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Review

LINC'ing form and function at the nuclear envelope

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ABSTRACT

The nuclear envelope is an amazing piece of engineering. On one hand it is built like a mediaeval fortress with filament systems reinforcing its membrane walls and its double membrane structure forming a lumen like a castle moat. On the other hand its structure can adapt while maintaining its integrity like a reed bending in a river. Like a fortress it has guarded drawbridges in the nuclear pore complexes, but also has other mechanical means of communication. All this is enabled largely because of the LINC complex, a multi-protein structure that connects the intermediate filament nucleoskeleton across the lumen of the double membrane nuclear envelope to multiple cytoplasmic filament systems that themselves could act simultaneously both like mediaeval buttresses and like lines on a suspension bridge. Although many details of the greater LINC structure remain to be discerned, a number of recent findings are giving clues as to how its structural organization can yield such striking dynamic yet stable properties. Combining double- and triple-helical coiled-coils, intrinsic disorder and order, tissue-specific components, and intermediate filaments enables these unique properties.

1. Introduction

Cellular architecture is largely organized by the cytoskeletal network, which provides connections all the way from the extracellular matrix to the genome. This cytoskeletal network consists of three principle filament systems defined by size that work together to achieve cell shape and stability: microfilaments, intermediate filaments and microtubules. The cytoskeleton also anchors and connects different cellular organelles that are delimited by lipid membranes and often their membranes are stabilized by some kind of 'scaffolding' protein. The nucleus stands out from other organelles in two major ways architecturally: (1) it is the only organelle membrane stabilized/reinforced by one of the three major cytoskeletal filament types – the intermediate filaments, and (2) it has a uniformly spaced moat-like double membrane.

The nuclear envelope (NE) is this double membrane system complete with a variety of transmembrane proteins, the nucleoskeleton lamins, and the nuclear pore complexes (NPCs) that are the gatekeepers directing molecular trafficking in and out of the nucleus. The outer nuclear membrane (ONM) is continuous with the ER and contains NE transmembrane proteins (NETs) that interact with cytoplasmic filament systems. The inner nuclear membrane (INM) is lined by the intermediate filament lamin polymer and both lamins and INM NETs interact with chromatin. Both ONM and INM connect where the NPCs are inserted [1]. In mammalian cells ONM and INM are separated by a roughly 50 nm wide lumen [2]. A subset of NETs from both the ONM and INM interact to connect the nucleus and the lamin nucleoskeleton to the cytoplasmic filament systems. Central to these connections are proteins of the LINC (linker of nucleo- and cytoskeleton) complex (Fig. 1) [3].

This complex is thought to provide a direct connection from the intermediate filament lamin polymer and its nuclear connections to the cytoplasmic filament systems and through these all the way to the extracellular matrix and adjacent cell connections. As such, in addition to its obvious mechanical stability role, this complex can be involved in signal mechanotransduction from extracellular signals to the genome [4]. It also maintains the spacing of the double membrane, at least in cells experiencing forces [3,5]. Although the knowledge about the structure, connections and role in human disease of the LINC complex is growing fast, there are still many open questions regarding its structural organization and mechanics as well as its tissue specific compositions/structures that may result in additional functions.

2. What the LINC complex connects

To understand the role of the LINC complex it is first necessary to understand the properties of what it connects — the nucleus and

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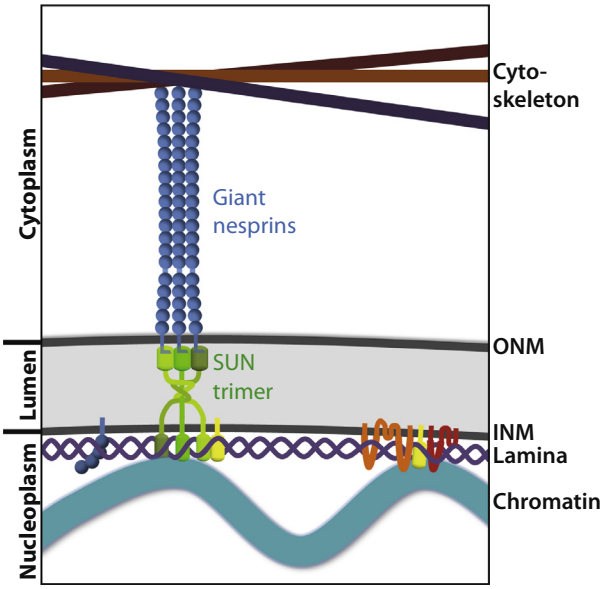

