Title

Building and breaking mechanical bridges between the nucleus and cytoskeleton: Regulation of LINC complex assembly and disassembly

Authors

McGillivary, Rebecca M

Starr, Daniel A

Luxton, GW Gant

Publication Date

2023-12-01

DOI

10.1016/j.ceb.2023.102260

Peer reviewed

HHS Public Access

Author manuscript

Curr Opin Cell Biol. Author manuscript; available in PMC 2024 February 11.

Published in final edited form as:

Curr Opin Cell Biol. 2023 December ; 85: 102260. doi:10.1016/j.ceb.2023.102260.

Building and breaking mechanical bridges between the nucleus and cytoskeleton: Regulation of LINC complex assembly and disassembly

Rebecca M. McGillivary,

Daniel A. Starr,

G. W. Gant Luxton

Department of Molecular and Cellular Biology, University of California, Davis, USA

Abstract

The nucleus is physically coupled to the cytoskeleton through LINC complexes, macromolecular bridges composed of SUN and KASH proteins that span the nuclear envelope. LINC complexes are involved in a wide variety of critical cellular processes. For these processes to occur, cells regulate the composition, assembly, and disassembly of LINC complexes. Here we discuss recent studies on the regulation of the SUNKASH interaction that forms the core of the LINC complex. These new findings encompass the stages of LINC complex assembly, from the formation of SUN-KASH heterooligomers to higher-order assemblies of LINC complexes. There is also new work on how components of the LINC complex are selectively dismantled, particularly by proteasomal degradation. It is becoming increasingly clear that LINC complexes are subject to multiple layers of regulation.

Introduction

The linker of nucleoskeleton and cytoskeleton (LINC) complex is an evolutionarily conserved nuclear envelopespanning macromolecular bridge that physically connects chromatin and the nuclear lamina to the cytoskeleton [1,2]. The LINC complex is composed of two types of type-II membrane proteins: Sad1/UNC-84 (SUN) proteins located in the inner nuclear membrane (INM), and Klarischt/ANC-1/SYNE homology (KASH) proteins that are embedded in the outer nuclear membrane (ONM) [3]. KASH proteins contain a conserved C-terminal transmembrane domain followed by a short (~10–32 residue), conserved luminal KASH peptide. Within the cytoplasm, KASH proteins interact with various cytoskeletal elements and signaling molecules [4,5]. KASH proteins are targeted to the nuclear envelope via a direct physical interaction between their KASH peptide and the conserved C-terminal SUN domain of SUN proteins in the perinuclear space of the nuclear envelope [3,6–8]. The KASH peptide is both necessary and sufficient to target proteins to

Corresponding authors: Starr, Daniel A. (dastarr@ucdavis.edu); Luxton, G.W. Gant (ggluxton@ucdavis.edu).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

the ONM [9]. Divergent SUN protein C-termini extend into the nucleoplasm, where they interact with lamins, chromatin-binding proteins, and other proteins [10]. The KASH-SUN interaction allows the LINC complex to transmit mechanical forces from the cytoplasm across the nuclear envelope and into the nucleoplasm. Consequently, LINC complexes are necessary for several fundamental cellular processes, including DNA damage repair, meiotic chromosome pairing, nuclear positioning, force transmission across the nuclear envelope, and the mechanoregulation of gene expression [10,11]. Mutations in KASH and SUN proteins are also associated with a variety of diseases [12,13*]. Regulating how, when, and where LINC complexes assemble and disassemble is therefore a fundamental task that the cell must perform to carry out a wide variety of cellular processes.

The LINC complex is not a singular complex – it is a modular class of complexes that consists of different combinations of SUN and KASH proteins. Mammals possess two ubiquitously expressed SUN proteins (SUN1 and SUN2) as well as three testes-specific SUN proteins (SUN3, SUN4, and SUN5) [10]. Mammals also have six KASH proteins: nuclear envelope spectrin-enriched protein (nesprin)-1, –2, –3, –4, KASH5 (a.k.a. CCDC155), and KASH6 (a.k.a. Jaw1/LRMP) [10,14]. It is currently unknown if and how the modularity of the LINC complex may contribute to its assembly and/or disassembly. For example, a variety of KASH proteins can bind to a single homotrimer of SUN1 or SUN2 [15,16]. There is also evidence that SUN1 and SUN2 can form heterotrimers [16,17*–20]. The two widely expressed SUN proteins and six KASH proteins allow for hundreds of possible unique LINC complexes. Given this large “state space” that LINC complexes can exist within, how do cells regulate LINC complex assembly and disassembly? Recent work has focused on the assembly of the core SUN-KASH interaction, the variety of structural configurations that the various SUN-KASH combinations can form, and the mechanisms cells use to selectively degrade particular SUN or KASH proteins. These mechanisms are summarized below and in Figures 1 and 2.

SUN protein homotrimerization

The process of LINC complex assembly begins with SUN protein homotrimerization [21]. One model is that SUN protein monomers adopt an autoinhibited state that prevents homotrimerization as they are being transported from the endoplasmic reticulum to the INM [22,23] (Figure 1b). This may be mediated by interactions between a portion of the SUN domain that, when active, clamps down on the KASH peptide called the KASH lid, and an immediately N-terminal coiled-coil (CC)-containing region known as the autoinhibitory domain (AID) [22,24]. SUN1 and SUN2 both possess two conventional CC-containing regions, CC1 and CC2, within their luminal domains (Figure 1a). In SUN2, the AID is a non-conventional CC, whereas the AID of SUN1 overlaps with CC2. The AID-SUN domain interaction locks SUN proteins in an inactive conformation that cannot homotrimerize or interact with KASH peptides. In contrast, CC1 is proposed to promote SUN protein homotrimerization [22,25]. While it is not known exactly how SUN proteins transition from monomeric to homotrimeric states, the homotrimerization process could be influenced by changes in the concentration of calcium ions within the perinuclear space; lower concentrations of calcium favor SUN protein homotrimerization at pH 8.0[25].

