doi.org/10.1074/jbc.M110.119651

Niwa, O., M. Shimanuki, and F. Miki. 2000. Telomere-led bouquet formation facilitates homologous chromosome pairing and restricts ectopic interaction in fission yeast meiosis. *EMBO J.* 19:3831–3840. http://dx.doi.org/10.1093/emboj/19.14.3831

O'Roak, B.J., P. Deriziotis, C. Lee, L. Vives, J.J. Schwartz, S. Girirajan, E. Karakoc, A.P. Mackenzie, S.B. Ng, C. Baker, et al. 2011. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat. Genet.* 43:585–589. http://dx.doi.org/10.1038/ng.835

Östlund, C., E.S. Folker, J.C. Choi, E.R. Gomes, G.G. Gundersen, and H.J. Worman. 2009. Dynamics and molecular interactions of linker of nucleoskeleton and cytoskeleton (LINC) complex proteins. *J. Cell Sci.* 122:4099–4108. http://dx.doi.org/10.1242/jcs.057075

Oza, P., S.L. Jaspersen, A. Miele, J. Dekker, and C.L. Peterson. 2009. Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev.* 23:912–927. http://dx.doi.org/10.1101/gad.1782209

Padmakumar, V.C., T. Libotte, W. Lu, H. Zaim, S. Abraham, A.A. Noegel, J. Gotzmann, R. Foisner, and I. Karakesisoglou. 2005. The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J. Cell Sci.* 118:3419–3430. http://dx.doi.org/10.1242/jcs.02471

Pare, G.C., J.L. Easlick, J.M. Mislow, E.M. McNally, and M.S. Kapiloff. 2005. Nesprin-1α contributes to the targeting of mAKAP to the cardiac myocyte nuclear envelope. *Exp. Cell Res.* 303:388–399. http://dx.doi.org/10.1016/j.yexcr.2004.10.009

Penkner, A.M., A. Fridkin, J. Gloggnitzer, A. Baudrimont, T. Machacek, A. Woglar, E. Csaszar, P. Pasierbek, G. Ammerer, Y. Gruenbaum, and V. Jantsch. 2009. Meiotic chromosome homology search involves modifications of the nuclear envelope protein Matefin/SUN-1. *Cell*. 139:920–933. http://dx.doi.org/10.1016/j.cell.2009.10.045

Petrie, R.J., H. Koo, and K.M. Yamada. 2014. Generation of compartmentalized pressure by a nuclear piston governs cell motility in a 3D matrix. *Science*. 345:1062–1065. http://dx.doi.org/10.1126/science.1256965

Phillips, C.M., and A.F. Dernburg. 2006. A family of zinc-finger proteins is required for chromosome-specific pairing and synapsis during meiosis in *C. elegans*. *Dev. Cell*. 11:817–829. http://dx.doi.org/10.1016/j.devcel.2006.09.020

Puckelwartz, M.J., E.J. Kessler, G. Kim, M.M. Dewitt, Y. Zhang, J.U. Earley, F.F. Depreux, J. Holaska, S.K. Mewborn, P. Pytel, and E.M. McNally. 2010. Nesprin-1 mutations in human and murine cardiomyopathy. *J. Mol. Cell. Cardiol.* 48:600–608. http://dx.doi.org/10.1016/j.yjmcc.2009.11.006

Rajgor, D., J.A. Mellad, F. Autore, Q. Zhang, and C.M. Shanahan. 2012. Multiple novel nesprin-1 and nesprin-2 variants act as versatile tissue-specific intracellular scaffolds. *PLoS ONE*. 7:e40098. http://dx.doi.org/10.1371/journal.pone.0040098

Rajgor, D., J.A. Mellad, D. Soong, J.B. Rattner, M.J. Fritzler, and C.M. Shanahan. 2014. Mammalian microtubule P-body dynamics are mediated by nesprin-1. *J. Cell Biol.* 205:457–475. http://dx.doi.org/10.1083/jcb.201306076