Fig. 1. Schematic representation of a LINC complex. ONM: outer nuclear membrane, INM: inner nuclear membrane. The core LINC complex consists of certain nesprin isoforms in the ONM and SUN proteins in the INM. LINC is also supported by additional proteins in the INM such as short nesprin isoforms (blue), emerin (yellow), LUMA (red) and NET5 (orange).

nuclear contents on one side and the cytoplasmic filaments and their myriad connections all the way to the extracellular matrix on the other side. On its outer face, the principle cytoplasmic filament systems are defined by size: microfilaments (7 nm), intermediate filaments (10 nm) and microtubules (25 nm). All three work together to largely define cell shape and stability, but also have a variety of more specific functions such as cytokinesis, pinocytosis and phagocytosis, intracellular transport, signaling pathways and cell migration [6]. Some functions and structures are highly tissue-specific such as the Z-bands of muscle, immune synapses, actin in the acrosomes of spermatozoa (in lower organisms), cilia and flagella and many others [6,7]. Microfilaments are built of oligomerized actin forming two strands wrapping around one another in a right-handed spiral whereas microtubules are built of α- and β-tubulin heterodimers that form linear protofilaments which can also interact laterally to form a sheet that circularizes into a 25 nm diameter hollow tube [6]. Intermediate filament are also assembled with the starting point of dimers, but they have very different properties from the other skeletal systems both by being insoluble due to their coiled-coil rod domain and by assembling into head-to-tail linear arrays that further layer to eventually form the roughly 10 nm diameter filaments with about 32 molecules in cross section [8,9]. Intermediate filaments also differ from other cytoskeletal systems in their dynamics *e.g.* actin and tubulin dynamically assemble and disassemble, are stabilized by tension and are highly polarized to support directional movement by motor proteins whereas intermediate filaments are much less dynamic, resist tension and have no polarity [6]. Each filament system connects to different plasma membrane protein assemblies that in turn connect it to the extracellular matrix.

On the inner face of the LINC complex it connects to the nucleoskeleton that is formed principally by a polymer of type-V intermediate filament lamins. Lamins differ from cytoplasmic intermediate filaments by having a longer coiled-coil region [10,11], a nuclear localization sequence [12,13], and a C-terminal CaaX box that is farnesylated [14,15]. All of these properties contribute to the translocation of lamins into the nucleus [16,17]. Mammals have three genes encoding lamins: *LMNA*, *LMNB1* and *LMNB2*. Alternative splicing of these genes gives rise to different isoforms [18,19], some of which are tissue specific. Examples are the spermatocyte-specific isoforms lamin C2 and B3 [20,21]. The full-length splice forms of *LMNA*, *LMNB1* and *LMNB2* are all post-translationally farnesylated. In the case of lamin A the last 18 amino acids containing the farnesyl moiety are cleaved during subsequent lamin A processing so that the farnesyl moiety only remains in the B-type lamins, potentially allowing an assembly closer to the INM due to the farnesyl integration into the membrane lipid bilayer. This predicts the formation of different lamin sublayers at the INM, which is beginning to gain experimental support [22]. In contrast to B-type lamins, that are present in all nucleated somatic cells, the expression of A-type lamins seems to be correlated with terminal differentiation; however, different tissues have distinct characteristic patterns for the relative expression of the different lamin subtypes [23]. Lamins directly interact with a variety of NETs and chromatin. Accordingly they have been implicated in DNA replication, chromatin organization, mechanical stabilization of the nucleus, positioning of nuclear pores and anchoring of nuclear membrane components [24]. Intriguingly, by being comprised of just intermediate filaments, this nucleoskeleton should be far more elastic, deformable, and capable of withstanding strong compression or stretch/tension forces compared to the combined cytoplasmic filament systems. These properties might be structurally necessary because of the interaction with DNA/chromosomes, the largest individual molecules in the cell with enormous force bearing and generating potential.

