KASH-domain proteins in nuclear migration, anchorage and other processes

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Summary

The nucleus in eukaryotic cells can move within the cytoplasm, and its position is crucial for many cellular events, including migration and differentiation. Nuclear anchorage and movement can be achieved through association of outer nuclear membrane (ONM) proteins with the three cytoskeletal systems. Two decades ago studies described *C. elegans* mutants with defects in such events, but only recently has it been shown that the strategies for nuclear positioning are indeed conserved in *C. elegans*, *Drosophila*, mammals and potentially all eukaryotes. The integral ONM proteins implicated in these processes thus far all contain a conserved Klarsicht/ANC-1/Syne homology (KASH) domain at their C-terminus that can associate with Sad1p/UNC-84 (SUN)-domain proteins of the inner nuclear membrane within the periplasmic space of the nuclear envelope (NE). The complex thus formed is responsible not only for association with cytoplasmic elements but also for the integrity of the NE itself.

Key words: Nuclear envelope, Outer nuclear membrane, KASH domain, Nesprin

Introduction

The contents of the nucleus are enclosed by two lipid bilayers, the inner (INM) and the outer (ONM) nuclear membrane, which together form the nuclear envelope (NE). The INM and the ONM fuse at the nuclear pore complexes (NPCs), and the lumen region between these membranes is called the periplasmic (or perinuclear) space (PS) (Gerace and Burke, 1988). Beneath the INM is the nuclear lamina, a web of intermediate filament (IF) proteins composed of A- and B-type lamins. These proteins give shape and stability to the NE, in addition to associating with proteins bound to chromatin (Gruenbaum et al., 2005). The ONM, by contrast, is continuous with the endoplasmic reticulum (ER) and, in fact, these two structures share a set of proteins as well as ribosomes (Gerace and Burke, 1988).

The nucleus and other organelles can move within the cytoplasm. The position of the nucleus is important for processes such as mitosis, meiosis, cell migration and polarization, and nuclear positioning occurs in the cells of most eukaryotes (Morris, 2000). Interactions with the three cytoskeletal filament systems [i.e. F-actin, IFs and microtubules (MTs)] anchor the nucleus or allow it to move in the cytoplasm. The different filament systems, in turn, are connected to each other through members of the plakin family of cytoskeletal cross-linkers (Fuchs and Karakesisoglou, 2001; Leung et al., 2002). Although defects in nuclear positioning have been observed for a relatively long time, the mechanisms responsible are only now becoming evident.

## Nuclear positioning in *Caenorhabditis elegans*

Nuclear migration and anchorage were initially studied in *C. elegans*. The positions of the nuclei within the developing embryo can be mapped in great detail because *C. elegans* has a transparent body (Sulston and Horvitz, 1977; Sulston et al., 1983). Nuclear migration has been extensively studied in hyp7-precursor and P-cells within the embryo and larvae, respectively. Prior to the cell fusion events that form the large, multi-nucleated hypodermal syncytium (the hyp7 syncytium), the hyp7 precursor cells go through a series of elongation and nuclear migration events, whereby the nuclei move past the dorsal midline to the opposing lateral side (Sulston et al., 1983; Williams-Masson et al., 1998). Similarly, the P cells, which ultimately develop into motor neurons and epithelial cells, have nuclei that migrate from the lateral sides of the newly hatched larva to form a single row in the ventral cord (Sulston, 1976; Sulston and Horvitz, 1977).

The first worm strains shown to have defects in nuclear migration and anchorage in these two cell types, which result in uncoordinated movement, were *unc-83* and *unc-84* (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Malone et al., 1999). The *unc-83*, and some *unc-84*, worms also display defective nuclear migration in cells of the intestinal primordium (Starr et al., 2001). In the majority of the *unc-84* mutants, the nuclei move slowly and do not migrate past the dorsal midline in the hyp7 precursor cells and float freely within the hyp7 syncytium. However, some *unc-84* worms only display the migratory defects. In P cells, the nuclei fail to migrate to the ventral cord and the cells ultimately apoptose (Malone et al., 1999). The *unc-83* worms have the same phenotype, except that the nuclei have never been observed to float freely within the cytoplasm of the hyp7 syncytium (Starr et al., 2001) (Fig. 1A).