Interestingly, another report demonstrated that *in vitro* SUN-KASH interactions were promoted by the addition of physiological levels of calcium [17]. How can calcium both inhibit LINC complex assembly by decreasing homotrimerization and promote assembly by increasing SUN-KASH interactions? One potential explanation for these differing results may stem from the use of the isolated luminal domain of SUN2 vs. a full-length SUN2 protein reconstituted in a supported lipid bilayer.

SUN-KASH interactions: intermolecular SUN-KASH disulfide bonds

SUN-KASH interactions often include an intermolecular disulfide bond between conserved cysteines in the SUN domain and in the KASH peptide (Figure 1a) [21]. Molecular dynamics simulations show that this covalent linkage enhances the LINC complex's ability to transmit forces [26,27]. In addition, this intermolecular disulfide bond is necessary for LINC complex-dependent processes, including ANC-1 dependent nuclear anchorage in the developing *C. elegans* hypodermis and rearward nuclear movement during centrosome orientation in fibroblasts polarizing for directional cell migration [27]. A mutation that prevents SUN2 from forming its intermolecular disulfide bond with KASH peptides reduces the levels of SUN2 protein in the cell [28*] – suggesting that regulation of this disulfide bond may regulate the overall stability of LINC complexes.

Thioredoxin related transmembrane protein 4 (TMX4) is a regulator of the SUN-KASH intermolecular disulfide bond. TMX4 is a protein disulfide isomerase (PDI), a family of over 20 proteins that modify disulfide bonds and largely reside in the endoplasmic reticulum [29]. TMX4 is one of a few PDI proteins that is enriched in the nuclear envelope [30**,31]. Nesprins are the clients of TMX4: TMX4 breaks the intermolecular SUN-KASH disulfide bond and forms a mixed disulfide with the nesprin [30**]. Further investigation of TMX4 function within the nuclear envelope will reveal if it plays a regulatory role in LINC complex-dependent processes or if it affects LINC complex stability. Future studies should investigate the potential roles of the remaining members of the PDI protein family during LINC complex regulation.

SUN-KASH interactions: binding modes

The next step after trimerization in LINC complex assembly is the SUN protein's SUN domain binding to the KASH protein's KASH peptide within the perinuclear space of the nuclear envelope (Figure 1b). The SUN-KASH binding interface that forms the core of the LINC complex was recently investigated in detail by several structural studies. The crystal structures of human SUN1 and SUN2 bound to the KASH peptides of human nesprin1–4 and KASH5 have been reported [15,21,32,33]. These structures allow us to more precisely identify the binding interactions that are most likely important for establishing the LINC complex-mediated mechanical linkage across the nuclear envelope.

The above-described work shows that different KASH peptides bind to SUN homotrimers in two distinct modes (Figure 2a and b). The C-terminal ten residues of KASH peptides bind within common interfaces formed between neighboring SUN protomers in a SUN protein homotrimer and under the KASH lids of SUN domains [15]. However, the way that the KASH peptide exits this binding pocket varies. Nesprin-1 and -2 bind SUN2 such

that their KASH peptides make a 90° turn at a conserved proline at position –11 residues from the C terminus and continues out towards the periphery of the SUN2 homotrimer [15] (Figure 2a). This positions the KASH peptide so that the conserved –23 cysteine can form an intermolecular disulfide bond with SUN2's conserved C583 [21]. Nesprin-1 binds to SUN1 homotrimers in a similar configuration [32].

In contrast, crystal structures suggest that nesprin-3, -4, and KASH5 bind to SUN2 homotrimers such that their KASH peptides extend up towards the KASH lid of the neighboring SUN protomer [15] (Figure 2b). Nesprin-4 and KASH5 binding to SUN1 homotrimers display a similar configuration [32]. Nesprin-3, −4, and KASH5 have a leucine instead of a proline at position −11, allowing the rest of the KASH peptide to extend in a relatively straight configuration away from the SUN domain interface [15].

These results show that the different KASH peptides exhibit two distinct binding modes: the outward turn of nesprin-1 and −2 bound to SUN homotrimers, or the upward extension of nesprin-3, −4, and KASH5 bound to SUN homotrimers. One thing not explained by these structures is that the KASH peptide of nesprin-3 has a conserved −23 cysteine like nesprin-1 and nesprin-2, but it adopts the same binding mode as nesprin-4 and KASH5 [15]. Therefore, it is unclear how the −23 cysteine of nesprin-3 is positioned to form a disulfide bond with SUN2. It is possible that the crystal structures are capturing just a subset of a few possible different binding modes for each SUN-KASH pair.