3. Building blocks of the LINC complex

The LINC complex itself can be subdivided into the core complex and other associated proteins that can give it greater specificity of function. The core complex consists of an INM SUN (Sad1/UNC84 homology) domain protein and an ONM KASH (Klarsicht/ANC-1/Syne homology) domain protein. The actual SUN and KASH domains within these proteins interact with one another in the lumen of the NE [25,26]. Some SUN proteins have been shown to oligomerize [3,26,27] and the variety of both SUN- and KASH-domain proteins enables some functional specificity even without its associated proteins.

So far five SUN proteins have been identified in human – encoded by the genes *SUN1*, *SUN2*, *SUN3*, *SPAG4* and *SUN5*. Of these five SUN domain-containing proteins only SUN1 and SUN2 seem to be widely expressed [26,28,29], whereas the expression of SUN3, SPAG4 and SUN5 seems to be restricted in a tissue specific manner, mostly testis-specific [30,31]. SUN1 and SUN2 are quite distinct by sequence and have some differences in identified partners, but for their main mechanical LINC complex function they appear to be largely redundant because individual knockout mice exhibited minimal phenotypes [32] and because it required a double SUN1/SUN2 knockdown to have any effect on the 50 nm spacing of the NE [3]. Their functional redundancy must necessarily reflect what they share and both proteins have an N-terminal lamin A binding domain in common, a coiled-coil dimer region just after the transmembrane span(s) in the lumen (Fig. 2B), bind short nesprin 1 and 2 isoforms, and interact with LINC-associated protein emerin [33]. For SUN1 a meiosis specific isoform, SUN1η, has been described that lacks the emerin- and short nesprin-binding domains [34].

Five human KASH domain-containing proteins associated with the LINC complex have been identified to date: nesprins 1–4 (nuclear envelope spectrin repeat proteins) and KASH5 (encoded by *SYNE1*, *SYNE2*, *SYNE3*, *SYNE4* and *CCDC155* respectively). All of these proteins have a short luminal C-terminal KASH domain and on the other side of the membrane nesprins contain a number of