The *unc-83* and *unc-84* genes encode an ONM protein, UNC-83, and an INM protein, UNC-84, respectively. UNC-83 has a conserved C-terminal Klarsicht/ANC-1/Syne-1 homology (KASH) domain, which contains a transmembrane

Fig. 1. Phenotypes of the *C. elegans* mutants *anc-1*, *unc-83*, *unc-84* and *zyg-12*. (A) In *C. elegans*, hyp7 precursor cells go through a series of elongation and alternating intercalation events on the dorsal side of the developing embryo. In the wild-type embryo, the nuclei (grey and black circles) then move to the opposite side of the embryo (S3). After fusion of the hyp7 precursors into the large multi-nucleated hyp7 syncytium, the nuclei are evenly anchored near the dorsal midline (DM) (S4). *Unc-83* and some *unc-84* mutant alleles result in failure of the nuclei to migrate past the DM (S3); these are therefore mispositioned in the syncytium, although they appear to be anchored (S4). In the *anc-1* worms, the nuclei migrate normally (S3) but are unanchored and typically form clumps in the syncytium (S4). The majority of the *unc-84* mutant alleles, and all the *unc-83 anc-1* double mutants, result in incorrect positioning and anchorage of the nuclei in the syncytium (S4). For simplicity, only a few of the hyp7 precursor cells are depicted. (B). In the single-celled embryo of *C. elegans*, MTs associated with the two centrosomes pull the two pronuclei (black ovals) together; these then fuse and the nuclear envelope breaks down. At the start of the first mitosis, MTs associated with the centrosomes pull the paired chromosomes apart. In the *zyg-12* worms, the centrosomes are detached from the pronuclei. As a result, the nuclei are not pulled together and do not fuse properly. The nuclear envelopes still break down and the chromosomes from each pronucleus still associate with MTs. Cytokinesis occurs but results in daughter cells that contain abnormal numbers of chromosomes.
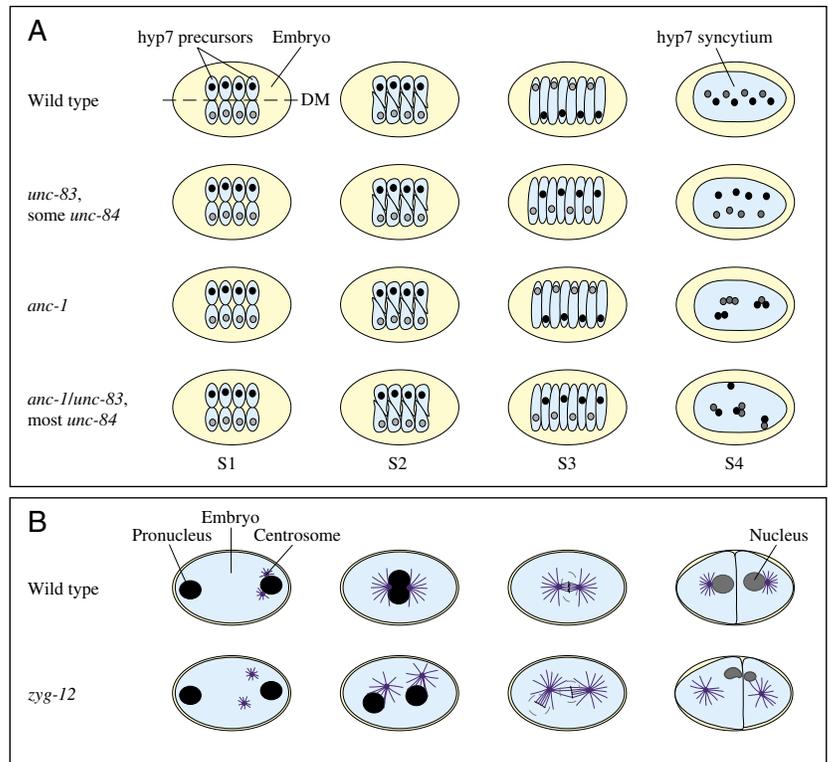

