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REVIEW

SUBJECT COLLECTION: EXPLORING THE NUCLEUS

Molecular models of LINC complex assembly at the nuclear envelope

Zeinab Jahed[1,2,*], Nya Domkam[3], Jessica Ornowski[3], Ghafar Yerima[3] and Mohammad R. K. Mofrad[3,4,*]

ABSTRACT

Large protein complexes assemble at the nuclear envelope to transmit mechanical signals between the cytoskeleton and nucleoskeleton. These protein complexes are known as the linkers of the nucleoskeleton and cytoskeleton complexes (LINC complexes) and are formed by the interaction of SUN and KASH domain proteins in the nuclear envelope. Ample evidence suggests that SUN–KASH complexes form higher-order assemblies to withstand and transfer forces across the nuclear envelope. Herein, we present a review of recent studies over the past few years that have shed light on the mechanisms of SUN–KASH interactions, their higher order assembly, and the molecular mechanisms of force transfer across these complexes.

KEY WORDS: Higher-order assembly, LINC complex, Mechanotransduction, Nuclear envelope, Nuclear mechanics, SUN

Introduction

The nuclear envelope (NE) consists of inner and outer nuclear membranes (INM and ONM), which act as a barrier between the genetic information inside the nucleus and other cell organelles (Fig. 1A). The discovery of conserved interactions between the SUN and KASH protein families, one anchored to the inner and the other to the outer nuclear membrane, revealed that the nuclear membranes are physically coupled (Haque et al., 2006; McGee et al., 2006; Crisp et al., 2006; Starr et al., 2001; Malone et al., 2003, 1999). SUN proteins interact with various elements of the nucleoskeleton, whereas KASH proteins bind to various cytoskeletal proteins through their large cytoplasmic domains. The transduction of physical signals between the cytoskeleton and nucleoskeleton ultimately depends on the molecular interactions between a short conserved KASH domain and the conserved SUN domain in the NE. Owing to their important roles in physically connecting the cytoplasm to the nucleus, SUN and KASH domain proteins are known as the main components of linkers of the nucleoskeleton and cytoskeleton complexes (LINC complexes) (Jahed and Mofrad, 2018; Luxton and Starr, 2014; Hao and Starr, 2019).

Here, we review the most recent advances in our understanding of how LINC complexes assemble in the NE to mediate force transfer. We will begin by introducing various SUN and KASH interacting pairs. Next, we will highlight current research on how different SUN and KASH protein pairs interact and transfer force at the molecular level. Finally, we will discuss proposed models for the higher-order assembly of LINC complexes in the NE to maximize the transfer of forces.

SUN and KASH protein families

At least five SUN proteins (SUN1 to SUN5; SUN4 is also known as SPAG4) and six KASH proteins [nuclear envelope spectrin repeat protein 1 to 4 (nesprin-1 to -4; symbols SYNE1–SYNE4), KASH5 and lymphoid-restricted membrane protein (LRMP; also known as IRAG2)] have been identified in mammals to date. SUN and KASH proteins are expressed in a tissue-specific manner, and their protein-encoding genes are subject to extensive alternative splicing (Katta et al., 2014; Jahed et al., 2016; Razafsky and Hodzic, 2009; Jahed and Mofrad, 2018; Jahed et al., 2014). SUN1 and SUN2 are expressed in a wide variety of cell types and bind at least four KASH proteins (nesprin-1 to -4) (Malone et al., 1999; McGee et al., 2006; Haque et al., 2006; Sosa et al., 2012; Crisp et al., 2006; Malone et al., 2003; Starr et al., 2001). SUN1 and SUN2 have shown partial functional redundancy in several cellular processes (see Jahed et al., 2018a,b for a detailed structural and functional comparison between SUN1 and SUN2). The other SUN proteins, SUN3, SUN4 and SUN5, are thought to be restricted to testis-specific cells (Malone et al., 1999; McGee et al., 2006; Haque et al., 2006; Crisp et al., 2006; Calvi et al., 2015; Gao et al., 2020; Sosa et al., 2012). The SUN domain of all SUN proteins resides in the space between the INM and ONM, known as the perinuclear space, where it interacts with the KASH domain (Fig. 1A). In addition, large domains of SUN proteins with a predicted coiled-coil structure span the NE and reach the INM where these proteins are anchored. The nucleoplasmic domains of SUN proteins interact with elements of the nucleoskeleton, and many of them directly interact with lamins, chromatin and other INM proteins through their N-terminus (Chang et al., 2015; Crisp et al., 2006; Luxton and Starr, 2014; Tapley et al., 2011; Bone et al., 2014; Fridolfsson et al., 2010).

In comparison, KASH proteins are tail-anchored to the ONM, and their C-terminus resides in the perinuclear space, where they interact with SUN proteins. Despite their large cytoplasmic domains, the luminal KASH domain is much shorter than the SUN domain with only 8 to 30 residues in the perinuclear space (Sosa et al., 2013). Currently six KASH proteins are recognized in humans (Jahed et al., 2016; Rothballer and Kutay, 2013b). Nesprin-1 and nesprin-2 are abundant in mammals (Lombardi et al., 2011; Hale et al., 2008; Rothballer and Kutay, 2013a; Haque et al., 2010). Several studies have shown that RNA splicing can generate multiple isoforms of nesprin-1 and nesprin-2 proteins (Rajgor et al., 2012; Rajgor and Shanahan, 2013). Specific isoforms of nesprin-1 and nesprin-2 contain a calponin homology domain, which binds directly to the actin cytoskeleton. However, several other cell types express nesprin-1 and nesprin-2 isoforms that lack this domain and hence lack the ability to bind actin (Zhang et al., 2002, 2007; Crisp

[1]Department of Chemistry, Stanford University, Stanford, CA 94305, USA. [2]Department of Nanoengineering, Jacobs School of Engineering, University of California, San Diego, CA 92039, USA. [3]Molecular Cell Biomechanics Laboratory, Departments of Bioengineering and Mechanical Engineering, University of California, Berkeley, CA 94720, USA. [4]Molecular Biophysics and Integrative Bioimaging Division, Lawrence Berkeley National Lab, Berkeley, CA 94720, USA.