It is currently unknown how these different binding modes influence the interaction of the LINC complex with the ONM. The length of the KASH peptide that protrudes out of the ONM and into the perinuclear space is relatively short, only 10–32 residues depending on the KASH protein [34]. For the longer KASH peptides, with conserved cysteines at position −23, there are 6–8 residues extending beyond the interaction with the SUN domain. These residues are usually acidic and are not captured in any crystal structures, but in molecular dynamic simulations, they can easily reach the ONM [35]. For the shorter KASH peptides, like KASH5 and *C. elegans* UNC-83, the linker is not long enough to reach the ONM after exiting the KASH lid of the SUN homotrimer without forcing the conserved hydrophobic patch present on outer surface of the KASH lid of the SUN domain into the lipid bilayer (Figure 2a and b) [35]. Further structural studies performed on full-length KASH and SUN proteins reconstituted in supported lipid bilayers are needed to understand how these different binding modes may affect LINC complex assembly.

Higher-order LINC complex assemblies

How, when, and why do multiple LINC complexes interact with each other? Higher-order LINC complex assemblies are known to form in cells and are necessary for performing biological functions. In fibroblasts polarizing for migration, SUN2 and nesprin-2G form linear arrays along dorsal perinuclear actin cables called transmembrane actin-associated nuclear (TAN) lines – this is necessary for rearward nuclear movement to occur [36,37]. SUN2 has also been found to form clusters as a part of the endoplasmic reticulum stress response [38]. In addition, higher-order SUN1-containing LINC complexes can form rings around nuclear envelope-associated meiotic telomeres, which are involved in meiotic chromosome pairing during meiotic prophase [39].

How do LINC complexes form higher-order assemblies, and how are the SUN and KASH proteins arranged in these assemblies? *In vivo* studies have shown that mouse SUN1 forms higher-order oligomers in the nuclear envelope [18,40]. Molecular dynamics simulations show that SUN1 homotrimers can interact laterally [41]. This has led to a model of SUN1 higher-order assemblies where multiple SUN1 homotrimers bound to KASH proteins in the ONM form an array in the nuclear envelope (Figure 2c). The oligomerization abilities of the germline-expressed SUN3–5 are less well understood than that of SUN1–2, though recent work suggests that SUN3 can form higher-order oligomers in the nuclear envelope, similar to SUN1 [42].

Recently, another model for higher-order LINC complex assemblies has been proposed: a 6:6 heterohexamer model, where the SUN protein homotrimers face each other in a "head-to-head" interaction (Figure 2d) [32]. The head-to-head model derives from the crystal structure of the human SUN domain of SUN1 or SUN2 bound to human KASH peptides, and is consistent with *in vitro* molecular weight and size determination data of purified SUN proteins and bound KASH peptides in solution [32]. In previously reported crystal structures of SUN2-KASH complexes, SUN homotrimers were packed in a head to head configuration within the crystals, with the hydrophobic patches present on the outside of their KASH lids of their SUN domains interacting with each other [15,21]. In a solution without a membrane present, it would be energetically favorable for these hydrophobic patches to be buried within the 6:6 interface, suggesting that this structure could represent an experimentally derived artifact. In the context of the nuclear envelope, these hydrophobic SUN domain residues would likely be brought into close proximity with the ONM (Figure 2b) [14]. Furthermore, fluorescence fluctuation spectroscopy (FFS) data and molecular dynamics simulations support a model where in the presence of a membrane, SUN1 homotrimers interact laterally within the nuclear envelope [40,41]. This allows the KASH peptides to easily reach the ONM (Figure 2c, inset). Dual-color FFS data of mouse SUN2 and the KASH peptide and transmembrane domain of mouse nesprin-2 support the model of the nesprin-2 KASH peptide binding to a homotrimer of SUN2 [43]. The head-to-head model also introduces a new issue: for the SUN domains to be angled for their head-to-head interaction, some KASH peptides would not be able to reach the ONM (Figure 2d, inset). Given the current data, we favor a model where SUN and KASH proteins interact in a 3:3 ratio with their interfaces immediately adjacent to the surface of the ONM. The discrepancies between these two models of higher-order LINC complex assembly highlight the need to further investigate the arrangement of LINC complexes in the nuclear envelope.

Regulation of LINC complex disassembly

Intact LINC complexes allow the cell to transmit forces from the cytoskeleton to the nucleus [3]. The regulation of LINC complex disassembly is one mechanism that the cell may use to tune force transmission across the nuclear envelope. For example, when human mesenchymal stem cells are placed under intense cyclic tensile strain, levels of SUN2 protein decrease, thus protecting the chromatin from these forces [44]. Increased mechanical force does not always result in reduced SUN protein levels - for example, increased cytoskeletal stiffness and increased extracellular matrix stiffness can promote SUN protein stability in some cell types [45,46]. The regulation of LINC complex assembly/disassembly

in response to various forces seems to depend on specific cellular contexts. In the case of LINC complex disassembly, there are a variety of models for how it is regulated. The AAA + ATPase torsinA is proposed to disassemble LINC complexes, though the exact aspect of LINC assembly it disrupts is unclear (Figure 1b) [47]. Increased calcium levels can also promote the disassembly of SUN homotrimers or promote the SUN-KASH interaction depending on the cellular context (Figue 1b) [17,25,48].

The proteasome has been shown to selectively degrade specific components of the LINC complex, including human SUN2 [49,50,51**–53]. SUN2 is a substrate of the Skip, Cullin, F-box-containing β-transducin repeat-containing ($SCF^{\beta TrCP}$) E3 Ligase (Figure 1a) [51**,54]. This degradation is specific to SUN2: the nucleoplasmic domain of SUN2, but not SUN1, contains a noncanonical recognition site for the $SCF^{\beta TrCP}$ ubiquitin ligase [51**]. Serines found near this site must be phosphorylated for SUN2 to be ubiquitinated [51**]. The phosphorylation of these serines is positively regulated by casein kinase 2 (CK2), and negatively regulated by the phosphatase C-terminal domain NE phosphatase 1 (CTDNEP1) (Figue 1a) [51**].