region and residues that lie within the PS, and a cytoplasmic N-terminus that does not show sequence similarity to any known protein (McGee et al., 2006). UNC-84 contains several potential transmembrane domains and a C-terminal region that is homologous to the yeast protein Sad1p and, accordingly, is called the Sad1p/UNC-84 (SUN) domain (Malone et al., 1999; McGee et al., 2006). The localization of UNC-84 to the INM is not dependent upon its SUN domain but rather on the presence of nuclear lamins, which probably interact with its N-terminus (Lee et al., 2002). Deletion of UNC-84, mutations in the UNC-84 SUN domain or mutations within the UNC-83 KASH domain can prevent the localization of UNC-83 at the ONM (McGee et al., 2006; Starr et al., 2001), presumably because of a loss of direct interaction between the UNC-83 KASH and UNC-84 SUN domains within the PS (McGee et al., 2006) (Fig. 2). This explains why the nuclear migration defects in *unc-83* and *unc-84* mutant worms are very similar (Malone et al., 1999). Curiously, UNC-83 is present at the NE in a limited number of cell types (including P, hyp7, intestinal, pharyngeal and uterine cells), unlike UNC-84, which is localized at the NE in nearly all cells (Starr et al., 2001).

UNC-83 and UNC-84 were originally proposed to tether the nucleus to centrosomes, which was hypothesized to drive nuclear migration (Malone et al., 1999; Reinsch and Gonczy, 1998); however, later studies showed a normal association between the centrosomes and nuclei in *unc-83* and *unc-84* mutant cells whose nuclei fail to migrate (Lee et al., 2002; Starr et al., 2001). The proteins that interact with the N-terminus of UNC-83 to facilitate nuclear migration have not been identified. Recently, another ONM protein, ZYG-12, was shown to mediate an association between the centrosomes and

the nucleus in *C. elegans*. In *zygote defective (zyg)-12* mutant worms, the centrosome detachment defect in the developing embryo results in death as a consequence of chromosome segregation defects (Fig. 1B). ZYG-12 is a member of the Hook family and localizes to both the centrosomes, which is MT dependent, and the NE. Interestingly, its recruitment to the NE is dependent upon the expression of the only other SUN-domain-containing protein in *C. elegans*, Matefin/SUN-1 (Fridkin et al., 2004; Malone et al., 2003). This is consistent with the results of experiments showing that in *unc-84*-null cells the centrosomes are correctly associated with the NE (Lee et al., 2002). Unlike the case of UNC-84, nuclear lamins are not involved in the retention of Matefin/SUN-1 at the INM (Fridkin et al., 2004). There are three splice variants of ZYG-12 (A, B and C), two of which contain a KASH domain (B and C). Localization of these two variants at the ONM probably depends upon an association between their C-termini and that of Matefin/SUN-1 (Fig. 2).

Defects in nuclear anchorage were first detected in the hyp7 syncytium. Five mutant alleles of the anchorage defective 1 (*anc-1*) gene were isolated from mutant worms in which unanchored nuclei float freely in the cytoplasm of the syncytium (Hedgecock and Thomson, 1982). In these worms, mitochondria also appear unanchored, which suggests that the same mechanism is responsible for the anchorage of nuclei and mitochondria. The ANC-1 protein contains two N-terminal calponin homology (CH) motifs, which together comprise an actin-binding domain (ABD), and a C-terminal KASH domain (Starr and Han, 2002). The presence of a cytoplasmic ABD indicated that the defects in nuclear and mitochondrial anchorage might be due to a loss of interaction with the actin