*Authors for correspondence (mofrad@berkeley.edu; zjahed@ucsd.edu)

M.R.K.M., 0000-0001-7004-4859

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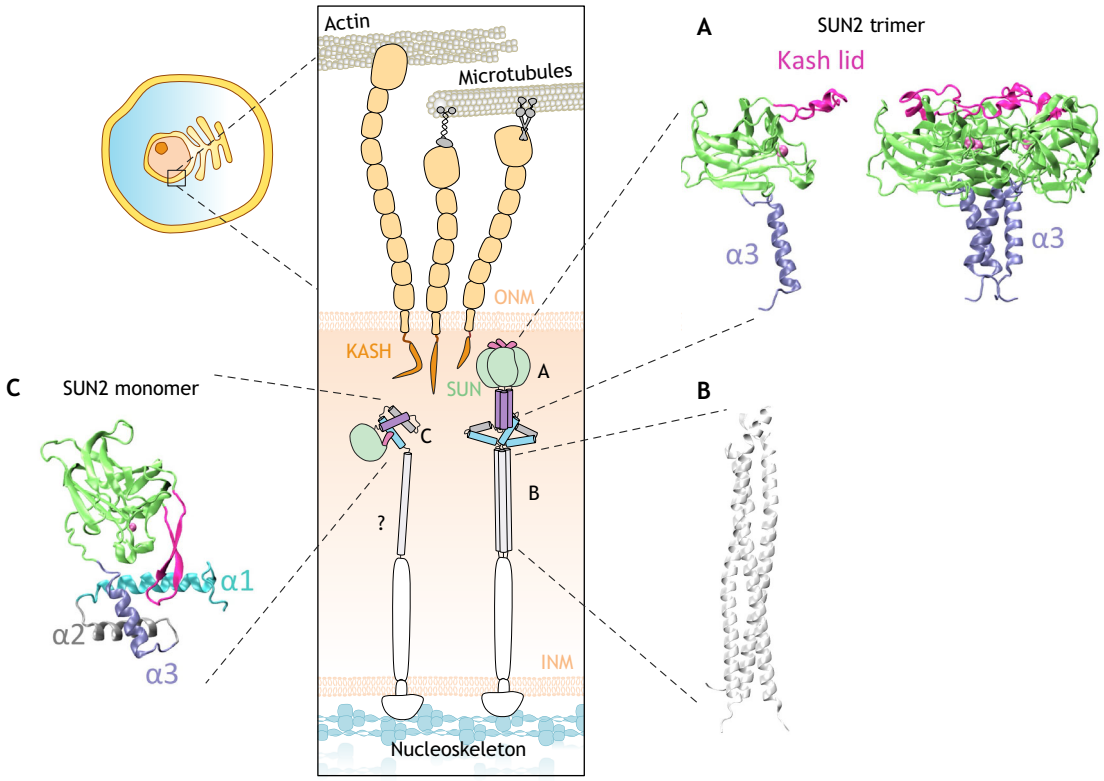

Fig. 1. LINC complexes at the nuclear envelope. In the middle, an overview of how SUN and KASH domains meet inside the perinuclear space between the inner and outer nuclear membrane (INM and ONM) is shown. The cytoplasmic domains of KASH-domain-containing proteins bind to the actin and microtubule cytoskeleton. (A) Crystal structures of the conserved SUN domain protomer (left) of a SUN trimer (right) (PDB ID: 4DXT). The main KASH-binding site on SUN is the KASH-lid shown in magenta. (B) Crystal structure of trimeric coiled coil domains of SUN2 (PDB ID: 5ED9). (C) Crystal structure of auto-inhibited SUN2 (PDB ID: 5ED8). In this structure the KASH-lid is bound between the α1, α2 and α3 helices. Adapted from Jahed et al. (2018b).

et al., 2006; Rajgor et al., 2012; Rajgor and Shanahan, 2013; Wilson and Holzbaur, 2015; Schneider et al., 2011). Additionally, some nesprin-2 isoforms may contain spectrin repeats, which mediate interactions with microtubules (Wilson and Holzbaur, 2015; Schneider et al., 2011). The third nesprin protein, nesprin-3, is smaller than most nesprin-1 and nesprin-2 isoforms and can bind to actin and intermediate filaments (Wilhelmsen, 2005; Ketema et al., 2007). Nesprin-4 is less common as it is found in a limited number of cell types, and it binds to microtubules through a kinesin protein (Roux et al., 2009). The fifth KASH protein is simply named KASH5 and is germ-cell specific (Horn et al., 2013; Morimoto et al., 2012). Another protein that has a domain with a sequence similar to that of KASH domains was identified as LRMP (Behrens et al., 1994; Shindo et al., 2010). One recent study suggests that LRMP plays an important role in positioning the nucleus by interacting with SUN proteins and microtubules (Kozono et al., 2018).

It is now widely accepted that SUN proteins form oligomers in the NE (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012). Crystal structures of different fragments of SUN2 and SUN1 have revealed that these proteins can at least form trimers *in vitro* (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012) (Fig. 1A,B). The conserved SUN domain of SUN2 consists of a protruding 'KASH-lid' that binds KASH proteins (Fig. 1A). Three α-helices of neighboring SUN2 protomers (α3) form a coiled-coil that precedes the SUN domain (Sosa et al., 2012). Crystal structures of other trimeric coiled-coil domains have also been solved for SUN2 (Fig. 1B) (Nie et al., 2016). Some studies suggest that α3 engages with two other α-helices (α1 and α2) to keep SUN1 and SUN2 proteins in an autoinhibited monomeric state that is unable to bind KASH proteins (Fig. 1C) (Jahed et al., 2018b; Nie et al., 2016; Xu et al., 2018). Although the mechanisms of activation remain unknown, several studies have shown that SUN1 and SUN2 must be activated (i.e. must oligomerize) to bind to KASH, as discussed below.