CTDNEP1 also regulates the proteasomal degradation of SUN2 through its ability to alter the lipid makeup of the INM [52]. When CTDNEP1 levels are decreased, the levels of diacylglycerol in the INM are also decreased, reducing lipid packing in the INM [52**]. This affects SUN2's association with the INM because SUN2 has an amphipathic helix in its nucleoplasmic domain [52**,55]. This helix binds to lipid membranes *in vitro* [52**]. Amphipathic helices tend to bind to membranes with packing defects, so the current model is that SUN2's weakened association with the INM promotes its degradation [52**]. This is also specific to SUN2, as SUN1 does not have this amphipathic helix [52**].

It is interesting that SUN2 is subjected to multiple layers of regulation, but there is little information about human SUN1 being regulated by proteasomal degradation. Given that SUN1 more readily forms higher-order assemblies in the nuclear envelope [18,40,41], one would expect that the cell would have a regulatory mechanism to tune the amount of SUN1 present within the nuclear envelope, and thus regulate when and where these larger assemblies would be able to form.

Proteasomal regulation is also applied to KASH proteins, including the giant isoform of nesprin-2 (nesprin-2G). When the nuclear envelope transiently ruptures during interphase in response to stress caused by cytoskeletal forces, the endosomal sorting complex required for transport (ESCRT) machinery binds to the rupture to re-seal the nuclear membranes [56,57]. BROX, a Bro1 domain protein that binds ESCRT III [58], is brought to the site of nuclear rupture, where it then ubiquitinates nesprin-2G [59**]. The proteasomal degradation of both SUN2 and nesprin-2G may assist in regulating the local forces that act upon the nuclear envelope. Degradation of nesprin-2G near nuclear envelope rupture sites allows for the rupture to be repaired more quickly – it is hypothesized that reducing the amount of mechanical stress at these sites by reducing nesprin-2G levels makes nuclear envelope repair easier [59**]. When the levels of $SCF^{\beta TrCP}$ E3 ligase are depleted, nuclei become misshapen [54]. Moreover, the expression of a non-degradable version of SUN2 results in the appearance of wrinkled nuclei [51**].

Degradation of LINC complex components is important for the cell to balance forces across the nuclear envelope. This type of regulation is not limited to human cells – the SUN protein Koi in Drosophila is also ubiquitinated and degraded by the proteasome [60]. Therefore, the specific degradation of LINC complex components is likely a general mechanism used by cells to regulate when and where specific LINC complexes are present within the nuclear envelope.

Conclusion

The LINC complex field is rapidly discovering an increasing array of regulatory mechanisms that cells use to control how LINC complexes are built and taken apart. This is generating many more questions about LINC complex assembly: How are the many different SUN and KASH proteins spatially arranged into functional complexes in the nuclear envelope? Which cellular conditions promote LINC complex assembly? How do cells dismantle or re-arrange LINC complexes? Uncovering new mechanisms, and deepening our understanding of currently studied mechanisms, will allow us to better understand the biology of the LINC complex and the many cellular processes it is involved in.

Acknowledgements

We apologize to our colleagues for not referencing all the primary literature due to space constraints. We thank members of the Starr-Luxton lab for helpful discussions. Research in the Starr-Luxton lab is supported by grant R35GM134859 to D.A.S. and GM129374 to G.W.G.L. from the National Institutes of Health and an Allen Distinguished Investigator Award, a Paul G. Allen Frontiers Group advised grant of the Paul G. Allen Family Foundation.

Data availability

No data was used for the research described in the article.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

* * of outstanding interest

1. Starr DA, Hermann GJ, Malone CJ, Fixsen W, Priess JR, Horvitz HR, Han M: unc-83 encodes a novel component of the nuclear envelope and is essential for proper nuclear migration. Development 2001, 128:5039–5050. [PubMed: 11748140]
2. Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, Burke B, Stahl PD, Hodzic D: Coupling of the nucleus and cytoplasm: role of the LINC complex. J Cell Biol 2006, 172:41–53. [PubMed: 16380439]
3. Starr DA, Fridolfsson HN: Interactions between nuclei and the cytoskeleton are mediated by SUN-KASH nuclear-envelope bridges. Annu Rev Cell Dev Biol 2010, 26:421–444. [PubMed: 20507227]
4. Chang W, Worman HJ, Gundersen GG: Accessorizing and anchoring the LINC complex for multifunctionality. J Cell Biol 2015, 208:11–22. [PubMed: 25559183]
5. Luxton GWG, Starr DA: KASHing up with the nucleus: novel functional roles of KASH proteins at the cytoplasmic surface of the nucleus. Curr Opin Cell Biol 2014, 28:69–75. [PubMed: 24704701]
6. Gundersen GG, Worman HJ: Nuclear positioning. Cell 2013, 152:1376–1389. [PubMed: 23498944]

7. Burke B, Roux KJ: Nuclei take a position: managing nuclear location. Dev Cell 2009, 17:587–597. [PubMed: 19922864]

8. Razafsky D, Hodzic D: Bringing KASH under the SUN: the many faces of nucleo-cytoskeletal connections. J Cell Biol 2009, 186:461–472. [PubMed: 19687252]

9. Starr DA, Han M: Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science 2002, 298:406–409. [PubMed: 12169658]

10. Meinke P, Schirmer EC: LINC'ing form and function at the nuclear envelope. FEBS Lett 2015, 589:2514–2521. [PubMed: 26096784]

11. Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R, Burridge K: Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. Nat Cell Biol 2014, 16:376–381. [PubMed: 24609268]

12. Méjat A, Misteli T: LINC complexes in health and disease. Nucleus 2010, 1:40–52. [PubMed: 21327104]

13*. Storey EC, Fuller HR: Genotype-phenotype correlations in human diseases caused by mutations of LINC complex-associated genes: a systematic review and meta-summary. Cells 2022, 11, 10.3390/cells11244065. This systematic review reveals associations between LINC complex proteins and LINC complex-associated proteins and human disease. This paper shows that many aspects of the LINC complex are important for human health, motivating further studies into the regulation of LINC complex assembly.

