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OPINION

SUN-domain proteins: 'Velcro' that links the nucleoskeleton to the cytoskeleton

Yonatan B. Tzur, Katherine L. Wilson and Yosef Gruenbaum

Abstract | The novel SUN-domain family of nuclear envelope proteins interacts with various KASH-domain partners to form SUN-domain-dependent 'bridges' across the inner and outer nuclear membranes. These bridges physically connect the nucleus to every major component of the cytoskeleton. SUN-domain proteins have diverse roles in nuclear positioning, centrosome localization, germ-cell development, telomere positioning and apoptosis. By serving both as mechanical adaptors and nuclear envelope receptors, we propose that SUN-domain proteins connect cytoplasmic and nucleoplasmic activities.

For a discussion of the SUN-domain (Sad1 and UNC-84 homology domain) family of proteins, we must first introduce the nuclear envelope. In all eukaryotic cells, the nuclear envelope separates the nucleus from the cytoplasm. The nuclear envelope is composed of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM). The two membranes join at nuclear pore complexes, which control the traffic of macromolecules between the nucleoplasm and the cytoplasm. In metazoans, the nuclear pore complexes and the INM are anchored to a structural network of lamin filaments[1] (BOX 1). Lamin polymers confer mechanical strength to the nucleus[2,3]. Many nuclear membrane and nucleoplasmic proteins interact with lamins[4], and lamin-associated protein complexes support a broad range of functions[4] (BOX 1). At least 80 unique integral membrane proteins were found to localize at the nuclear envelope in mammalian cells[5]. It is assumed that most of these proteins interact directly or indirectly with lamins[4]. Among these nuclear membrane proteins are three families, each of which is characterized by a distinct motif (specifically, LEM, SUN or KASH). LEM-domain proteins (named after LAP2, emerin and MAN1) are reviewed elsewhere[4]. We will discuss proteins that contain the SUN domain and their partners, many of which contain the KASH (named after Klarsicht, ANC-1 and SYNE1 homology) domain[6–8].

Most (but not all) SUN-domain proteins are localized at the INM. The situation is more complicated for KASH-domain proteins, most individual isoforms of which are localized on either the ONM or the INM[8,9]. However, some isoforms lack the KASH domain, do not localize to the nuclear envelope and are instead proposed to tether other organelles to the cytoskeleton[8]. Several INM-localized SUN-domain and KASH-domain proteins can interact with lamins, and in certain cases this interaction is required for their nuclear envelope localization (see below).

This perspective will focus primarily on SUN-domain proteins: we will depict their structural organization (known and hypothetical) in complexes that traverse the nuclear envelope, and discuss their roles in nuclear positioning, centrosome attachment to the ONM, links to the cytoskeleton, and telomere positioning during meiosis. We will propose a model in which SUN-domain proteins serve as mechanical 'Velcro' to interconnect the cytoskeleton and nucleoskeleton, and suggest that SUN-domain proteins have further, non-mechanical roles as specialized nuclear envelope receptors.

SUN-domain and KASH-domain proteins
Both the SUN domain and regions that are upstream of the SUN domain interact directly with the KASH domain of KASH-domain proteins[10,11]. This interaction is required for the cellular functions of both types of protein.

Domain organization of SUN-domain proteins. Malone and colleagues[12] coined the term SUN domain when they discovered a motif of ~120 residues in the C terminus of the *Caenorhabditis elegans* UNC-84 protein. This protein has significant homology to a region in the *Schizosaccharomyces pombe* Sad1 protein and several uncharacterized mammalian proteins. Genome-database

searches indicated that UNC-84 is conserved and that the number of SUN-domain proteins has increased over the course of evolution (FIG. 1). For example, the *S. pombe* genome contains a single SUN-domain gene, whereas *C. elegans* and *Drosophila melanogaster* each have two, and mammals have at least four SUN-domain genes, *SUN1*, *SUN2*, *SUN3* and *SPAG4* (REF. 8).

Besides the SUN domain, members of this family share other structural features to varying extents (FIG. 2). Most SUN-domain proteins have at least one predicted transmembrane domain. Several span the membrane multiple times, which is a potentially useful property for proteins that are proposed to anchor mechanical-load-bearing structures and transmit mechanical force. Human SUN1 spans the membrane three times[13]. SUN1 has an additional hydrophobic region that is conserved in SUN2, but is not membrane inserted in either protein; the function of this hydrophobic region is unknown[13,20]. *Caenorhabditis elegans* UNC-84, which is localized at the INM, is predicted to have as many as nine hydrophobic domains (FIG. 2), but how many actually span the INM is unknown. Strangely, both of the SUN-domain proteins in *D. melanogaster*, which are uncharacterized, seem to lack transmembrane domains. Last, most SUN-domain proteins have at least one coiled-coil domain, which in human SUN1 is proposed to mediate homodimerization[13]. There is also the unexplored possibility that SUN-domain proteins might form heterodimers, as depicted speculatively in FIG. 3.