The interactions between various SUN and KASH pairs provides a physical linkage between the cytoskeleton and nucleoskeleton, and allows the direct transfer of mechanical forces across the NE. In the next section, we will discuss the molecular features of SUN–KASH complexes.

Molecular features of SUN–KASH pairs

The first clues into the molecular mechanisms of force transfer across the LINC complex were revealed when the crystal structure of the conserved SUN2–KASH1 and SUN2–KASH2 (where KASH1 and KASH2 refer to the KASH domains of nesprin-1 and -2) interaction was solved in 2012 (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012). The details of this interaction have since been reviewed in detail (Sosa et al., 2013; Jahed and Mofrad, 2018; Hao and Starr, 2019). Briefly, it was shown that SUN2 forms trimers that can bind to three KASH peptides simultaneously, forming an overall hexameric complex (Sosa et al., 2012) (Fig. 2A). In these complexes, each KASH peptide is sandwiched between two neighboring SUN protomers. In the SUN2–KASH2 complex, the KASH2 peptide interacts with a protruding KASH lid on one SUN2 protomer (residues 0 to −17) and the globular core of a neighboring

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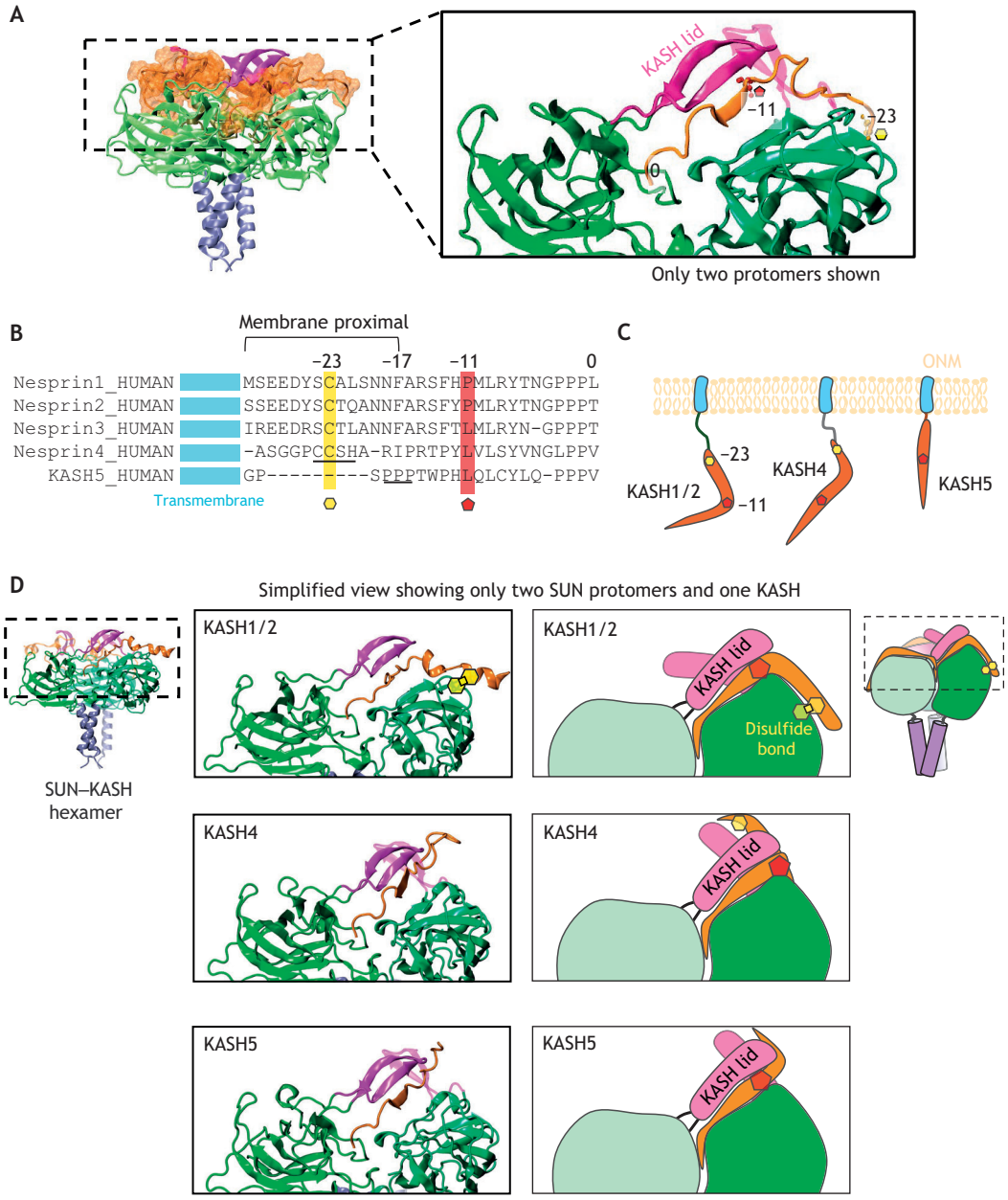