14. Jahed Z, Domkam N, Ornowski J, Yerima G, Mofrad MRK: Molecular models of LINC complex assembly at the nuclear envelope. J Cell Sci 2021, 134, 10.1242/jcs.258194.

15. Cruz VE, Esra Demircioglu F, Schwartz TU: Structural analysis of different LINC complexes reveals distinct binding modes. J Mol Biol 2020, 432:6028–6041. [PubMed: 33058875]

16. Stewart-Hutchinson PJ, Hale CM, Wirtz D, Hodzic D: Structural requirements for the assembly of LINC complexes and their function in cellular mechanical stiffness. Exp Cell Res 2008, 314:1892–1905. [PubMed: 18396275]

17*. Majumder S, Hsu Y-Y, Moghimianavval H, Andreas M, Giessen TW, Luxton GWG, Liu AP: In vitro synthesis and reconstitution using mammalian cell-free lysates enables the systematic study of the regulation of LINC complex assembly. Biochemistry 2022, 61:1495–1507. [PubMed: 35737522] This paper utilizes an *in vitro* synthetic biology platform and artificial nuclear membrane to investigate LINC complex assembly mechanisms using full-length SUN proteins. It shows that increased levels of calcium promote the SUN-KASH interaction. It also shows that the intermolecular SUN-KASH disulfide bond is important for stabilizing the SUNKASH interaction.

18. Lu W, Gotzmann J, Sironi L, Jaeger V-M, Schneider M, Lüke Y, Uhlén M, Szigyarto CA-K, Brachner A, Ellenberg J, et al. : Sun1 forms immobile macromolecular assemblies at the nuclear envelope. Biochim Biophys Acta 2008, 1783:2415–2426. [PubMed: 18845190]

19. Xiong H, Rivero F, Euteneuer U, Mondal S, Mana-Capelli S, Larochelle D, Vogel A, Gassen B, Noegel AA: Dictyostelium Sun-1 connects the centrosome to chromatin and ensures genome stability. Traffic 2008, 9:708–724. [PubMed: 18266910]

20. Wang Q, Du X, Cai Z, Greene MI: Characterization of the structures involved in localization of the SUN proteins to the nuclear envelope and the centrosome. DNA Cell Biol 2006, 25:554–562. [PubMed: 17132086]

21. Sosa BA, Rothballer A, Kutay U, Schwartz TU: LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins. Cell 2012, 149:1035–1047. [PubMed: 22632968]

22. Nie S, Ke H, Gao F, Ren J, Wang M, Huo L, Gong W, Feng W: Coiled-coil domains of SUN proteins as intrinsic dynamic regulators. Structure 2016, 24:80–91. [PubMed: 26688217]

23. Turgay Y, Ungricht R, Rothballer A, Kiss A, Csucs G, Horvath P, Kutay U: A classical NLS and the SUN domain contribute to the targeting of SUN2 to the inner nuclear membrane. EMBO J 2010, 29:2262–2275. [PubMed: 20551905]

24. Xu Y, Li W, Ke H, Feng W: Structural conservation of the autoinhibitory domain in SUN proteins. Biochem Biophys Res Commun 2018, 496:1337–1343. [PubMed: 29408528]