The dimerization idea is important, because each SUN-domain dimer could directly anchor two KASH-domain partners and thereby significantly enhance the mechanical stability of protein complexes that bridge the nuclear envelope. Furthermore, assuming one-to-one binding of the SUN and KASH domains, SUN dimers could interact with two KASH proteins in the same membrane (for example, the ONM), or on opposite membranes (the ONM and the INM).

Domain organization of KASH-domain proteins. *Caenorhabditis elegans* encode three known KASH-domain proteins (ANC-1, UNC-83 and ZYG-12), all of which have a C-terminal transmembrane domain followed by the ~35-residue KASH domain. The KASH domain also is present in several vertebrate and invertebrate proteins, including those encoded by *MSP300* and *Klarsicht* in *D. melanogaster* and the *Nesprin-1* (also known as *CPG2*, *syne-1*, *myne-1* and

Box 1 | **The lamins**

Lamins are type-V intermediate filament proteins. They are the main structural constituents of the metazoan nuclear lamina, a protein meshwork that lies between the inner nuclear membrane and chromatin. Lamins are also present in the nucleoplasm[1,48]. Lamins are divided into A and B types on the basis of their expression patterns and protein structure. B-type lamins are essential proteins that are encoded by two genes in mammals (*LMNB1* and *LMNB2*) and are present in all metazoan cells. By contrast, A-type lamins (for example, lamin A and C) are derived by alternative splicing of a single gene (*LMNA* in human) that is found only in more complex metazoans; this gene is expressed mainly in differentiated cells and is not essential for cell viability[49]. B-type lamins and lamin A (but not lamin C) are translated as precursors with a C-terminal CAAX motif. The cysteine residue in the CAAX motif is farnesylated, the last three residues (AAX) are subsequently removed by a zinc-metalloprotease and the cysteine is then methyl-esterified. To produce mature lamin A, the last 15 residues are then cleaved by the zinc-metalloprotease Zmste24 (REF. 50).

Lamin polymers confer mechanical strength to the nucleus[2,3]. Lamins also interact with many different nuclear proteins[51] and are involved in chromatin organization, DNA replication, transcription, assembly and disassembly of the nuclear envelope, spacing of nuclear pore complexes and apoptosis[52]. Mutations in the human *LMNA* gene affect muscle, adipose, bone, nerve and skin cells and are responsible for at least 12 heritable diseases, collectively termed laminopathies, that include muscular dystrophies and premature ageing[49,53,54].

Enaptin), *Nesprin-2* (also known as *syne-2* and *NUANCE*) and *Nesprin-3* genes in mammals[8]. Many KASH-domain proteins are enormous (>1 MDa)[9]. They are also diverse; for example, the *Nesprin-1* and *Nesprin-2* genes each produce more than 12 protein isoforms (of which some are small or lack the KASH domain) through alternative transcription initiation and transcription termination, or alternative pre-mRNA splicing[9]. Nesprin proteins also have numerous spectrin-repeat domains, which are thought to confer an extended configuration; the largest isoforms at the nuclear envelope are estimated to extend up to 500 nm into the cytoplasm[8]. Nesprins and other KASH-domain proteins have recently been reviewed in detail[8,9]. With a few exceptions, our discussion is limited to Nesprin isoforms for which SUN-domain interactions have been characterized.

Subcellular localization

Current evidence indicates that INM-positioned SUN-domain proteins interact with ONM-positioned KASH-domain proteins, and in this manner create protein 'bridges' that span both nuclear membranes[8,14]. Other SUN-domain and KASH-domain proteins might be associated with other organelles[8,13,15]. So, a precise knowledge of the localization and topology for each protein is needed to understand their roles.