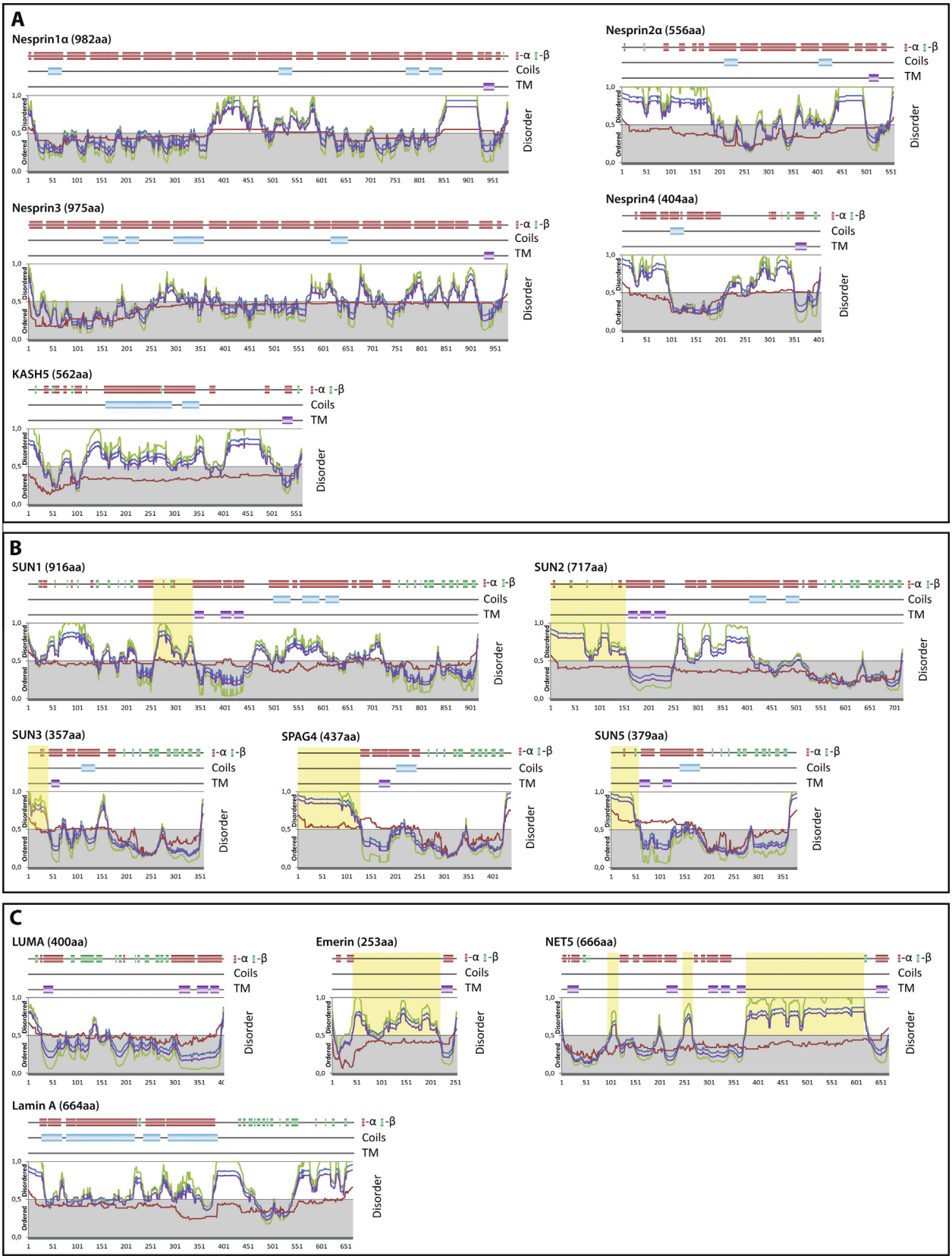

Fig. 2. Prediction of secondary structure, coiled-coils, transmembrane domains and protein disorder for (A) KASH-domain proteins, (B) SUN-domain proteins and (C) other LINC associated proteins. Top line: Jpred4 (http://www.compbio.dundee.ac.uk/jpred/index.html) was used to predict alpha helices (red) and beta sheets (green). Line 2: coiled coils as predicted using Jpred4 (blue). Line 3: transmembrane domains (violet) were predicted using THMM (http://www.cbs.dtu.dk/services/TMHMM) and – in case of known domains that failed to predicted – TMPred (http://www.ch.embnet.org/software/TMPRED_form.html). Disorder prediction: GeneSilico Metadisorder service (http://iimcb.genesilico.pl/metadisorder/FKYa138760caq/) was used to predict intrinsic disorder using 4 different algorithms (METADISORDERMD2 – blue; METADISORDER3D – red; METADISORDER – green; METADISORDERMD – violet). Values below 0.5 are predicted as ordered, above as disordered. Areas of predicted disorder that are not entirely contradicted by the structure prediction are displayed in a yellow field.

spectrin repeats [35–38] whereas KASH5 has a large coiled-coil region (Fig. 2A). Individual nesprin genes also have a wide range of splice variants that can have very many or very few spectrin repeats as well as lack the KASH domain [39]. All nesprins can interact with cytoplasmic filament systems via their N-terminal domain. This is generally cytoplasmic as most nesprin isoforms are too big to enter the nucleus through the peripheral channels of the NPCs; however, it could also be nucleoplasmic for the very short nesprin isoforms [40,41]. In fact, small tissue-specific isoforms of nesprin 1α and nesprin 2α have been described in muscle [41] that interact in the nucleoplasm with lamin A, emerin and SUN1 [25,42]. The cytoplasmic regions of the larger nesprin 1 and 2 giant isoforms interact directly with actin, but both can also bind the motor protein kinesin through which it is thought that they can also tether microtubules to the NE. The kinesin interaction occurs via a conserved LEWD motif that is essential for nuclear distribution in myotubes [43]. Interestingly the LEWD motif is also present in the shorter nesprin 1α and nesprin 2α isoforms, though whether this indicates a mitotic role or that these proteins have both INM and ONM roles remains to be clarified. Nesprin 4 also interacts with kinesin [36] whereas KASH5, which is germ-cell specific, interacts with the dynein-dynactin complex providing yet another mechanism for linking to the cytoplasmic microtubule network [44]. Nesprin 3 by contrast interacts with plectin, which is best known for connecting intermediate filaments but can act as a link to all three major cytoplasmic filament systems [45]. Thus, between the different KASH-domain proteins the LINC complex can connect to all three major cytoplasmic filament systems; however, because different gene products have different properties and preferences for particular filaments, their relative expression could confer unique properties to NE-cytoplasmic filament interactions.