25. Jahed Z, Vu UT, Fadavi D, Ke H, Rathish A, Kim SCJ, Feng W, Mofrad MRK: A molecular model for LINC complex regulation: activation of SUN2 for KASH binding. Mol Biol Cell 2018, 29:2012–2023. [PubMed: 29995584]
26. Jahed Z, Shams H, Mofrad MRK: A disulfide bond Is required for the transmission of forces through SUN-KASH complexes. Biophys J 2015, 109:501–509. [PubMed: 26244732]
27. Cain NE, Jahed Z, Schoenhofen A, Valdez VA, Elkin B, Hao H, Harris NJ, Herrera LA, Woolums BM, Mofrad MRK, et al. : Conserved SUN-KASH Interfaces mediate LINC complexdependent nuclear movement and positioning. Curr Biol 2018, 28:3086–3097.e4. [PubMed: 30245107]
28*. Sharma R, Hetzer MW: Disulfide bond in SUN2 regulates dynamic remodeling of LINC complexes at the nuclear envelope. Life Sci Alliance 2023, 6, 10.26508/lsa.202302031. This paper shows that the intermolecular SUN-KASH disulfide bond is important for SUN2 stability – mutant versions of SUN2 that cannot form this bond have accelerated protein turnover. This study shows that the disulfide bond is not just important for the mechanical strength of the LINC complex, but also plays a role in SUN2 protein turnover. This paper also reports a conformation-specific antibody that recognizes unbound SUN2 molecules – this is a valuable tool for investigating LINC complex assembly in cells.
29. Galligan JJ, Petersen DR: The human protein disulfide isomerase gene family. Hum Genom 2012, 6:6.
30* *. Kucińska MK, Fedry J, Galli C, Morone D, Raimondi A, Soldà T , Förster F, Molinari M: TMX4-driven LINC complex disassembly and asymmetric autophagy of the nuclear envelope upon acute ER stress. Nat Commun 2023, 14:3497. [PubMed: 37311770] This paper identifies TMX4 as a regulator of the intermolecular SUNKASH disulfide bond. This is the first study showing that a protein disulfide isomerase acts on nesprins to break the intermolecular SUNKASH disulfide bond.
31. Cheng L-C, Baboo S, Lindsay C, Brusman L, Martinez-Bartolomé S, Tapia O, Zhang X, Yates JR 3rd, Gerace L: Identification of new transmembrane proteins concentrated at the nuclear envelope using organellar proteomics of mesenchymal cells. Nucleus 2019, 10:126–143. [PubMed: 31142202]
32. Gurusaran M, Davies OR: A molecular mechanism for LINC complex branching by structurally diverse SUN-KASH 6:6 assemblies. Elife 2021, 10, 10.7554/eLife.60175.
33. Wang W, Shi Z, Jiao S, Chen C, Wang H, Liu G, Wang Q, Zhao Y, Greene MI, Zhou Z: Structural insights into SUN-KASH complexes across the nuclear envelope. Cell Res 2012, 22:1440–1452. [PubMed: 22945352]
34. Sosa BA, Kutay U, Schwartz TU: Structural insights into LINC complexes. Curr Opin Struct Biol 2013, 23:285–291. [PubMed: 23597672]
35. Jahed Z, Hao H, Thakkar V, Vu UT, Valdez VA, Rathish A, Tolentino C, Kim SCJ, Fadavi D, Starr DA, et al. : Role of KASH domain lengths in the regulation of LINC complexes. Mol Biol Cell 2019, 30:2076–2086. [PubMed: 30995155]
36. Luxton GWG, Gomes ER, Folker ES, Vintinner E, Gundersen GG: Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. Science 2010, 329:956–959. [PubMed: 20724637]
37. Luxton GWG, Gomes ER, Folker ES, Worman HJ, Gundersen GG: TAN lines: a novel nuclear envelope structure involved in nuclear positioning. Nucleus 2011, 2:173–181. [PubMed: 21818410]
38. Vidak S, Serebryannyy LA, Pegoraro G, Misteli T: Activation of endoplasmic reticulum stress in premature aging via the inner nuclear membrane protein SUN2. Cell Rep 2023, 42:112534. [PubMed: 37210724]
39. Ding X, Xu R, Yu J, Xu T, Zhuang Y, Han M: SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. Dev Cell 2007, 12:863–872. [PubMed: 17543860]
40. Hennen J, Saunders CA, Mueller JD, Luxton GWG: Fluorescence fluctuation spectroscopy reveals differential SUN protein oligomerization in living cells. Mol Biol Cell 2018, 29:1003–1011. [PubMed: 29514929]

41. Jahed Z, Fadavi D, Vu UT, Asgari E, Luxton GWG, Mofrad MRK: Molecular Insights into the mechanisms of SUN1 oligomerization in the nuclear envelope. Biophys J 2018, 114:1190–1203. [PubMed: 29539404]

42. Hennen J, Hur K-H, Karuka SR, Luxton GWG, Mueller JD: Protein oligomerization and mobility within the nuclear envelope evaluated by the time-shifted mean-segmented Q factor. Methods 2019, 157:28–41. [PubMed: 30268407]

43. Hennen J, Hur K-H, Kohler J, Reddy Karuka S, Angert I, Luxton GWG, Mueller JD: Identifying heteroprotein complexes in the nuclear envelope. Biophys J 2020, 118:26–35. [PubMed: 31839257]

44. Gilbert HTJ, Mallikarjun V, Dobre O, Jackson MR, Pedley R, Gilmore AP, Richardson SM, Swift J: Nuclear decoupling is part of a rapid protein-level cellular response to high-intensity mechanical loading. Nat Commun 2019, 10:4149. [PubMed: 31515493]

45. Belaadi N, Pernet L, Aureille J, Chadeuf G, Rio M, Vaillant N, Vitiello E, Lafanechère L, Loirand G, Guilluy C: SUN2 regulates mitotic duration in response to extracellular matrix rigidity. Proc Natl Acad Sci USA 2022, 119, e2116167119. [PubMed: 36322767]

46. Mu X, Tseng C, Hambright WS, Matre P, Lin C-Y, Chanda P, Chen W, Gu J, Ravuri S, Cui Y, et al. : Cytoskeleton stiffness regulates cellular senescence and innate immune response in Hutchinson-Gilford Progeria Syndrome. Aging Cell 2020, 19, e13152. [PubMed: 32710480]

47. Saunders CA, Harris NJ, Willey PT, Woolums BM, Wang Y, McQuown AJ, Schoenhofen A, Worman HJ, Dauer WT, Gundersen GG, et al. : TorsinA controls TAN line assembly and the retrograde flow of dorsal perinuclear actin cables during rearward nuclear movement. J Cell Biol 2017, 216:657–674. [PubMed: 28242745]

48. Lombardi ML, Jaalouk DE, Shanahan CM, Burke B, Roux KJ, Lammerding J: The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton. J Biol Chem 2011, 286:26743–26753. [PubMed: 21652697]