SUN-domain proteins. The endogenous *S. pombe* Sad1 protein localizes *in vivo* to the spindle pole body (SPB; the yeast microtubule-organizing centre)[16]. However, when ectopically expressed, Sad1 also localizes at the nuclear envelope[16]. In metazoan

cells, most tested endogenous SUN-domain proteins localize at the nuclear envelope during interphase (see below). Their exact topology within the envelope is the focus of much interest, as therein lies the key to their proposed nuclear envelope 'bridging' activities[14,17]. Immunogold electron microscopy (EM) analysis of the *C. elegans* matefin (also known as SUN-1) protein localized its N terminus to the INM, but its exact topology was not determined[18]. The N-terminal domain of mouse and human SUN1 is sufficient to target SUN1 to the nuclear envelope[11,15,19] and also confers direct binding to A-type lamins[13,19], which hints at a potential localization mechanism. However, SUN1 localization seems to be lamin A independent *in vivo*[11,13,15,19] (see below). The N-terminal domain of human SUN2 also binds A-type lamins[13], and its C-terminal SUN domain has been localized to the lumenal space between the INM and the ONM in human HeLa cells[20]. These findings indicate that SUN1 and SUN2 have similar topologies, with their N-terminal domains in the nucleoplasm and their SUN domains in the lumen of the nuclear envelope (FIG. 3). Whether this topology applies to other SUN-domain proteins is not known.

All studied metazoan SUN-domain proteins colocalize with lamins, as determined by indirect immunofluorescence[11,13,14,18,20]. Whereas SUN1 and SUN2 can bind lamins directly[13,19], others cannot (for example, *C. elegans* UNC-84 (REF. 14)). Paradoxically, the second *C. elegans* SUN-domain protein, matefin/SUN-1, can bind Ce-lamin (a B-type lamin) directly *in vitro* but does not seem to depend on Ce-lamin for its nuclear envelope localization *in vivo*[18].
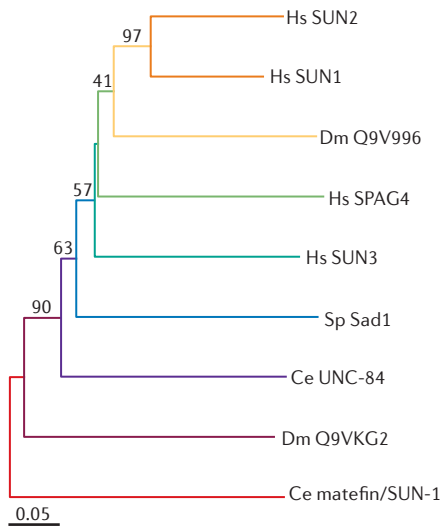
Figure 1 | **Phylogenetic relationships among SUN-domain proteins.** Phylogenetic analysis of SUN-domain amino-acid sequences from human (Hs), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce) and *Schizosaccharomyces pombe* (Sp) shows several statistically significant branching events. Human SUN-domain proteins fall into two subgroups. One includes the inner-nuclear-membrane-localized SUN1 and SUN2 (which probably originated from a close common ancestor) and the other more ancient group includes SPAG4 and SUN3, which localize in the endoplasmic reticulum and the outer nuclear membrane. *Drosophila melanogaster* Q9V996 is related to both sets of human proteins and might represent the shared ancestor of human SUN-domain proteins. *Caenorhabditis elegans* UNC-84 and *S. pombe* Sad1 are significantly closer to the human proteins than to *D. melanogaster* Q9VKG2 or *C. elegans* matefin/SUN-1. This analysis was done by aligning the amino-acid sequences of each SUN domain using ClustalW[55] with default alignment parameters. Neighbour-joining phylogenetic analysis was applied to the conserved core of these alignments (corresponding to residues 675–810 of human SUN1) using default parameters of Bootstrap tree analysis (1,000 trials). The tree was plotted using 'njplot' and processed with Adobe Illustrator 11.0.0. The bootstrap values for 1,000 trials are shown. Numbers at the branch points indicate the percentage bootstrap support calculated from 1,000 trees. The scale bar indicates genetic distance and is equivalent to 5% amino-acid diversity.

Are lamins sufficient to localize SUN1 at the nuclear envelope? The answer to this question has been surprisingly elusive. Mammalian SUN1 remained localized at the nuclear envelope in mouse fibroblasts that were devoid of A-type lamins (*Lmna*-knockout cells), but this could have been due to weak interactions with B-type lamins[11]. SUN1 also remained localized in cells that