Fig. 2. Proposed protein complexes spanning the NE in *C. elegans*, *Drosophila* and mammals. The nuclear lamins of several species are proposed to interact with the N-terminal regions of the SUN-domain proteins of the INM. Within the PS, the SUN-domains (or regions immediately upstream of the SUN-domains) associate with the KASH domains of ONM proteins. Within the cytoplasm, the N-terminal regions of these proteins associate with the microtubule-organizing center (MTOC), MTs, F-actin or plectin (which can directly bind to the IFs). Thus, there is a continuous protein scaffold from the nuclear lamina, through the NE, to the different cytoskeletal systems in several different species. The SUN-domain proteins are shown as dimers. SUN1 crosses the INM three times, whereas the other SUN-domain proteins cross it at least once. The two *Drosophila* SUN-domain proteins identified in database searches (labeled Dm-SUN in this depiction) do not contain any recognizable membrane spanning regions, although these may not represent full-length sequences.
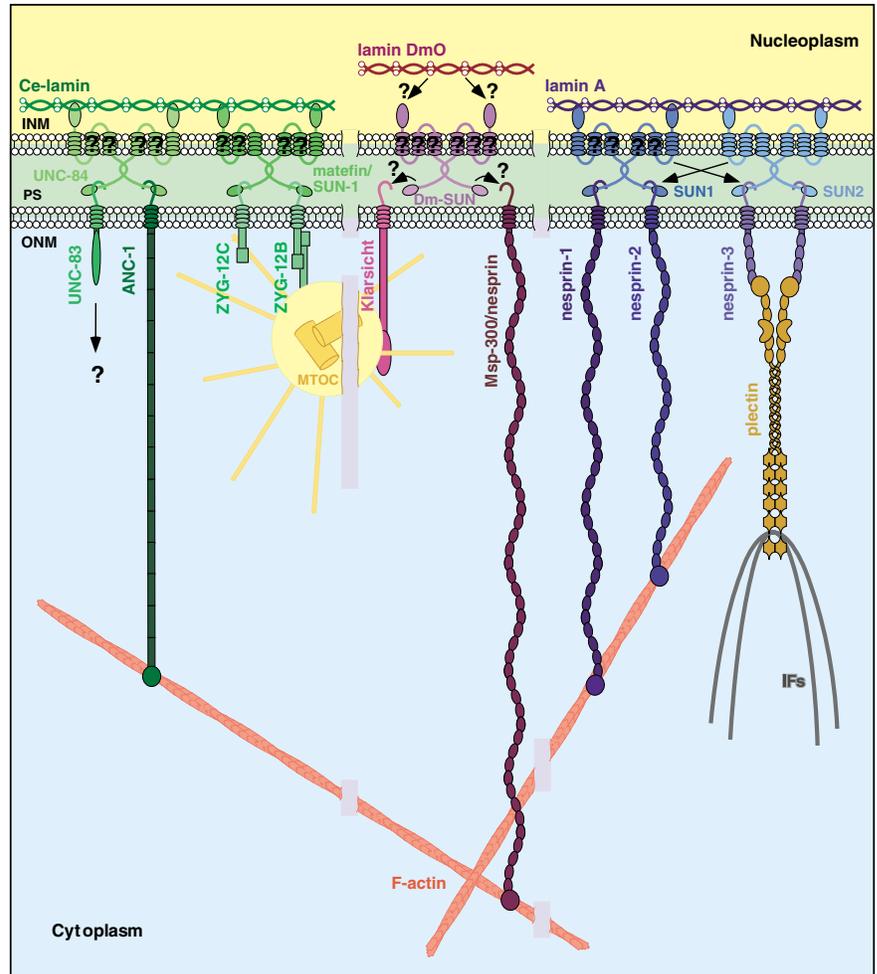
cytoskeleton. Indeed, a direct association with F-actin has been demonstrated in vitro and an N-terminal fragment of ANC-1, containing the ABD, specifically decorates F-actin in cells (Starr and Han, 2002). ANC-1 is an enormous molecule of ~950 kDa. This size is due to a region of repetitive structural elements between the ABD and KASH domain. Therefore, it seems that a relatively large distance needs to be maintained between the nucleus and F-actin. The KASH domain of ANC-1 is necessary for its localization at the ONM and mutations within the SUN domain of UNC-84 prevent the proper localization of ANC-1 to the NE (Starr and Han, 2002). Additionally, overexpression of a construct containing the KASH domain alone results in nuclear anchorage defects in the hypodermal syncytium, which are probably due to a competition with endogenous ANC-1 for a limited number of UNC-84-binding sites (Starr and Han, 2002). Interestingly, an *unc-84*-null mutation has no effect on mitochondrial anchorage in muscle cells (Starr and Han, 2002). The SUN domain of UNC-84 is thus also responsible for the recruitment of ANC-1 to the ONM through its KASH domain, although note that in this case a direct interaction has not been detected (Starr and Han, 2002) (Fig. 2).