Fig. 2. The SUN–KASH interaction. (A) Crystal structure of the SUN2–KASH2 hexamer (PDB ID: 4DXS; KASH peptides are shown in orange). The simplified zoomed view of the interaction shows only one interacting unit (between one KASH and two SUN protomers) for clarity. (B) Sequence alignment between the transmembrane and luminal KASH domains of different human KASH-domain-containing proteins. Position 0 represents the C terminus of the KASH domain, which resides in the perinuclear space. Position −11 is occupied by a proline residue in the KASH domain of nesprin-1 (KASH1) and KASH2, and a leucine residue in KASH3, KASH4 and KASH5 (red pentagon). KASH1–KASH4 contain a cysteine residue in position −23 (yellow hexagon). KASH4 and KASH5 contain unique CCSH and PPP domains, respectively (underlined). (C) Schematic representation of KASH domains in the perinuclear space. The proline in position −11 generates a 90° kink in KASH1 and KASH2 (KASH1/2), which is not seen in the solved structures of the KASH3–KASH5 domains. (D) The KASH domains of KASH1 or KASH2, KASH4 and KASH5 bind distinctly to SUN protomers. The interaction between KASH1 or KASH2 (KASH1/2) with SUN1 or SUN2 terminates at an intermolecular disulfide bond formed by a cysteine residue at position −23 (yellow hexagon). This disulfide bond is missing in KASH4 despite the conservation of this cysteine, as well as in KASH5, owing to the lack of this residue. The state of the cysteine residue at –23 in KASH3 has not been determined.

SUN2 protomer [residues −18 to −23; note that the negative numbering of KASH peptides was originally proposed (Sosa et al., 2012, 2013) with the argument that since all KASH domains identified to date are found at the very C terminus of proteins, the KASH domain is numbered starting with 0 as the C terminus (last residue of the protein), followed by negative numbers up to the transmembrane domain] (Fig. 2A). By using molecular dynamics simulations, we showed that the SUN2–KASH2 hexameric complex is extremely stable under mechanical forces and forces on KASH2 peptides are directly transferred to the coiled coil regions of SUN2 (Jahed et al., 2015; Cain et al., 2018).

Most molecular studies in the past few years focused on the two major KASH proteins, KASH1 and KASH2, and the molecular mechanisms of force transfer across other KASH proteins [i.e. KASH3, KASH4 (KASH domains of nesprin-3 and -4) and KASH5] have not yet been explored. Despite the presence of some

conserved SUN-binding residues, the domains in KASH1 to KASH5 exhibit key differences in their lengths and sequences (Fig. 2B). In a recent study, we showed that swapping the KASH domains or shortening the length of KASH domains affects LINC-complex-dependent nuclear anchorage in *Caenorhabditis elegans* (Jahed et al., 2019). Additionally, through molecular dynamics simulations, we showed that shortening the length of KASH2 peptides to that of KASH5 results in lower force transfer across the complex (Jahed et al., 2019). More recently, the structures of SUN1 in complex with KASH4 and KASH5 were also solved, revealing distinct binding modes for these compared with the complex containing KASH1 or KASH2 (Gurusaran and Davies, 2021). A second independent study solved the structure of SUN2 bound to KASH3, KASH4 and KASH5 peptides (Cruz et al., 2020).

Based on these studies, several key differences have been identified between the different KASH proteins, which suggest that they adopt distinct mechanisms to stabilize their interactions with SUN proteins for maximal force transfer as discussed below.

First, two interesting residues that distinguish the binding modes of KASH1 and KASH2 from that of KASH3, KASH4 and KASH5 are the proline at position −11 and the cysteine residue at position −23 (Fig. 2B,C). The proline at −11 in KASH1 and KASH2 allows these KASH proteins to form a kink, so the residues following −11 (residues −11 to −23) can interact with the globular core of the neighboring SUN protomer (Fig. 2D) (Cruz et al., 2020; Gurusaran and Davies, 2021). The interaction of KASH1 and KASH2 with the globular core of SUN ends with a cysteine residue at position −23, which can form an intermolecular disulfide bond with a perfectly positioned cysteine residue in the globular core of SUN2 (Fig. 2D). Using molecular dynamics simulations, we showed that this intermolecular disulfide can mediate maximal force transfer across the SUN2–KASH2 complex (Jahed et al., 2015; Cain et al., 2018). Interestingly, although the cysteine at position −23 is conserved in KASH4, replacing the proline at position −11 with a leucine moves KASH4 away from the globular core of the neighboring SUN protomer, therefore inhibiting the intermolecular disulfide formation (Fig. 2D) (Cruz et al., 2020; Gurusaran and Davies, 2021). Instead, in KASH4, the cysteine at position −23 is involved in the coordination of an ion as discussed in the following sections. Furthermore, instead of interacting with the globular core, the remaining residues following position −11 (residues −11 to −23) in KASH4 interact with the top surface of the neighboring KASH-lid (Fig. 2D) (Cruz et al., 2020). Finally, KASH5, which is much shorter than the other KASH proteins, also contains a proline at position −11, but lacks the cysteine at position −23 (Fig. 2D). Similar to KASH4, KASH5 does not kink to bind to the core of the neighboring SUN protomer and binds to the neighboring KASH-lid instead (Fig. 2D); it also does not contain a cysteine to form a disulfide bond with the SUN protein (Cruz et al., 2020; Gurusaran and Davies, 2021).

Our molecular dynamics simulations showed that the lack of the disulfide bond between SUN2 and KASH2 (Jahed et al., 2015; Cain et al., 2018), or the shortening of KASH2 (Jahed et al., 2019), would interfere with force transfer across that SUN–KASH complex. However, based on the recently revealed binding modes of KASH4 and KASH5 as discussed above, these KASH proteins appear to adopt alternative mechanisms for stabilizing the SUN–KASH interaction and maximizing force transfer (Cruz et al., 2020; Gurusaran and Davies, 2021).