are downregulated for both A-type lamins and lamin B1 (REF. 15), but this might be explained by the incomplete knockdown of lamin expression, compensatory binding to lamin B2 encoded by a different gene (BOX 1), or a putative lamin-independent retention mechanism. SUN2 also remained localized in HeLa cells that are downregulated for A-type lamins. However, it was frequently lost from the nuclear envelope of *Lmna*-knockout fibroblasts[13]. SUN2 was also identified as a predicted stable component of the nuclear lamina network in a large-scale proteomic analysis of mammalian nuclear envelope proteins[5], and biochemical extraction experiments show that SUN2 is not extracted by detergent or 8 M urea alone, but can be extracted by detergent and 8 M urea. These results are consistent with binding to lamins but do not rule out other anchoring mechanisms[20]. The retention mechanisms for SUN-domain proteins might hold some surprises, as 'rescue' experiments indicate that lamin interactions alone are insufficient[13], and cells might therefore use a novel mechanism(s) to retain SUN-domain proteins at the nuclear envelope. The human SUN3 (REF. 13) and SPAG4 (also known as SUN4)[15] proteins, when expressed from tagged transgenes, did not localize specifically at the nuclear envelope but instead localized throughout the endoplasmic reticulum (ER)[13,15]. The functions of ER-localized SUN-domain proteins are unknown. However, we speculate that ER/ONM-localized (the ONM is part of the ER network) SUN proteins might also form nuclear-envelope-bridging complexes, in combination with INM-localized KASH-domain proteins (see below).

KASH-domain proteins. Different Nesprin isoforms localize to different parts of the nuclear envelope[9]. In immunogold EM experiments, the KASH domain of Nesprin-1 isoforms localized to the nuclear membranes, but this analysis did not discriminate between INM-localized versus ONM-localized isoforms[21]. In a similar analysis, the KASH domain of Nesprin-2 isoforms localized to both the INM and the ONM[22,23], which is consistent with Nesprin-2 isoforms that extend into either the cytoplasm or nucleoplasm[24]. Immunogold localization of green fluorescent protein (GFP) in keratinocytes that expressed GFP fused to the N-terminal domain of Nesprin-3 localized this protein to the ONM[25].

The localization of KASH-domain proteins at the ONM requires their SUN-domain partners on the INM. For example, the KASH domain of ANC-1 localizes to the

ONM in an UNC-84-dependent manner, because mutations in UNC-84 displaced ANC-1 from the ONM[26]. Likewise, the localization of the Nesprin-2 giant isoform at the nuclear envelope requires both human SUN1 and SUN2 (REFS 11,13). The localization of Nesprin-2 giant and Klarsicht at the ONM also depends, at least indirectly, on A-type lamins, as these KASH-domain proteins each mislocalize in cells in which A-type lamins are downregulated[13,22,27,28]. The KASH domain is both essential to target large KASH-domain proteins to the nuclear envelope, and sufficient to localize to the nuclear envelope when expressed alone[10,21].

The Nesprin-1α isoform localizes to the INM[29]. Its nucleoplasmic domain binds lamin A *in vitro*, and its nuclear envelope localization requires A-type lamins[30]. Nesprin-1α also has very high (4 nM) affinity for the nuclear membrane protein emerin *in vitro*[29], the localization of which also requires A-type lamins[31]. This INM-localized KASH-domain protein adds another dimension to the nucleoskeletal side of nuclear-envelope-bridging complexes, as it binds the nuclear lamina network both directly (by binding lamin A) and indirectly (by binding emerin). However, we do not know how Nesprin-1α at the INM connects to SUN-domain proteins. Indeed, current evidence does not exclude a potential dual localization of Nesprin-1α also on the ONM. In cardiomyocytes, Nesprin-1α associates with a nuclear-envelope-localized signalling complex that includes both the muscle-specific A-kinase anchoring protein (mAKAP) and the ryanodine receptor, an ER/ONM-localized Ca^{2+} channel that is essential for muscle-cell contraction. Whether Nesprin-1α is emerin associated, or localizes to the INM versus the ONM, was not determined in cardiomyocytes[32]. Endogenous Nesprin-2 also requires A-type lamins to localize at the nuclear envelope, as it mislocalizes in human SW-13 cells, which naturally lack A-type lamins[23].

Linking the nucleus and the cytoskeleton
SUN-domain proteins regulate the position of the nucleus in the cell[10,26,33,34]. The hypodermis tissue of *C. elegans* arises from the fusion of precursor cells to form a multinucleated syncytium. In this syncytium, nuclei are evenly spaced and remain separated even when the worm is motionless. Mutations in either UNC-84 or its binding partner, the KASH-domain protein ANC-1, disrupt nuclear attachment to the actin cytoskeleton, and these untethered nuclei move freely in the shared cytoplasm

until they clump together[26]. UNC-84 and another KASH-domain partner, UNC-83, are also required for developmentally regulated nuclear migrations in hyp7 cells, gut primordial cells and P-cell precursors[12]. However, in these cases, the mutations probably disrupt an interaction between the nucleus and microtubule-dependent motors, which are required for directed movement of the nucleus. Nuclear migration failure in P-cells eventually leads to P-cell death and uncoordinated movement[35]. So, a single SUN-domain protein in *C. elegans*, UNC-84, links the nuclear envelope to two different elements of the cytoskeleton. The simple explanation for this dual function is that UNC-84 can bind independently to either of two KASH-domain partners, ANC-1 and UNC-83, that in turn link to actin and microtubules, respectively.