Worms with mutations in both *anc-1* and *unc-83* have a compound phenotype. The nuclei within the syncytium are misplaced as well as unanchored because they fail to migrate prior to fusion and are unable to interact with the actin cytoskeleton (Hedgecock and Thomson, 1982). Most of the *unc-84* mutant alleles similarly produce nuclear migration and anchorage defects, but some only lead to defects in nuclear migration (Fig. 1A). The worms that show both defects either lack UNC-84 or have mutations in regions in or near the SUN domain; those that only display defects in migration have mutations within the nucleoplasmic N-terminus of UNC-84 (Malone et al., 1999), indicating that anchorage may not

entirely depend only upon an interaction with the nuclear lamins.

The data thus support a model in which UNC-84 becomes localized at the INM by binding to the nuclear lamins with its N-terminus, which leaves its periplasmic SUN domain available for association with the KASH domain of either ANC-1 or UNC-83 to retain these proteins at the ONM. ANC-1 is responsible for nuclear anchorage through interactions with the actin cytoskeleton, whereas UNC-83 is necessary for nuclear migration, although how this is achieved is currently unknown (Fig. 2).

Nuclear positioning in *Drosophila melanogaster*
Two KASH-domain proteins have been identified in *Drosophila*, Klarsicht and Msp-300/nesprin. Klarsicht, originally called Marbles, is important for the development of the compound eye in *Drosophila*. Mutation of the *klarsicht* gene results in the failure of the nuclei in photoreceptors to migrate to the apex of the developing eye imaginal disc and, therefore, most nuclei remain at the basal side, which results in oddly shaped photoreceptors (Fischer-Vize and Mosley, 1994). Importantly, this nuclear migration defect coincides with the detachment of the centrosome from the nucleus (Patterson et al., 2004) (Fig. 3A). Klarsicht is also involved in the transport of lipid droplets in *Drosophila* embryos (Welte et al., 1998), but this involves a different C-terminal splice variant
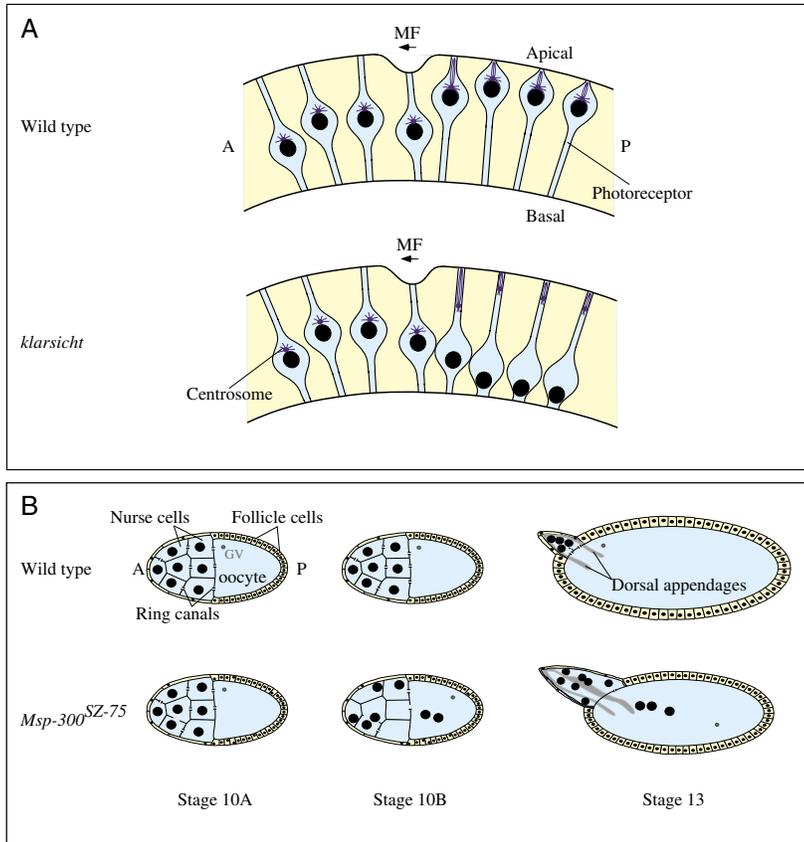