The LINC core can be supplemented with additional proteins to contribute further unique properties to the complex. Emerin, NET5 (Samp1) and LUMA (TMEM43) are all NETs that have been shown to interact with some LINC cores in some cell types. Emerin, encoded by the *EMD* gene, is located principally in the INM [46], but has also been reported in the ONM, ER, plasma membrane, and at intercalated discs [47–52]. Thus it is hard to distinguish which population may be responsible for its reported roles in nuclear structural integrity, cellular susceptibility to mechanical stress damage, alterations in gene expression, cell proliferation and differentiation [53]. Nonetheless, emerin has been shown to interact with lamin A, SUN1 and nesprins (through interaction with spectrin repeats [42]) and some of these reported roles could reflect its influence on LINC function and with having been found also in the ONM it could potentially contribute from both sides of the membrane. LUMA, which is encoded by *TMEM43*, is another NET that has been shown to interact with lamins A and B as well as emerin [29] and SUN2 [54] and is suggested to function as a membrane organizer [29]. However, like emerin it has been found not only in the INM but also in the ONM and ER [29] and as such it could also potentially impact on LINC function from either end of the core complex. Finally, NET5 (Samp1, encoded by *TMEM201*) is also associated with LINC complexes. Not only does NET5 interact with SUN1 [55] and lamin A [56], but a more direct function with LINC has been described in contributing to a more specialized LINC complex associated with TAN-lines [57]. TAN-lines serve as tracks for nuclear migration and positioning within the cell, particularly to keep the nucleus following the leading edge during fibroblast movements [58].

At least one pair of LINC core SUN- and KASH-domain proteins is found in all higher eukaryotes and, though homologs are not always readily apparent in lower eukaryotes, they have been found where a focused search has been undertaken [59]. During evolution multicellular eukaryotes have amplified both core components, but not necessarily in a linear fashion. For example, mammals tend to have 4–6 SUN-domain containing and 4–6 KASH-domain containing proteins whereas fish have twice this number of KASH-domain nesprins but only half as many SUN proteins and chickens have increased the number of SUN protein genes by 3–4-fold. This together with both the identified and probably many as yet unidentified LINC associated proteins yields a manifold of possible interactions that could support LINC complex functionalities specific for different tissues, developmental stages and organisms [60]. Indeed, during mouse sperm development, the existence of distinctive LINC complexes has been shown. They consist either of spermatogenesis-specific SUN3 and nesprin 1 or SUN1η and nesprin 3. These two LINC complexes can contribute distinct functional specificities even within the same cells, localizing to opposite poles of the spermatid [34].

4. Form and function

Many of the functions of LINC complex proteins such as in nuclear positioning, cell division and the organization of the cytoskeleton [60] are likely indirect effects reflecting its core function in connecting the genome and nucleoskeleton to cytoplasmic filaments and the extracellular matrix. For this core function the molecular structure of the SUN–KASH interface would have to be designed for both extreme load bearing and tension forces to be buffered within the NE lumen while maintaining a tight binding interface impervious to disruption. The design would also need to provide a counter force to prevent the complex being pulled out of the membrane on either end.

Moreover, LINC complex components have been shown to be important for specific functions such as telomere positioning [61] and for association of the centrosome with the nuclear membrane [49]. Both of these more specific functions represent the tethering of an enormous mass. The telomere tethering is thought to hold an entire chromosome — the largest individual molecule in a cell — in place while lining it up with its sister for meiotic recombination. The centrosome tethering not only reflects the mass of this large complex, but also the entire microtubule network that reaches out from it. Thus the molecular structure for these specialized interactions likely includes even more stabilizing forces and partner proteins. Another specific function is the positioning of nuclei in muscle fibers. Nesprin 1 and nesprin 2 double knockout mice fail to recruit synaptic nuclei to the neuromuscular junction in skeletal muscle [62] while SUN1 and SUN2 double knockout mice have abnormal synaptic nuclei [32].

Finally, it appears that some LINC complexes take the principal structural function while others are more specialized for transducing mechanical signals to the nucleus and thus activating mechano-sensitive genes [63]. Those involved in mechanotransduction would require a design that would prevent any small pressures on the nucleus from activating them or else the dynamically active cell would be constantly activating mechanosensitive genes.