The other *C. elegans* SUN-domain protein, matefin/SUN-1, links the nuclear envelope to a third cytoskeletal element — the centrosome or microtubule-organizing centre — through ZYG-12, a nuclear-envelope-localized KASH-domain protein. ZYG-12 also binds centrosomes, and its interaction with matefin/SUN-1 is required to attach centrosomes to the nuclear envelope during embryogenesis[36]. Matefin/SUN-1 is expressed only in the germ line; the gene product is deposited maternally and the protein persists in the nuclear envelope through to late embryogenesis[18]. Matefin/SUN-1 is probably not required for centrosome attachment later in development, as centrosomes localize normally in the germ cells of worms that are homozygous for a *mtf-1/sun-1* deletion (A. Fridkin and Y.G., unpublished observations).

KASH-domain proteins bind the cytoskeleton. In addition to their KASH and spectrin-repeat domains, large KASH-domain proteins also have a functional domain that confers direct binding to a specific element of the cytoskeleton. ANC-1, MSP-300, Nesprin-1 giant and Nesprin-2 giant each have two calponin-homology domains at their N terminus, which bind actin filaments in the cytoplasm[8] (FIG. 3). Most small isoforms of Nesprin-1 and Nesprin-2 lack this domain and presumably do not bind actin. Interestingly, Nesprin-3 also lacks the actin-binding domain but instead binds plectin, a protein that links cytoplasmic intermediate filaments to filamentous (F)-actin[25]. Klarsicht, a *D. melanogaster* Nesprin protein, also lacks an actin-binding domain, but instead binds microtubules[27]. So, KASH-domain proteins collectively
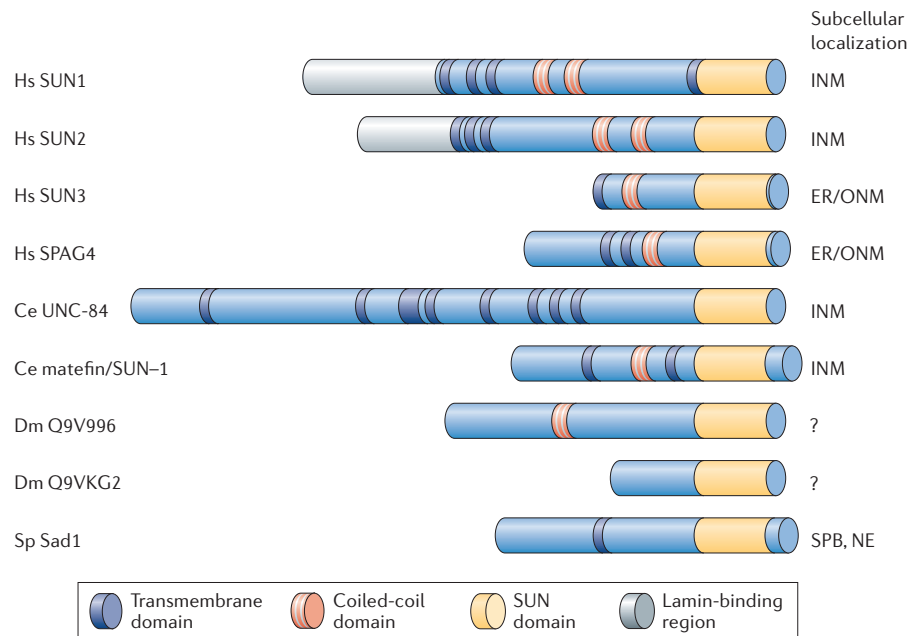

Figure 2 | **Known functional domains in SUN-domain proteins.** Each protein and its predicted domains are depicted to scale. SUN domains and the coiled-coil domains were predicted using the SMART database. The lamin-binding regions in SUN1 and SUN2 (REF. 13) are indicated as well as the subcellular localizations[11,13,15,16,18–20]. Transmembrane domains were predicted using TMPRED with the lowest threshold, which therefore probably includes hydrophobic regions that do not act as transmembrane domains *in vivo*. The number of actual transmembrane domains and the roles of non-embedded hydrophobic domains are important areas of current research on SUN-domain proteins. Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; ER, endoplasmic reticulum; Hs, human; INM, inner nuclear membrane; NE, nuclear envelope; ONM, outer nuclear membrane; Sp, *Schizosaccharomyces pombe*; SPB, spindle pole body.

interact with all three major elements of the cytoskeleton. The SUN-domain partner for Nesprin-3 is not yet known. However, based on the overlapping roles of SUN1 and SUN2 in anchoring Nesprin-2 giant[13], we speculate that even if SUN-domain proteins have 'preferred' KASH-domain partners, they might recognize other KASH-domain proteins.