Second, a new model for LINC complex assembly was put forward positing that two SUN–KASH hexamers interact head-on to form a dodecameric complex consisting of a 6:6 ratio of SUN and KASH (Gurusaran and Davies, 2021). Previously solved structures of the Apo (unbound) and KASH-bound SUN2, as well as docking models of SUN1 and SUN2 had also shown a head-on interaction between two SUN–KASH hexamers where the ONM-facing sides interact (Wang et al., 2012; Jahed et al., 2018a,b). However, until now, these interactions had mostly been dismissed as physiologically irrelevant crystal contacts, owing to poorly packing in the case of the SUN2–KASH2 and SUN2–KASH1 complexes (Sosa et al., 2012; Wang et al., 2012; Zhou et al., 2012; Cruz et al., 2020). However, the authors of the recent study observed that SUN1 forms 6:6 complexes with KASH1, KASH 4 and KASH 5 (Fig. 3A–C), all containing conserved interactions that stabilize the head-on hexamer-hexamer interactions (Gurusaran and Davies, 2021). In the case of the SUN1–KASH1 and SUN1–KASH2 6:6 complexes, the KASH-lids of the opposing hexamers are the only interacting regions, and the hexamers pack rather poorly in the head-on interaction (Cruz et al., 2020) (Fig. 3A). SUN1–KASH4 is the more interesting complex of the three structures. As mentioned previously, KASH4 does not kink towards the globular core of the neighboring SUN1 protein to form a disulfide bond at C-23 and instead interacts with the ONM-facing, top region of the neighboring KASH-lid (Gurusaran and Davies, 2021) (Fig. 2D). The KASH lids of SUN1 are therefore not involved in the head-on interactions in the SUN1–KASH4 complex in contrast to what is found for the SUN1–KASH1 and SUN1–KASH2 complexes. Instead of the KASH lids, in the 6:6 complex, the three KASH4 peptides contribute to the head-on interaction and coordinate a Zn^{2+} ion between a CCSH motif (underlined in Fig. 2B), which comprises the cysteine residues at position −23 on pairs of opposing KASH4 peptides (Fig. 3B). Together, the KASH4 peptides coordinate three Zn^{2+} ions between the SUN1–KASH4 hexamers, which stabilizes this dodecameric complex (Fig. 3B). The SUN1–KASH5 complex also shows extensive head-on interactions that are mediated by interactions between a PPP motif (underlined in Fig. 2B) on opposing KASH5 peptides (Gurusaran and Davies, 2021) (Fig. 3C). Through the head-on interactions, the SUN1–KASH4 and SUN1–KASH5 complexes can become dodecameric complexes with a 6:6 ratio of SUN and KASH proteins, which are higher-order oligomers compared with the hexameric complexes consisting of 3:3 ratio of SUN and KASH. The higher-order head-on interactions in the SUN1–KASH4 and SUN1–KASH5 complexes may be a distinct mechanism through which these complexes withstand and transmit higher forces, while they lack the intermolecular disulfide bonds found in the complexes formed between SUN1 or SUN2 and KASH1 or KASH2.

Cruz et al. show that the Apo SUN2 trimers can also interact head-on, resulting in a significant change in the conformations of the KASH-lids in that several hydrophobic residues are buried to generate a stable interacting surface between two opposite SUN2 trimers (Cruz et al., 2020).

Taken together, the above studies determined the molecular details of local interactions between SUN and KASH domains *in vitro*. However, the oligomer state and orientation of SUN and KASH complexes *in vivo*, in the NE, remains unknown. Based on these findings, in the following section, we discuss the likely models of LINC assembly and their higher-order oligomerization in the NE of living cells.

Current models of LINC complex assembly in the NE

Linear SUN-trimer model

Since their discovery, the most widely accepted model of LINC complex interactions in the NE has been one in which a SUN trimer is

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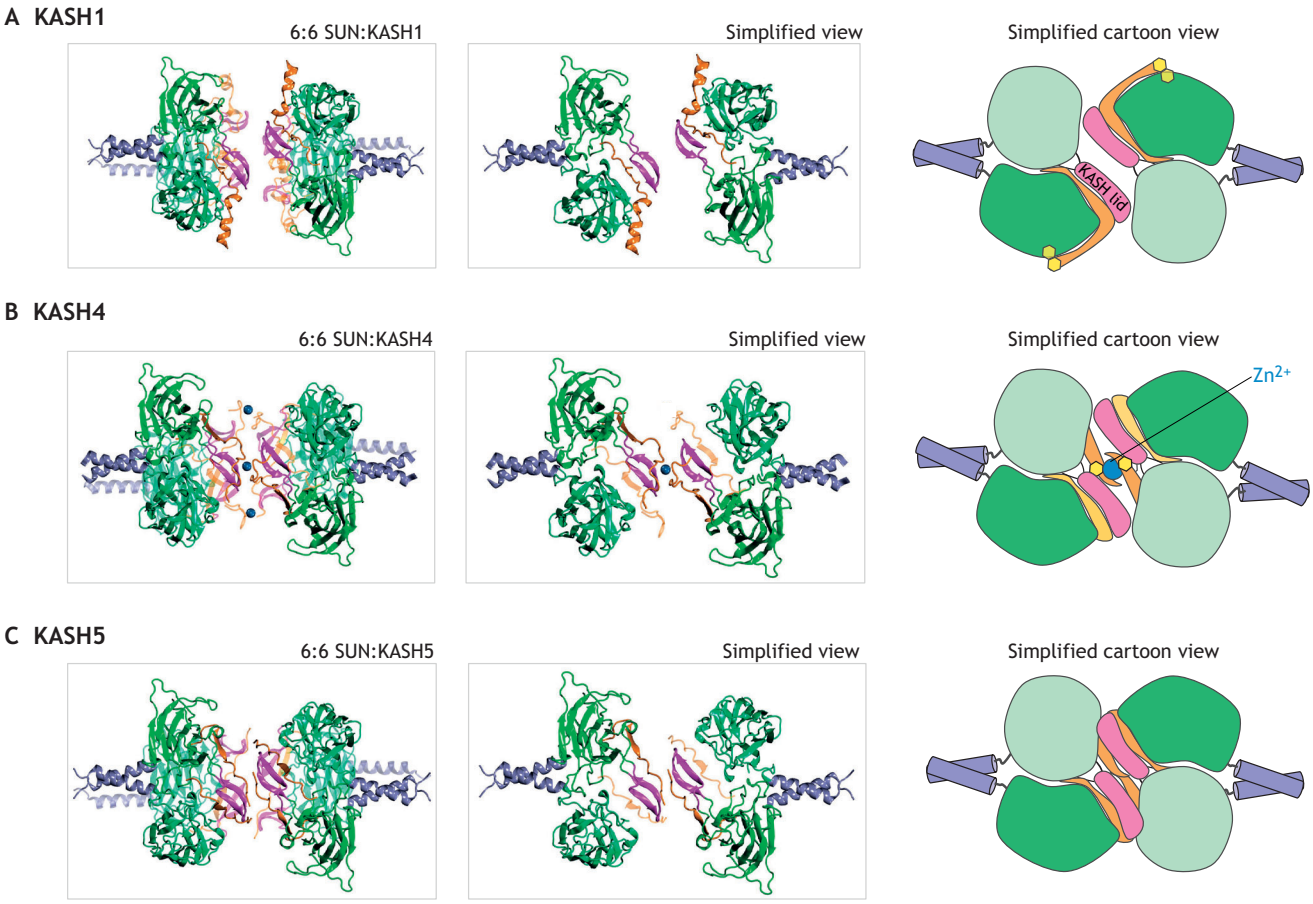