49. Ji J, Shen J, Xu Y, Xie M, Qian Q, Qiu T, Shi W, Ren D, Ma J, Liu W, et al. : FBXO2 targets glycosylated SUN2 for ubiquitination and degradation to promote ovarian cancer development. Cell Death Dis 2022, 13:442. [PubMed: 35525855]

50. Loveless TB, Topacio BR, Vashisht AA, Galaang S, Ulrich KM, Young BD, Wohlschlegel JA, Toczyski DP: DNA damage regulates translation through β-TRCP targeting of CReP. PLoS Genet 2015, 11, e1005292. [PubMed: 26091241]

51* *. Krshnan L, Siu WS, Van de Weijer M, Hayward D, Guerrero EN, Gruneberg U, Carvalho P: Regulated degradation of the inner nuclear membrane protein SUN2 maintains nuclear envelope architecture and function. Elife 2022, 11, 10.7554/eLife.81573. This paper shows that the proteasomal degradation of SUN2 is dependent upon the phospohorylation of non-canonical recognition sites present within SUN2's nucleoplasmic domain for the $SCF^{\beta TrCP}$ E3 Ligase. This study provides new details about the process of proteasomal regulation of SUN2.

52* *. Lee S, Carrasquillo Rodrõ Guez JW, Merta H, Bahmanyar S: A membrane-sensing mechanism links lipid metabolism to protein degradation at the nuclear envelope. J Cell Biol 2023, 222, 10.1083/jcb.202304026. This paper shows that the lipid composition of the INM affects the stability of SUN2, but not SUN1, promoting the degradation of SUN2 by the proteasome. This opens future avenues of investigation into how the lipids that compose the nuclear envelope affect LINC complex assembly.

53. Kim TY, Siesser PF, Rossman KL, Goldfarb D, Mackinnon K, Yan F, Yi X, MacCoss MJ, Moon RT, Der CJ, et al. : Substrate trapping proteomics reveals targets of the βTrCP2/FBXW11 ubiquitin ligase. Mol Cell Biol 2015, 35:167–181. [PubMed: 25332235]

54. Coyaud E, Mis M, Laurent EMN, Dunham WH, Couzens AL, Robitaille M, Gingras A-C, Angers S, Raught B: BioID-based identification of skp Cullin F-box (SCF)β-TrCP1/2 E3 ligase substrates. Mol Cell Proteomics 2015, 14:1781–1795. [PubMed: 25900982]

55. Majumder S, Willey PT, DeNies MS, Liu AP, Luxton GWG: A synthetic biology platform for the reconstitution and mechanistic dissection of LINC complex assembly. J Cell Sci 2018, 132, 10.1242/jcs.219451.

56. Raab M, Gentili M, de Belly H, Thiam HR, Vargas P, Jimenez AJ, Lautenschlaeger F, Voituriez R, Lennon-Duménil AM, Manel N, et al. : ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. Science 2016, 352:359–362. [PubMed: 27013426]

57. Denais CM, Gilbert RM, Isermann P, McGregor AL, te Lindert M, Weigelin B, Davidson PM, Friedl P, Wolf K, Lammerding J: Nuclear envelope rupture and repair during cancer cell migration. Science 2016, 352:353–358. [PubMed: 27013428]

58. Ichioka F, Takaya E, Suzuki H, Kajigaya S, Buchman VL, Shibata H, Maki M: HD-PTP and Alix share some membranetraffic related proteins that interact with their Bro1 domains or proline-rich regions. Arch Biochem Biophys 2007, 457:142–149. [PubMed: 17174262]

59* *. Wallis SS, Ventimiglia LN, Otigbah E, Infante E, Cuesta-Geijo MA, Kidiyoor GR, Carbajal MA, Fleck RA, Foiani M, Garcia-Manyes S, et al. : The ESCRT machinery counteracts Nesprin-2G-mediated mechanical forces during nuclear envelope repair. Dev Cell 2021, 56:3192–3202.e8. [PubMed: 34818527] This study shows how cells can selectively degrade nesprin-2G in response to nuclear damage caused by mechanical forces. Nuclear ruptures are sensed by ESCRT machinery, which recruits the protein BROX that subsequently ubiquitinates nesprin-2G. This paper identified a new pathway by which cells can regulate the amount and composition of their LINC complexes.

60. Tan KL, Haelterman NA, Kwartler CS, Regalado ES, Lee P-T, Nagarkar-Jaiswal S, Guo D-C, Duraine L, Wangler MF, University of Washington Center for Mendelian Genomics, et al. : Ari-1 regulates myonuclear organization together with parkin and Is associated with aortic aneurysms. Dev Cell 2018, 45:226–244.e8. [PubMed: 29689197]