Bridging the nuclear envelope
We are still far from understanding SUN-domain and KASH-domain proteins at the molecular level. Crisp *et al.*[13] propose that SUN1 and SUN2 are embedded in the INM as homodimers (and possibly as heterodimers), with each dimer binding two KASH-domain proteins. We further propose that SUN-domains might be flexibly hinged, such that an INM-localized SUN-domain protein might be free to interact with KASH-domain proteins that are located either on the opposite membrane (the ONM) or, potentially, the same membrane (the INM) (see the 'flexible hinge' in FIG. 3). This possibility provides a mechanism for INM-localized SUN-domain proteins to anchor INM-localized KASH-domain proteins (for example, Nesprin-1α)[37]. In its most extreme form,

this model would allow one SUN-protein dimer to link KASH proteins on opposite membranes (for example, see Nesprin-1α and Nesprin-1 giant in FIG. 3).

We propose an additional possibility that does not require a flexible hinge: INM-localized KASH-domain proteins might be anchored by SUN3 or SPAG4, the relatively uncharacterized SUN-domain proteins that are proposed to localize in the ER and the ONM[13]. In this model, ONM-localized SUN-domain proteins could serve as 'reverse anchors' for INM-localized KASH-domain proteins (FIG. 3), in addition to their unknown roles in the ER. Although highly speculative, these models are worth considering because they suggest mechanisms by which SUN-protein dimers might collectively distribute mechanical force bidirectionally at the nuclear envelope via attachments to KASH-domain proteins on both the INM and the ONM. Also worth considering is the possibility that some KASH-domain proteins, such as Nesprin-1α, might have SUN-domain-independent anchoring mechanisms, for example, through direct binding to lamins and emerin[37].
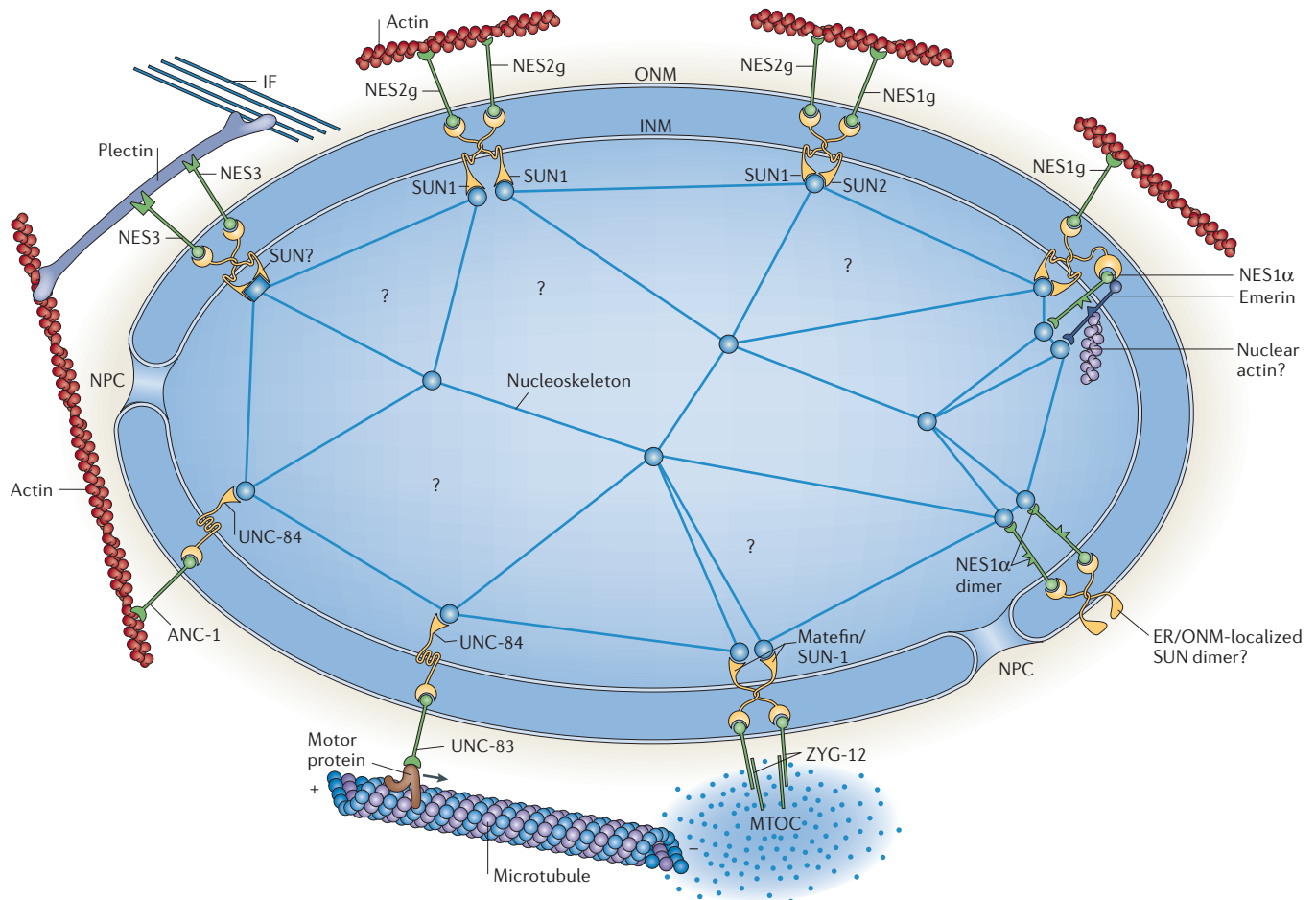
Figure 3 | **Models for mechanical bridging of the nuclear envelope by SUN-domain proteins.** Schematic depiction (not to scale) of the interactions between SUN-domain proteins (yellow) and KASH-domain proteins (green) at the inner and outer nuclear membranes (INM and ONM, respectively) of the nuclear envelope, and their interactions with specific elements of the cytoskeleton, a generic nucleoskeleton network (light blue), and the INM protein emerin (dark blue). *Caenorhabditis elegans* SUN-domain proteins (UNC-84, matefin/SUN-1) and KASH-domain proteins (ANC-1, UNC-83 and ZYG-12) are depicted on the lower left. UNC-84 can bind either ANC-1, which binds actin, or UNC-83, which binds microtubules via an unidentified microtubule-dependent motor protein. Matefin/SUN-1 binds ZYG-12 dimers, which bind the microtubule-organizing centre (MTOC). Human proteins SUN1 and SUN2 anchor Nesprin-2 giant (NES2g) at the ONM. NES2g and the giant isoform of Nesprin-1 (NES1g) each bind actin. Nesprin 3 (NES3) binds plectin, which links cytoplasmic intermediate filaments (IFs) to actin. SUN-domain proteins with at least one predicted coiled-coil domain (see FIG. 2) are depicted as homodimers or heterodimers. This diagram includes two blatant speculations. First, relatively uncharacterized endoplasmic reticulum (ER)/ONM-localized SUN proteins (for example, SUN3), might provide 'reverse anchors' for INM-localized KASH-domain proteins (for example, NES1α). Second (the 'flexible hinge' model), INM-localized SUN dimers might interact with KASH-domain proteins that are located either on the ONM (for example, NES1g) or the INM (for example, NES1α), or on both simultaneously. The question marks indicate that the actual structure of the nucleoskeleton is unknown. NPC, nuclear pore complex.