Fig. 3. SUN–KASH hexamers can interact head-to-head to form 6:6 SUN–KASH assemblies. (A) Crystal structure of the SUN1–KASH1 dodecamer (PDB ID: 6R15) consisting of two SUN1 trimers and six KASH1 peptides (left). A simplified view is presented in the middle, showing only two SUN protomers (of a SUN trimer) and one KASH interacting head-to-head. A cartoon view of the simplified head-to-head interaction is shown on the right. There is a small head-to-head interaction in the case of the SUN1–KASH1 complex. The SUN1–KASH2 and SUN3–KASH2 complexes might also adopt a similar structure to SUN1–KASH1. (B) Dodecameric SUN1–KASH4 complex (PDB ID: 6R16). Each dodecamer can coordinate three Zn^{2+} ions through head-to-head interactions of KASH peptides on opposing hexamers. (C) In the SUN1–KASH5 complex (PDB ID: 6R2I), the SUN1 and KASH5 hexamers also packs tightly through the interactions of opposing KASH proteins.

oriented towards the ONM where it binds to ONM-anchored KASH protein (Fig. 1 and Fig. 4A). Because SUN-domain proteins consist of several coiled coil regions between their transmembrane domain at the INM and their SUN domain, this model allows them to linearly span the NE and reach the short KASH domain at the ONM. Evidence of a monomeric auto-inhibited state, in which the KASH-lid is bound between an α-helix bundle formed by α1, α2 and α3, has been shown *in vitro* for SUN1 and SUN2 (Jahed et al., 2018a,b; Nie et al., 2016; Xu et al., 2018). If SUN monomers in fact adopt this conformation *in vivo*, the KASH lids of SUN would be positioned away from the ONM and be unable to bind KASH (Fig. 1) (Jahed et al., 2018b; Nie et al., 2016; Xu et al., 2018). Trimerization mediated by the coiled coil regions of SUN may activate SUN1 and SUN2 for KASH binding and position their KASH lids close to the ONM where they can bind to KASH (Figs 1 and 4A).

Higher-order SUN–KASH network model

Unlike the linear model of LINC assembly, the recent structural data propose a head-on interaction of KASH-bound SUN domains, which would orient the SUN domains away from the ONM (Gurusaran and Davies, 2021). Similarly, apo-SUN trimers can also associate head-on through their SUN domains, which would position the SUN domains parallel to the membrane (Fig. 4B) (Cruz et al., 2020). If these two interacting SUN trimers could indeed bind to six KASH proteins simultaneously, a network of SUN–KASH complexes would span the NE. However, in this model, it is unclear how the short ONM-anchored KASH domain would reach the SUN domains furthest away from the ONM. Regarding this question, small angle X-ray scattering (SAXS)-based rigid-body modeling has shown that, in addition to the head-on interaction, the interacting KASH lids of two hexamers can act as hinges allowing the SUN domains to also interact laterally at an angle (Gurusaran and Davies, 2021). This would orient the SUN domain towards the ONM and expose the KASH lids for KASH binding (Fig. 4C). We also showed through molecular docking and molecular dynamics simulations that each SUN1 trimer may interact laterally with three neighboring SUN1 trimers (Jahed et al., 2018a; Hennen et al., 2017, 2018), forming lateral networks of SUN proteins, and potentially SUN–KASH complexes (Fig. 4C). Finally, earlier studies on SUN proteins have revealed that there are cysteine residues in predicted CC domains of SUN near the inner nuclear membrane (Fig. 4D) that are capable of forming interchain disulfide bonds (Lu et al., 2008). These interchain disulfides would allow the formation of large macromolecular assemblies of SUN in the NE (Lu et al., 2008; Jahed et al., 2018b; Jahed and Mofrad, 2018).