5. LINC molecular structure

Although there are many distinct SUN- and KASH-domain containing proteins, there is only high resolution structural information for part of the most common LINC core of SUN2 and nesprin 1. In early 2012 the crystal structure of the SUN-domain of SUN2 was solved. The SUN domain forms a trimer resembling a cloverleaf sitting on a stem (Fig. 3C and D) instead of a dimer as had been previously assumed because of the coiled-coil dimer prediction after the transmembrane region [64]. A trimer would greatly enhance stability and also provides in this cloverleaf interface a potential grabbing/locking mechanism if the KASH domain of nesprins could somehow insert within the trimer. However, the

authors noted that algorithms predicting the oligomerization [65] strongly predicted the first coiled-coil (Fig. 2) to be dimeric while the second coiled-coil had only a modest probability to be trimeric, raising the possibility that the trimer could be a crystallization artefact. Nonetheless, evidence for this structure was greatly strengthened when later in 2012 the structure of the SUN2 SUN-domain in complex with the nesprin KASH-domain was solved showing a hexameric complex of three SUN protomers and three KASH peptides with a disulfide bond covalently linking SUN and KASH [66]. The binding interface comprises two antiparallel beta-sheets from SUN2, named as KASH-lid, sticking out from the body of the SUN domain that lay over a beta sheet from the KASH domain of nesprin 1 as well as nesprin 2 (Fig. 3A and B).

Interestingly, the KASH-lid region is unstructured in the monomeric form of the SUN domain without the KASH peptide in the original Zhou et al. 2012 structure, but incorporates an alpha helix in the later Sosa et al. 2012 study [66] (Fig. 3A). Thus the KASH-lid appears to be quite dynamic until binding to KASH, which could facilitate the insertion of the KASH domain into the SUN domain trimer. Moreover the ability to achieve distinct conformations in the different structures might reflect the ability to form connections to other proteins in the ONM besides KASH-domain proteins. All in all the combined structure resembles a locking mechanism whereby turning one protein trimer in relation to the other captures projections of the one protein under a solid physical projection from the other so that forces pulling the two apart would meet maximum resistance. Sedimentation equilibrium of a SUN2 fragment containing both predicted coiled-coils led to the conclusion that the trimeric form is the physiologically relevant oligomerization state of human SUN2 [66]. Moreover, it has been shown in several studies that the second predicted coiled-coil domain that forms the triple helical coiled-coil in the trimer is necessary to achieve a SUN–KASH interaction [3,26,67].

While the locking mechanism seems clear, the rest of the molecular structure resides in the realm of speculation. Going outwards from the NE the KASH domain is at the C-terminus of all KASH-domain proteins and follows almost immediately the single transmembrane spanning segment of this protein family (Fig. 1). Thus this lock occurs almost immediately under the ONM and the insertion of three KASH-domain proteins together in the membrane and then spanning out just under the membrane should further stabilize the structure by including the membrane itself in its force bearing function. On the outside of the ONM the nesprins have many spectrin repeats and groups of spectrin repeats are interspersed with short regions containing many prolines, which would enable considerable bending within these regions. Thus, just as the KASH domains would spread outwards in the NE lumen, the longer regions in the cytoplasm could also spread outwards. This makes particular sense for nesprins because there are no indications that any of the spectrin repeat domains interact to form dimers or trimers. In the case of KASH5, its coiled-coil region might dimerize to achieve a different type of interface with cytoplasmic filaments, although, again, no evidence has been presented regarding whether this might form a coiled-coil interface for two or three KASH5 molecules or an interface with another protein. Interestingly, all KASH-domain containing proteins also have multiple short regions of predicted intrinsic disorder throughout the long cytoplasmic region; however, in the case of nesprins these tend to overlap with regions also strongly predicted as alpha helical (Fig. 2A) so that the structure remains unclear. Nonetheless, between the likely bending properties and the possible intrinsic disorder, we predict that these regions can be stretched by pulling forces or collapse upon compression forces so that they would buffer a considerable amount of the forces from the cytoplasmic filaments. This should considerably reduce the force taken at the SUN–KASH interface just under the ONM.