It is easy to comprehend how SUN-domain proteins in the INM could link the nuclear envelope to specific elements of the cytoskeleton via their binding to KASH-domain proteins in the ONM, given the binding of KASH-domain proteins to actin, microtubules, plectin and centrosomes. The picture of nucleoskeleton organization is less clear, and indeed begs the question of whether nuclear-envelope-bridging complexes are symmetric in their mechanisms of attachment to the cytoskeleton and to the nucleoskeleton. Given that mechanical networks are only as strong as their weakest link, we must consider the contacts that are made by KASH-domain proteins embedded in the INM. Might these KASH-domain proteins link to a correspondingly diverse group of mechanical elements inside the nucleus, and if so, what are these elements (besides lamins and emerin)?

Nesprin-1α and several small Nesprin-2 isoforms interact with the nuclear membrane protein emerin and A-type lamins[23,29], whereas other Nesprin-2 isoforms colocalize with heterochromatin and have calponin-homology domains that presumably bind actin polymers in the nucleus[23]. Nuclear actin polymers are conformationally distinct from cytoplasmic F-actin, and probably serve various roles in the nucleus, some of which are structural[38,39]. Actin and nuclear spectrin are proposed to form an INM cortical network that is anchored by emerin[40], the same protein to which both Nesprin-1α and the intranuclear Nesprin-2 isoform can bind directly[23,29] (FIG. 3). In theory, an INM-localized KASH-domain protein that can bind actin would be appealingly symmetric if it could link directly to nucleoskeletal actin, but such an isoform remains hypothetical. One can also consider potential symmetry in the mechanisms by which INM-localized compared to ER/ONM-localized SUN-domain proteins

themselves are anchored. In human cells, INM-localized SUN-domain proteins bind directly to lamins. Perhaps ER/ONM-localized ones bind cytoplasmic intermediate filaments.