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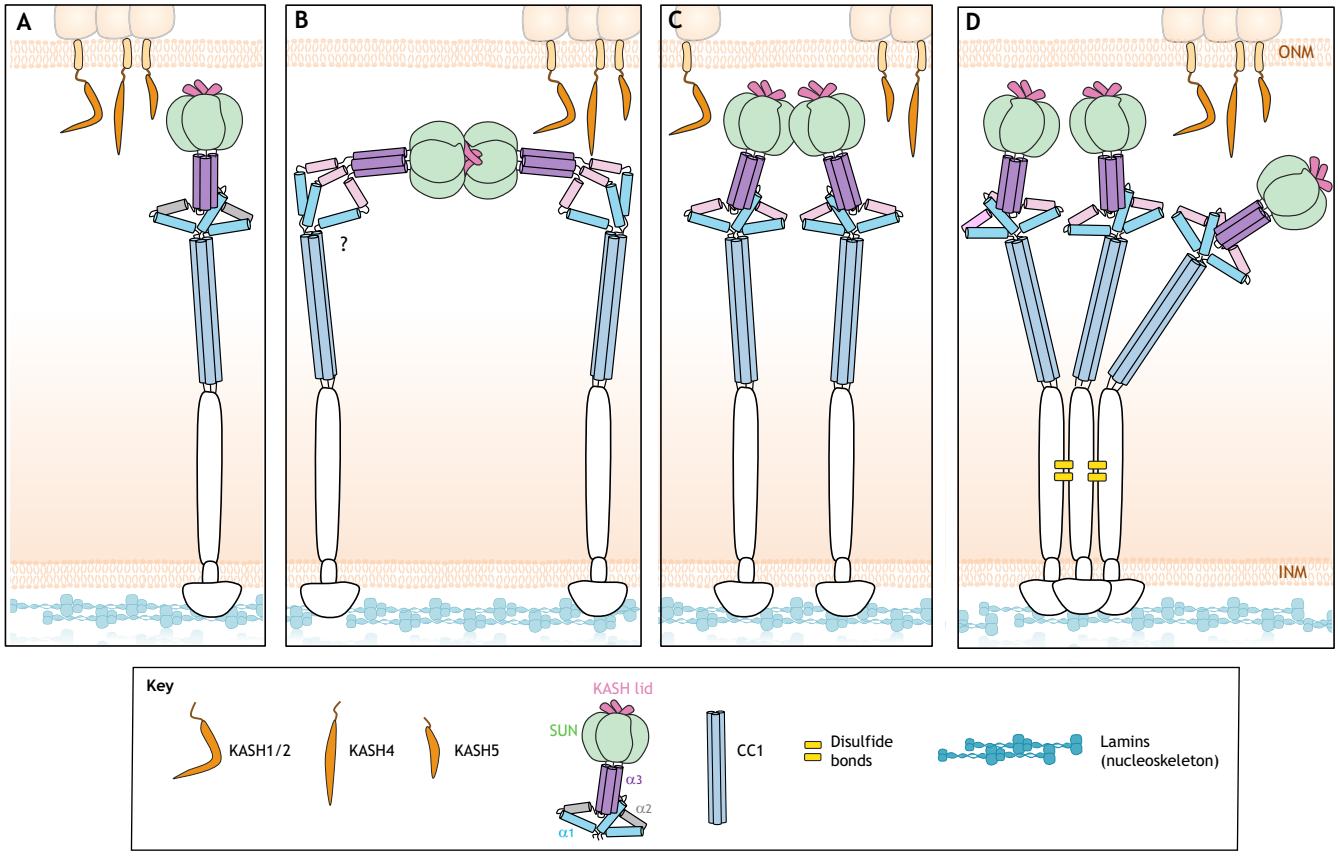

Fig. 4. Proposed hypothetical models for the orientation of SUN and KASH proteins at the nuclear envelope. (A) Linear assembly model. SUN proteins form linear trimers that span the nuclear envelope; their KASH lids then face the ONM where they can bind to the short ONM-anchored KASH proteins. (B) Higher-order assembly model. SUN trimers can interact with each other head-on through their SUN domains; in this scenario, neighboring KASH lids would be engaged with each other facing away from the ONM where the KASH domains reside, and therefore would be unable to bind to KASH. (C) SUN trimers may also interact with each other laterally or at an angle through their SUN domain and form higher-order assemblies. The KASH lids of these laterally interacting SUN domains would then be able to meet the KASH domains at the NE and potentially bind to six KASH domains simultaneously. (D) SUN proteins may form even higher oligomers through lateral interactions with other domains near the inner nuclear membrane, such as interchain disulfide bonds mediated by the coiled-coil domains of SUN1. In this scenario, the KASH lid could be either facing away from or directed towards the ONM as shown, depending on the extent of these interactions.

Higher-order assemblies of SUN–KASH complexes could potentially transmit large amounts of force across the NE, as discussed next.

Force transfer across LINC complexes in the different models

It is widely accepted that SUN proteins oligomerize to bind to KASH proteins. Specifically, it has been shown that mammalian SUN1 and SUN2 proteins must form trimers to simultaneously bind to at least three KASH proteins to form highly stable hexameric complexes (Wang et al., 2012; Zhou et al., 2012; Sosa et al., 2012). As discussed above, more recent studies suggest that these SUN–KASH hexamers can further associate and form higher-order assemblies in the NE. Furthermore, a SUN trimer can bind to different KASH peptides in solution (Cruz et al., 2020); if this is also the case *in vivo*, since the KASH domain of different KASH proteins can bind to the microtubule and actin cytoskeleton, one SUN trimer could be bound simultaneously to actin and microtubules (for example, via nesprin-2 and KASH5, respectively) and potentially be pulled in different directions. Considering that LINC complexes likely experiences various types of mechanical loading in the NE, their higher-order oligomerization would be an elegant mechanism to withstand higher-magnitude, and more complex loads, including a combination of compressive and tensile stresses, as well as shear forces (Jahed and Mofrad, 2018; Jahed et al., 2018b; Gurusaran and Davies, 2021). For a review of cellular processes in which the SUN–KASH complex experiences these types of forces, please refer to our recent reviews (Jahed and Mofrad, 2018, 2019).