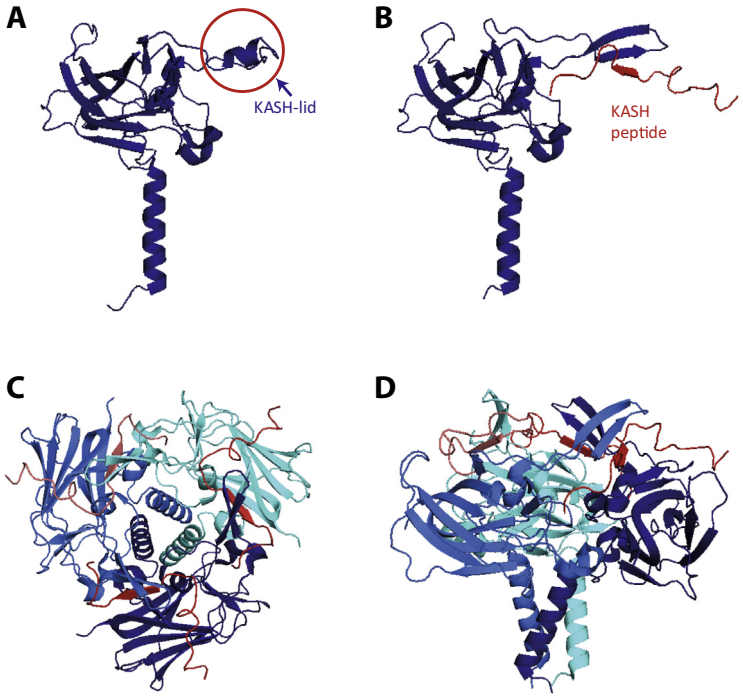

Fig. 3. Overview of the human SUN domain of SUN2, a SUN2–KASH domain complex and the human SUN–KASH interaction trimer complex. (A) The structural views of the apo-SUN form published in Sosa et al. [66] (PDB entry 4DXT) is shown as ribbon diagram in blue. The KASH-lid is indicated by a red circle. (B) The structure of the human SUN2–KASH1 complex (4DXR; Sosa et al. [66]) is indicated with the SUN-domain shown in blue and the KASH peptide in red. (C) Top view and (D) side view of the SUN2–KASH1 interaction trimer complex (4DXR; Sosa et al. [66]) are shown as trimers with the SUN-domains colored in blue and the KASH peptides in red. The structural views were modified using the PyMOL Molecular Graphics System, version 1.3 (Schrödinger, LLC).

Going in the other direction from the SUN–KASH interface, the SUN proteins tend to have considerable beta sheet prediction in the SUN domain consistent with the crystal structure, but the rest of the luminal region tends to strongly predict for alpha helical structure with poor if any intrinsic disorder prediction (Fig. 2B). In all cases this region also contains 1–3 short predicted coiled-coils. If these structural predictions are accurate they raise one extremely critical question about the entire LINC complex as currently envisioned. LINC is thought to span from SUN proteins embedded in the INM all the way across to the interface just under the ONM; however, with ⅓ to ½ of the luminal mass forming the trimeric globular SUN domain for the SUN–KASH interface it is hard to envision physically the SUN protein spanning the entire 50 nm luminal space without highly stretchable intrinsic disordered regions. After all, the linear lamin coiled-coil dimer rod domain is 52 nm and it is more than 100 amino acids longer than the luminal length of SUN2 apart from the globular SUN domain and as a trimer the SUN protein would be much shorter due to the larger wrapping diameter for a triple-helical coiled-coil. Thus either (1) despite the lack of prediction there are intrinsically disordered regions, or (2) the alpha helices after the one just under the SUN domain do not form a triple helical coiled-coil and are extremely stretched and under considerable tension, or (3) one SUN protein is embedded in the ONM to interact with KASH and another SUN protein in the INM dimerizes with the ONM SUN protein either directly through the predicted coiled-coil dimer or connects indirectly via this predicted coiled-coil to an as yet unidentified LINC component in the lumen. Though the latter possibility may be viewed as unlikely and heretical, the physical distance and lack of clarity of the remaining SUN protein molecular structure highlights the importance of focusing efforts on clarifying this question. Regardless, the fact that what is known does not fit with typical known structures indicates that the molecular architecture of this unique interface will be new and intriguing.