Roux, K.J., M.L. Crisp, Q. Liu, D. Kim, S. Kozlov, C.L. Stewart, and B. Burke. 2009. Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization. *Proc. Natl. Acad. Sci. USA*. 106:2194–2199. http://dx.doi.org/10.1073/pnas.0808602106

Sato, A., B. Isaac, C.M. Phillips, R. Rillo, P.M. Carlton, D.J. Wynne, R.A. Kasad, and A.F. Dernburg. 2009. Cytoskeletal forces span the nuclear envelope to coordinate meiotic chromosome pairing and synapsis. *Cell*. 139:907–919. http://dx.doi.org/10.1016/j.cell.2009.10.039

Schmitt, J., R. Benavente, D. Hodzic, C. Höög, C.L. Stewart, and M. Alsheimer. 2007. Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope. *Proc. Natl. Acad. Sci. USA*. 104:7426–7431. http://dx.doi.org/10.1073/pnas.0609198104

Schneider, M., W. Lu, S. Neumann, A. Brachner, J. Gotzmann, A.A. Noegel, and I. Karakesisoglou. 2011. Molecular mechanisms of centrosome and cytoskeleton anchorage at the nuclear envelope. *Cell. Mol. Life Sci.* 68:1593–1610. http://dx.doi.org/10.1007/s00018-010-0535-z

Schoppmann, S.F., U. Vinatzer, N. Popitsch, M. Mittlböck, S. Liebmann-Reindl, G. Jomrich, B. Streubel, and P. Birner. 2013. Novel clinically relevant genes in gastrointestinal stromal tumors identified by exome sequencing. *Clin. Cancer Res.* 19:5329–5339. http://dx.doi.org/10.1158/1078-0432.CCR-12-3863

Shibuya, H., and Y. Watanabe. 2014. The meiosis-specific modification of mammalian telomeres. *Cell Cycle*. 13:2024–2028. http://dx.doi.org/10.4161/cc.29350

Shimanuki, M., F. Miki, D.-Q. Ding, Y. Chikashige, Y. Hiraoka, T. Horio, and O. Niwa. 1997. A novel fission yeast gene, kms1$^+$, is required for the formation of meiotic prophase-specific nuclear architecture. *Mol. Gen. Genet.* 254:238–249. http://dx.doi.org/10.1007/s004380050412

Simpson, J.G., and R.G. Roberts. 2008. Patterns of evolutionary conservation in the nesprin genes highlight probable functionally important protein domains and isoforms. *Biochem. Soc. Trans.* 36:1359–1367. http://dx.doi.org/10.1042/BST0361359

Sjöblom, T., S. Jones, L.D. Wood, D.W. Parsons, J. Lin, T.D. Barber, D. Mandelker, R.J. Leary, J. Ptak, N. Silliman, et al. 2006. The consensus coding sequences of human breast and colorectal cancers. *Science*. 314:268–274. http://dx.doi.org/10.1126/science.1133427

Sosa, B.A., A. Rothballer, U. Kutay, and T.U. Schwartz. 2012. LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins. *Cell*. 149:1035–1047. http://dx.doi.org/10.1016/j.cell.2012.03.046

Sosa, B.A., U. Kutay, and T.U. Schwartz. 2013. Structural insights into LINC complexes. *Curr. Opin. Struct. Biol.* 23:285–291. http://dx.doi.org/10.1016/j.sbi.2013.03.005

Starr, D.A., and M. Han. 2002. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. *Science*. 298:406–409. http://dx.doi.org/10.1126/science.1075119

Starr, D.A., and H.N. Fridolfsson. 2010. Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. *Annu. Rev. Cell Dev. Biol.* 26:421–444. http://dx.doi.org/10.1146/annurev-cellbio-100109-104037

Sullivan, T., D. Escalante-Alcalde, H. Bhatt, M. Anver, N. Bhat, K. Nagashima, C.L. Stewart, and B. Burke. 1999. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* 147:913–920. http://dx.doi.org/10.1083/jcb.147.5.913