Fig. 3. Phenotypes of the *Drosophila* mutants *klarsicht* and *Msp-300*^SZ-75^. (A) In the wild-type eye imaginal disc, passage of the morphogenetic furrow (MF) initiates the differentiation of photoreceptors. Normally, the nuclei (black circles) are pulled up to the apical side through an association with the centrosome and MTs. In the *klarsicht* mutant flies, the centrosomes are no longer attached to the nuclei. As a result, the nuclei remain at the basal side. (B) At the start of stage 10B in wild-type *Drosophila* ovaries, the nurse cells (NCs) start to dump their cytoplasmic contents into the oocyte through the ring canals. By stage 13, most NCs, and their nuclei (large black ovals), have disappeared and the dorsal appendages are extruded at the anterior end. In *Msp-300*^SZ-75^ flies, the NC nuclei become detached once cytoplasmic dumping occurs and either form multi-nucleated cells, enter the oocyte or become mispositioned within individual cells. The germinal vesicle (GV) (the oocyte nucleus) (grey oval) is also mispositioned. For simplicity, only some of the NCs are depicted. A, anterior; P, posterior.

(Klarsicht-β, rather than Klarsicht-α and Klarsicht-γ) (Guo et al., 2005). Interestingly, *klarsicht*-null mutants are viable and fertile and display major defects only in eye morphology (Mosley-Bishop et al., 1999).

Klarsicht-α is ~251 kDa, whereas Klarsicht-γ is ~62 kDa. Both contain a C-terminal KASH domain and are localized at the NE in several cell types (Klarsicht-β does not have a KASH domain) (Guo et al., 2005). Klarsicht also associates with MTs in photoreceptors (Fischer et al., 2004; Patterson et al., 2004); the N-terminal region of Klarsicht, which is not present in Klarsicht-γ and shows no sequence similarity to other known proteins, is responsible (Fischer et al., 2004). Analyses of fly phenotypes associated with mutations in the *Drosophila* B-type lamin Dm0 indicate that Klarsicht and lamin Dm0 are part of the same pathway (Patterson et al., 2004). Lamin Dm0 may therefore be required for the localization of an UNC-84-like

protein to the INM and the consequent retention of Klarsicht at the ONM. The *Drosophila* genome encodes two uncharacterized SUN-domain proteins, although these are not predicted to have transmembrane regions (Malone et al., 2003; Starr and Han, 2003) (Fig. 2). Moreover, overexpression of the Klarsicht KASH domain in photoreceptors does not result in a mutant phenotype (Fischer et al., 2004). This suggests that it does not compete with the endogenous protein for a limited number of binding sites at the NE, in contrast to the ANC-1 KASH domain (Starr and Han, 2002).