Interestingly, on the other side of the INM in the nucleoplasm the remaining SUN protein mass contains very strongly predicted regions of intrinsic disorder (Fig. 2B). This may enable its snaking through the lamin polymer with which it also interacts to facilitate interactions with chromatin and, similar to the disorder or bending predicted for KASH protein mass in the cytoplasm, provide a buffer for forces coming from chromatin on the other side. Although specific chromatin partners have not been specified for SUN proteins, their functional importance in tethering telomeres to the NE in meiosis [68,69] together with the fact that their binding partners including lamins, emerin and NET5 all bind chromatin proteins [70–74] indicate that they contribute to a physical connection of LINC with the genome. As the long DNA strands at the core of chromosomes are the largest individual molecules in the cell, they have the potential to also contribute to buffering forces exerted on the nucleus while at the same time being highly deformable, like the innards of a pillow [75–77]. Thus, just as the large chromosome mass can stabilize the microtubule spindle in mitosis [78] they could contribute to stabilizing forces from the cytoskeleton on the nucleus in interphase. This idea also may shed light on the logic of the protein nucleoskeleton persisting almost exclusively from intermediate filaments and associated NETs. The extreme elasticity and tensile strength of the intermediate filament lamin polymer enables it to stretch under considerable force without breaking while the many individual connections between NETs and lamins on the one hand and NETs and chromatin on the other hand provide an overall strong and distributed connection between the membrane and the chromatin from which LINC can itself exert its functions. Not surprisingly, after observations of structured and unstructured regions of SUN proteins, the associated proteins known to function with LINC — emerin, NET5, LUMA and lamin A — exhibit a mixture of characteristics with some fully structured and others largely intrinsically disordered (Fig. 2C). This mixture of properties could add to the buffering capacity of the INM to forces from either side.

6. Implications and directions

That the LINC complex has a principally structural role is supported from its strong involvement in Emery-Dreifuss muscular dystrophy (EDMD), a rare, genetically heterogeneous neuromuscular disorder characterized by progressive skeletal muscle wasting and weakness, early contractures and cardiac arrhythmia that can evolve to cardiomyopathy [79]. Mutations in *LMNA* [80] and the LINC complex components *EMD* [81], *SYNE1*, *SYNE2*, *SUN1* and *SUN2* can all cause EDMD [41,82]. Mutations in lamin A and point mutations in emerin perturb interactions with SUN1 and SUN2 [33]. Point mutations in nesprins 1 and 2 result in nuclear morphology changes and mislocalization of SUN2 and emerin [41]. Mutations in SUN and nesprin proteins can act as modifiers resulting in a more severe phenotype [82–84] and altered NE elasticity is typical in cells from EDMD patients [85]. All of these defects point to a weakening of the LINC complex in EDMD pathophysiology. Furthermore, truncating mutations in *SYNE4* cause autosomal recessive deafness. The mutant protein lacked the KASH domain and failed to localize at the NE. In a mouse model for this disease nuclei of outer hair cells in *SYNE4* and also in *SUN1* knockout mice failed to maintain the basal position they occupy in wild type cells [86]. Finally, *SYNE1* nonsense mutations resulting in a loss of the KASH domain cause autosomal recessive cerebellar ataxia, a disease characterized by impaired walking and a lack of coordination of gait and limbs. Nuclei at neuromuscular junctions in patients appeared to be displaced to the periphery [87]. In all cases, disease pathology appears to be caused by defects in the structural integrity of the LINC complex and its connections.

These disease links further indicate the importance of determining all molecular details of LINC complex structure and finding all remaining partner proteins, particularly the tissue-specific ones that could contribute to the focusing of pathology in particular tissues. Indeed, finding both testis and muscle specific isoforms of nesprins already demonstrates that tissue-specific LINC complexes exist. That nesprin mutations also cause a cerebellar ataxia [87] argues for at least additional neuron-specific forms. The recent discovery of many SUN-related and SUN-associated proteins in plants that were not evident by a straightforward BLAST analysis [88] also indicates that there are many other proteins likely contributing to LINC yet to be discovered. Perhaps these additional proteins will be able to explain how LINC mediates the regular uniform spacing of the NE lumen and the biggest mystery: how this spacing can be maintained at 50 nm between human and Xenopus despite significant differences in SUN protein length and differ by 20 nm between human and yeast despite that the SUN proteins are closer in length. Clearly there is much more to be discovered about how the cell has engineered the critically important force bearing and transmitting interface of LINC.

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