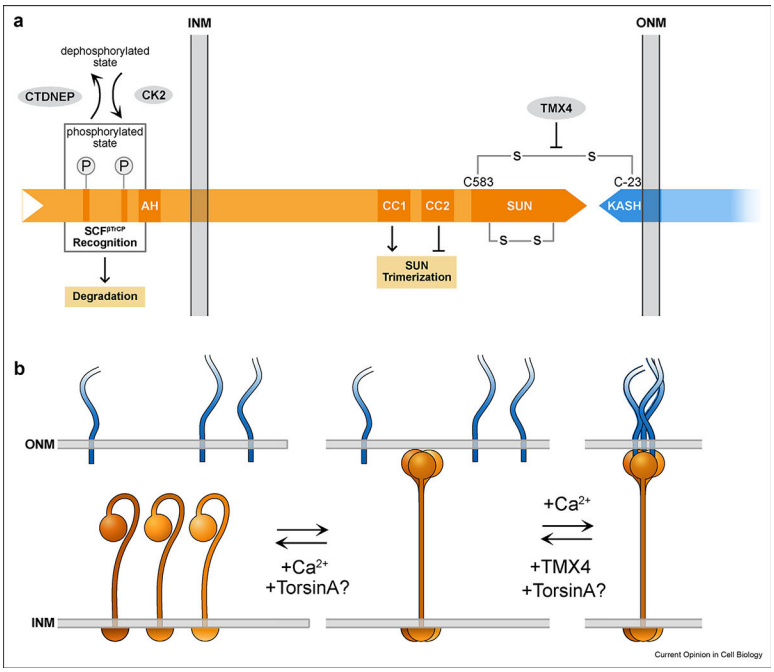

Figure 1. Points of regulation for LINC complex assembly and disassembly

(**a**) Diagram of the SUN-KASH interaction's points of regulation. The domains of human SUN2 are in orange, and the KASH peptide is in blue. Proteins are drawn as arrows pointing from the N- to the C-terminus. The inner nuclear membrane (INM) and the outer nuclear membrane (ONM) are drawn as grey bars. The nucleoplasmic portion of SUN2 contains regulatory sites for proteasomal degradation, including two phosphorylation sites and an amphipathic helix (AH). The luminal portion of SUN2 contains coiled-coil regions (CC1 and CC2) that are important for the regulation of SUN trimerization. The SUN-KASH interaction includes an inter-molecular disulfide bond between SUN2's C583 and the KASH peptide's cysteine at position −23 (23 amino acids from the C-terminus). (**b**) Model of LINC complex assembly. The SUN domains (orange spheres) can be in an autoinhibited state by interacting with the rest of its luminal domain (orange stalk). The second step of LINC assembly is trimerization of the SUN proteins. The core of LINC complex assembly is complete with the KASH peptides bind to the SUN trimers. In some contexts, increased levels of calcium lead to decreased trimerization of SUN proteins. TorsinA is thought to break apart LINC complexes, though the stage it acts upon is unknown. TMX4 breaks the inter-molecular disulfide bond between SUN and KASH, weakening their interaction.

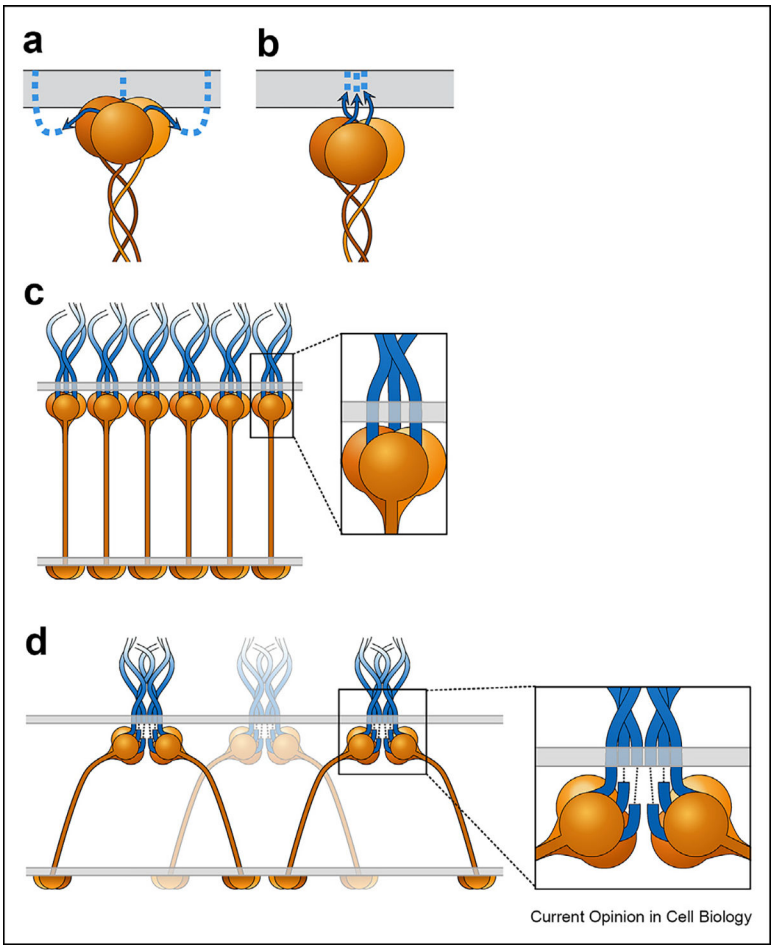

Figure 2. LINC complex binding modes and higher-order assembly models

(**a**) Binding mode 1. Here the N-terminus of the KASH peptide turns downwards (towards the SUN N-terminus) as it extends past the binding interface. (**b**) Binding mode 2. The N-termini of these KASH proteins do not make a turn, and instead extend up towards the outer nuclear membrane. (**c**) Higher-order LINC assembly. In this model, multiple LINC complexes associate laterally. This configuration of LINC complexes allows the KASH peptide to reach its binding interface in the SUN trimer while still being inserted into the INM (inset). (**d**) Head-to-head model. In this model the SUN trimers of neighboring LINC complexes face each other and bind in a “head-to-head” orientation. This SUN-to-SUN interaction as well as the binding of three KASH peptides to each SUN trimer forms a hetero-hexamer. With the SUN trimer angled away from the ONM, the KASH peptides are likely too short to both bind to the SUN domain and to have their transmembrane domains inserted into the ONM. This discrepancy in KASH peptide lengths is shown as dashed lines (inset).