Although Klarsicht and ZYG-12 are both necessary for the association of the centrosome with the nucleus, these two proteins are not related except in the KASH domain. In *Drosophila*, only two proteins show some sequence similarity to ZYG-12. One is a member of the Hook family, dHk (Kramer and Phistry, 1996; Kramer and Phistry, 1999), and the other is a recently identified Hook-related protein, dHkRP (Simpson et al., 2005). Both contain a putative N-terminal MT-binding domain, but do not contain a KASH domain or localize to the NE. dHk is cytosolic and reported to play a role in endocytic trafficking (Kramer and Phistry, 1996; Kramer and Phistry, 1999). The Hook proteins and ZYG-12 therefore do not appear to have similar functions in the two species, although Klarsicht and ZYG-12 seem to have acquired analogous functions in some cells, given their retention at the NE by a KASH domain and their ability to tether the nucleus to the centrosome.

Msp-300/nesprin is another KASH-domain protein present in *Drosophila*. Msp-300 (muscle-specific protein 300 kDa) was originally identified in a search for muscle-specific genes expressed during embryonic myotube migration and attachment (Volk, 1992). Given its localization to actin-rich muscle-attachment sites, Z-lines and the leading edge of migrating myotubes, Msp-300 was hypothesized to be crucial for muscle morphogenesis in the developing *Drosophila* embryo (Volk, 1992). Indeed, an embryonic lethal mutation in *Msp-300* leads to defects in some of these processes. In the *Msp-300*^SZ-75^ mutant flies, the myotubes cannot extend towards to the epidermal attachment sites and, as a result, the ability of the embryonic somatic muscle cells to contract is severely compromised (Rosenberg-Hasson et al., 1996).

Ten years after Msp-300 was characterized, a gene immediately downstream of *Msp-300*, provisionally called *nesprin* (nuclear envelope spectrin repeat), was shown to encode a KASH domain (Zhang et al., 2002). Careful analysis revealed that *Msp-300* and *nesprin* are in fact part of the same enormous gene spanning about 80 kb. *Msp-300* encodes the N-terminal portion of the full-length protein and *nesprin* encodes the C-terminal region. A repetitive coding region lies between the two and, therefore, the complete gene can potentially encode a gigantic molecule of ~1300 kDa: Msp-300/nesprin

(Zhang et al., 2002). Like ANC-1, Msp-300/nesprin contains a C-terminal KASH domain and an N-terminal ABD, which binds directly to F-actin in vitro and decorates these filaments in vivo (Rosenberg-Hasson et al., 1996; Volk, 1992). The region in between comprises a series of spectrin repeats (SRs) (Fig. 2) – three-helix bundles that can give proteins length and elasticity and mediate protein interactions, including homodimerization (Djinovic-Carugo et al., 2002; Mislow et al., 2002a). Interestingly, the repetitive regions in ANC-1 are not related to SRs, although they apparently have an analogous function. Msp-300/nesprin appears to be localized predominantly at the NE in nurse cells and the oocyte, but some also appears to be present in the cytoplasm (Yu et al., 2006). Curiously, expression of a GFP-KASH domain fusion protein does not have any obvious detrimental defects on the position of the nuclei in these cells. This suggests that the docking sites for Msp-300/nesprin, like those of Klarsicht, are not limited at the NE.

During oogenesis in *Drosophila*, one of the cells in the egg chamber becomes the oocyte; the other 15 become the supporting polyploid nurse cells. Eventually, the nurse cells 'dump' their cytoplasmic contents into the oocyte through ring canals and then undergo apoptosis (Spradling, 1993). The positions of the nuclei are thought to be crucial during this process because if they were to become detached they would block the canals (Yu et al., 2006). Since actin structures are important for nuclear anchorage in the nurse cells during dumping (Guild et al., 1997), Yu et al. investigated whether Msp-300/nesprin plays a role in this process by generating flies carrying the *Msp-300^{SZ-75}* allele only in the germ line (these flies are viable but do not contribute any wild-type Msp-300/nesprin to the developing *Msp-300^{SZ-75}* egg chamber) (Yu et al., 2006). Indeed, the egg chambers in these flies exhibit defects in cytoplasmic dumping and nuclear positioning (Yu et al., 2006) (Fig. 3B). Interestingly, they also have defective actin structures, which suggests that Msp-300/nesprin also has a role in actin organization (i.e. bundling and/or cross-linking), a role attributed to other ABD-containing proteins (Winder and Ayscough, 2005).