Sur, I., S. Neumann, and A.A. Noegel. 2014. Nesprin-1 role in DNA damage response. *Nucleus*. 5:173–191. http://dx.doi.org/10.4161/nucl.29023

Swartz, R.K., E.C. Rodriguez, and M.C. King. 2014. A role for nuclear envelope-bridging complexes in homology-directed repair. *Mol. Biol. Cell*. 25:2461–2471. http://dx.doi.org/10.1091/mbc.E13-10-0569

Talamas, J.A., and M.W. Hetzer. 2011. POM121 and Sun1 play a role in early steps of interphase NPC assembly. *J. Cell Biol.* 194:27–37. http://dx.doi.org/10.1083/jcb.201012154

Tamura, K., K. Iwabuchi, Y. Fukao, M. Kondo, K. Okamoto, H. Ueda, M. Nishimura, and I. Hara-Nishimura. 2013. Myosin XI-i links the nuclear membrane to the cytoskeleton to control nuclear movement and shape in *Arabidopsis*. *Curr. Biol.* 23:1776–1781. http://dx.doi.org/10.1016/j.cub.2013.07.035

Taranum, S., E. Vaylann, P. Meinke, S. Abraham, L. Yang, S. Neumann, I. Karakesisoglou, M. Wehnert, and A.A. Noegel. 2012. LINC complex alterations in DMD and EDMD/CMT fibroblasts. *Eur. J. Cell Biol.* 91:614–628. http://dx.doi.org/10.1016/j.ejcb.2012.03.003

Tikhonenko, I., V. Magidson, R. Gräf, A. Khodjakov, and M.P. Koonce. 2013. A kinesin-mediated mechanism that couples centrosomes to nuclei. *Cell. Mol. Life Sci.* 70:1285–1296. http://dx.doi.org/10.1007/s00018-012-1205-0

Tulgren, E.D., S.M. Turgeon, K.J. Opperman, and B. Grill. 2014. The Nesprin family member ANC-1 regulates synapse formation and axon termination by functioning in a pathway with RPM-1 and β-Catenin. *PLoS Genet.* 10:e1004481. http://dx.doi.org/10.1371/journal.pgen.1004481

Turgay, Y., L. Champion, C. Balazs, M. Held, A. Toso, D.W. Gerlich, P. Meraldi, and U. Kutay. 2014. SUN proteins facilitate the removal of membranes from chromatin during nuclear envelope breakdown. *J. Cell Biol.* 204:1099–1109. http://dx.doi.org/10.1083/jcb.201310116

Wanat, J.J., K.P. Kim, R. Koszul, S. Zanders, B. Weiner, N. Kleckner, and E. Alani. 2008. Csm4, in collaboration with Ndj1, mediates telomere-led chromosome dynamics and recombination during yeast meiosis. *PLoS Genet.* 4:e1000188. http://dx.doi.org/10.1371/journal.pgen.1000188

Wang, W., Z. Shi, S. Jiao, C. Chen, H. Wang, G. Liu, Q. Wang, Y. Zhao, M.I. Greene, and Z. Zhou. 2012. Structural insights into SUN-KASH complexes across the nuclear envelope. *Cell Res.* 22:1440–1452. http://dx.doi.org/10.1038/cr.2012.126

Warren, D.T., T. Tajsic, J.A. Mellad, R. Searles, Q. Zhang, and C.M. Shanahan. 2010. Novel nuclear nesprin-2 variants tether active extracellular signal-regulated MAPK1 and MAPK2 at promyelocytic leukemia protein nuclear bodies and act to regulate smooth muscle cell proliferation. *J. Biol. Chem.* 285:1311–1320. http://dx.doi.org/10.1074/jbc.M109.032557

Wilhelmsen, K., S.H. Litjens, I. Kuikman, N. Tshimbalanga, H. Janssen, I. van den Bout, K. Raymond, and A. Sonnenberg. 2005. Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. *J. Cell Biol.* 171:799–810. http://dx.doi.org/10.1083/jcb.200506083