Why do cells use SUN-domain proteins rather than nuclear pore complexes to mechanically bridge the nuclear envelope? Pore complexes link the INM and the ONM, are highly stable and are strongly anchored to the nuclear lamina network[41]. Although pore complexes cannot currently be excluded as load-bearing elements of the nuclear envelope, indirect evidence indicates that their core activity — nucleo-cytoplasmic transport — is vulnerable to mechanical deformation[42]. We speculate that the evolution of a specific bridging mechanism, based on SUN-domain and KASH-domain proteins, provided a versatile mechanism to anchor many cytoskeletal and nucleoskeletal structures to one integrative 'mechanical syncytium' at the nuclear envelope. Consistent with this idea, the links between SUN-domain and KASH-domain proteins are needed to maintain the uniform spacing between the INM and the ONM[13]. The importance of integrating mechanical connections is intuitively obvious when one considers that chromosomes are dense and must be hauled about without damaging either the nuclear envelope or the cell.

New roles for SUN-domain proteins

Other, non-mechanical roles for SUN-domain proteins are also emerging. In *S. pombe*, telomeres become clustered at the nuclear envelope near the SPB during meiosis. This clustering facilitates the alignment of homologous chromosomes and promotes their pairing and recombination[43]. Telomere clustering is mediated by the SUN-domain protein Sad1, which binds a protein named Bqt1, which joins with Bqt2 to bind the telomere-associated protein Rap1 (REF. 44). Sad1 also interacts with the KASH-domain protein Kms1 (REF. 45); interestingly, this interaction is required to maintain Sad1 at the SPB. So, the yeast SUN-domain and KASH-domain proteins have roles in meiosis that involve chromosome (telomere) tethering to the nuclear envelope.

In *C. elegans*, besides disrupting nuclear migration during development, mutations in the SUN-domain protein UNC-84 cause additional phenotypes (such as improper migration of gonad distal tip cells, egg-laying defects and reduced fat levels[12,35,46]) that are not reported for its known KASH-domain partners UNC-83 or ANC-1.

So, UNC-84 might have interesting novel partners. How or why a nuclear-envelope-associated SUN-domain protein influences fat levels is unknown. However, we note that certain mutations in A-type lamins cause partial lipodystrophy in humans[47].

Novel functions are also emerging for the second *C. elegans* SUN-domain protein, matefin/SUN1. Most embryos in which matefin/SUN-1 was downregulated died at the ~300-cell stage with phenotypes that cannot be attributed to centrosome detachment[18]. Worms that are homozygous for a *mtf-1/sun-1*-deletion allele show that this gene is essential for germline maturation and survival[18], with late larval phenotypes that hint at it having fundamental roles in germline cell proliferation or germ-cell maintenance, or both[18]. Matefin/SUN-1 also binds the apoptotic activator CED-4 and is required for apoptosis (Y.Z. and Y.G., unpublished observations).

Concluding remarks and outlook

On the basis of current evidence, we propose that SUN-domain proteins are the 'Velcro' in the nuclear envelope that mechanically links the cytoskeleton to the nucleoskeleton. SUN-domain proteins that localize in the ER and the ONM might have similar roles in spacing and reinforcing the ER-membrane network, while also potentially 'reverse-anchoring' INM-localized KASH-domain proteins (FIG. 3). We are also beginning to appreciate potential non-mechanical roles for SUN-domain proteins during apoptosis, meiosis and germ-cell maintenance.

The proposed mechanical roles of SUN-domain proteins in bridging the nuclear envelope raise exciting new questions about the nature and purpose of these attachments, not only during nuclear migration in specialized cell types, but in everyday interphase nuclei. SUN-domain proteins have the potential to directly transduce mechanical signals from the cytoplasm to the nucleus and back, indicating that they might serve as the 'integrins' of the nuclear envelope. Many open questions remain, however, including whether human cells use KASH-domain proteins to anchor centrosomes (that is, are they equivalent to ZYG-12 in *C. elegans*), and why both predicted SUN-domain proteins in *D. melanogaster* seem to lack transmembrane domains. As more binding partners are discovered, a better understanding of their connections will be achieved, and we will better comprehend the importance of these bridges.

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