A single, linear hexameric SUN–KASH complex with long coiled coil domains is ideal for tensile loads (Jahed and Mofrad, 2018). Coiled coil domains have unique elastic properties and can extend up to several times their lengths under tension (Schwaiger et al., 2002; Jahed et al., 2015). However, LINC complexes assembled according to the linear hexameric model would likely be unstable and may fail under compressive and shear forces due to the high diameter-to-length aspect ratio of their coiled-coil domains. Hence, a higher-order LINC complex assembly would explain how cells maintain the mechanical integrity of the NE and the transfer of forces to the nucleus. However, several questions remain unanswered. First, the oligomeric state of KASH-domain proteins has yet to be determined, and it remains unclear how the large cytoplasmic domains of several KASH proteins could cluster at the ONM to bind to SUN trimers. Second, the luminal KASH domain of KASH proteins is a very short peptide anchored to the ONM (Figs 1–3); therefore, any type of SUN–SUN interactions that would

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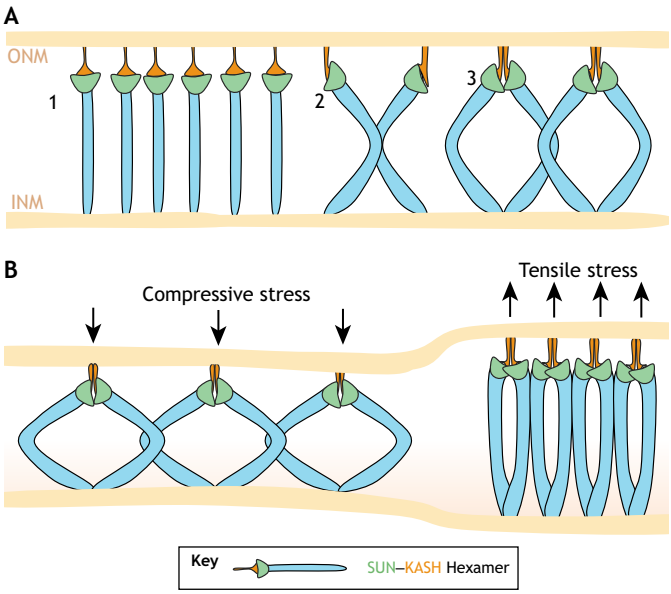

Fig. 5. Hypothetical model of LINC complex assembly and load bearing at the nuclear envelope. (A) Two models of LINC complex assembly. (1) Linear SUN trimer model. Here, SUN trimers bind three KASH-domain proteins and form linear arrays of SUN–KASH hexamers in the NE. In this model, the KASH-binding pockets on the SUN domain (green) face the ONM and KASH proteins (orange). (2,3) Higher-order SUN–KASH network model. Following SUN–KASH complex formation, lateral associations of SUN proteins through their coiled-coil domains may induce conformational changes that orient neighboring SUN–KASH hexamers in a way that they can interact with each other head-on (2). Lateral associations of several SUN–KASH hexamers creates large mesh-like networks of dimers of SUN–KASH hexamers in the NE (3). (B) The mesh-like networks proposed could be responsive to compressive and tensile forces on the NE, and withstand such forces without a loss of their integrity.

result in a movement of the SUN domains away from the ONM could inhibit KASH binding. For example, if the SUN–KASH hexamers interact head on, it is unclear how the KASH peptides would reach the KASH lids, which would be facing away from the ONM (Fig. 4B). One explanation may be that SUN trimers first face the ONM where they bind to three KASH proteins and form linear SUN-KASH hexamers spanning the NE (Fig. 5A). Lateral association with other SUN trimers or other proteins, and mechanical forces, could induce some conformational changes in the coiled-coil regions of SUN, bending the molecule and positioning the KASH-bound SUN domains to interact either head-on or at an angle to form dodecameric SUN–KASH complexes that laterally interact with neighboring dodecamers (Fig. 5A). We also discussed previously that some studies on SUN proteins have revealed potential interchain disulfide bonds between coiled-coil domains of SUN, which would allow the formation of large macromolecular assemblies (Lu et al., 2008; Jahed et al., 2018a,b; Jahed and Mofrad, 2018). Therefore the head-on and lateral associations would then allow SUN–KASH complexes to form a mesh-like network of SUN and KASH proteins in the NE (Fig. 5A). As opposed to the complexes proposed by the linear trimer model, such mesh-like networks of SUN–KASH complexes would maintain the integrity of the NE by responding to compressive and tensile stresses on the NE (Fig. 5B). Additionally, these networks could comprise a heterogeneous mix of complexes containing different SUN and KASH proteins.

Conclusions and perspectives

Our understanding of LINC complex assembly at the NE has evolved significantly over the past few years. Recent structural studies have suggested that SUN1 and SUN2 proteins adopt distinct binding modes for various KASH proteins. How these unique binding modes relate to force transfer and the distinct functions of LINC complexes formed by various SUN–KASH pairs remains to be studied. Additionally, recent structural studies show that LINC complexes may form higher-order assemblies through head-on interactions forming mesh-like networks, which challenges the original simple view of LINC complexes as linear assemblies. However, the details of any higher-order assembly of LINC complexes *in vivo* remains to be studied. If the head-on interactions are relevant *in vivo*, it would be very interesting to understand why various KASH proteins adopt distinct mechanisms for this head-on interaction. For example, what is the relevance of Zn^{2+} ion coordination, which seems to be unique to SUN1–KASH4? One can speculate that the distinct head-on interactions may be correlated with the proper activation and function of various SUN–KASH pairs. Additionally, most of the crystal structure data showing the head-on SUN–KASH interactions were specific to SUN1 (Gurusaran and Davies, 2021) and SUN2 (Cruz et al., 2020), and it remains unclear whether other SUN proteins can form such interaction.

We argue here that these mesh-like networks would potentially be more suitable for withstanding more-complex forces compared with linear assemblies that can transfer tensile force across the NE. However, further experimental and molecular modeling studies are required to determine how these mesh-like networks respond to forces, such as tension, compression and shearing. Finally, although there are several studies on the oligomeric state of SUN proteins, it remains unclear whether and how KASH proteins oligomerize so that six KASH proteins with large cytoplasmic domains are able to bind to two SUN proteins.

Competing interests

The authors declare no competing or financial interests.

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