Worman, H.J. 2012. Nuclear lamins and laminopathies. *J. Pathol.* 226:316–325. http://dx.doi.org/10.1002/path.2999

Wynne, D.J., O. Rog, P.M. Carlton, and A.F. Dernburg. 2012. Dynein-dependent processive chromosome motions promote homologous pairing in *C. elegans* meiosis. *J. Cell Biol.* 196:47–64. http://dx.doi.org/10.1083/jcb.201106022

Yoshida, M., S. Katsuyama, K. Tateho, H. Nakamura, J. Miyoshi, T. Ohba, H. Matsuhara, F. Miki, K. Okazaki, T. Haraguchi, et al. 2013. Microtubule-organizing center formation at telomeres induces meiotic telomere clustering. *J. Cell Biol.* 200:385–395. http://dx.doi.org/10.1083/jcb.201207168

Young, K.G., and R. Kothary. 2008. Dystonin/Bpag1 is a necessary endoplasmic reticulum/nuclear envelope protein in sensory neurons. *Exp. Cell Res.* 314:2750–2761. http://dx.doi.org/10.1016/j.yexcr.2008.06.021

Yu, J., K. Lei, M. Zhou, C.M. Craft, G. Xu, T. Xu, Y. Zhuang, R. Xu, and M. Han. 2011. KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development. *Hum. Mol. Genet.* 20:1061–1073. http://dx.doi.org/10.1093/hmg/ddq549

Yu, T.W., M.H. Chahrour, M.E. Coulter, S. Jiralerspong, K. Okamura-Ikeda, B. Ataman, K. Schmitz-Abe, D.A. Harmin, M. Adli, A.N. Malik, et al. 2013. Using whole-exome sequencing to identify inherited causes of autism. *Neuron*. 77:259–273. http://dx.doi.org/10.1016/j.neuron.2012.11.002

Zhang, Q., J.N. Skepper, F. Yang, J.D. Davies, L. Hegyi, R.G. Roberts, P.L. Weissberg, J.A. Ellis, and C.M. Shanahan. 2001. Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues. *J. Cell Sci.* 114:4485–4498.

Zhang, Q., C.D. Ragnauth, J.N. Skepper, N.F. Worth, D.T. Warren, R.G. Roberts, P.L. Weissberg, J.A. Ellis, and C.M. Shanahan. 2005. Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle. *J. Cell Sci.* 118:673–687. http://dx.doi.org/10.1242/jcs.01642

Zhang, Q., C. Bethmann, N.F. Worth, J.D. Davies, C. Wasner, A. Feuer, C.D. Ragnauth, Q. Yi, J.A. Mellad, D.T. Warren, et al. 2007. Nesprin-1 and -2 are involved in the pathogenesis of Emery Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum. Mol. Genet.* 16:2816–2833. http://dx.doi.org/10.1093/hmg/ddm238

Zhang, X., K. Lei, X. Yuan, X. Wu, Y. Zhuang, T. Xu, R. Xu, and M. Han. 2009. SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron*. 64:173–187. http://dx.doi.org/10.1016/j.neuron.2009.08.018

Zhou, X., K. Graumann, D.E. Evans, and I. Meier. 2012a. Novel plant SUN-KASH bridges are involved in RanGAP anchoring and nuclear shape determination. *J. Cell Biol.* 196:203–211. http://dx.doi.org/10.1083/jcb.201108098

Zhou, Z., X. Du, Z. Cai, X. Song, H. Zhang, T. Mizuno, E. Suzuki, M.R. Yee, A. Berezov, R. Murali, et al. 2012b. Structure of Sad1-UNC84 homology (SUN) domain defines features of molecular bridge in nuclear envelope. *J. Biol. Chem.* 287:5317–5326. http://dx.doi.org/10.1074/jbc.M111.304543

Zhou, X., K. Graumann, L. Wirthmueller, J.D. Jones, and I. Meier. 2014. Identification of unique SUN-interacting nuclear envelope proteins with diverse functions in plants. *J. Cell Biol.* 205:677–692. http://dx.doi.org/10.1083/jcb.201401138