Interestingly, the *Msp-300^{SZ-75}* mutation is lethal, unlike the deletion of ANC-1 in *C. elegans*. Larvae die because they do not hatch from the chorion owing to the previously mentioned defects in muscle attachment and contraction and not nuclear anchorage defects (Rosenberg-Hasson et al., 1996). By contrast, ANC-1 seems not to have crucial functions besides those associated with nuclear and mitochondrial anchorage.

The mammalian nesprins

The mammalian nesprins were originally identified in yeast two-hybrid screens for binding partners of a tyrosine kinase of the postsynaptic membrane in muscle (MuSK) (which yielded nesprin-1) (Apel et al., 2000) and for the cytoskeletal cross-linker protein plectin (which yielded nesprin-3) (Wilhelmsen et al., 2005). Nesprin-1, originally named synaptic nuclear
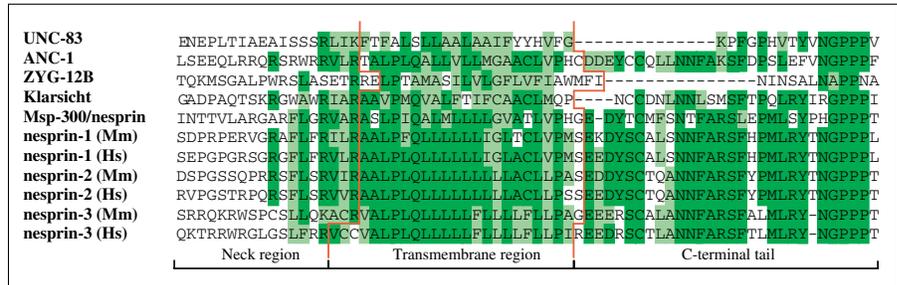

Fig. 4. Alignment of KASH domains from various proteins. The amino acid residues present in the predicted transmembrane regions are enclosed by red lines. The Zyg-12B KASH domain sequence is used because the Zyg-12C KASH domain sequence in the database lacks 16 residues encompassing part of the neck and transmembrane regions. Dark green, conserved amino acids; light green, amino acids with similar chemical properties in more than half of the KASH domains. Mm, *Mus musculus*; Hs, *Homo sapiens*.

envelope 1 (Syne-1) because of its presence at postsynaptic nuclei of neuromuscular junctions (Apel et al., 2000), is actually localized at the ONM of various cell types (Zhang et al., 2001; Zhang et al., 2002). It is also referred to as myocyte nuclear envelope 1 (Myne-1) (Mislow et al., 2002b) or enaptin (Padmakumar et al., 2004). Nesprin-2 was identified in database searches for sequences related to nesprin-1 or the α-actinin ABD (Apel et al., 2000; Zhen et al., 2002). It is also known as Syne-2 (Apel et al., 2000) or NUANCE (Zhen et al., 2002).

Nesprin-1 and nesprin-2 are giant proteins of ~976 kDa and ~764 kDa, respectively (Zhang et al., 2002), and each contains an N-terminal ABD (Padmakumar et al., 2004; Zhen et al., 2002). Nesprin-3 is much smaller, ~110 kDa, and instead binds to plectin at its N-terminus to connect it to IFs (Wiche, 1998; Wilhelmsen et al., 2005). All three contain a series of homologous SRs and a C-terminal KASH domain (Fig. 4). In vitro binding assays and localization studies with the isolated N-terminal regions indicate that the nesprins can indeed interact with actin filaments and IFs (Fischer et al., 2004; Padmakumar et al., 2004; Starr and Han, 2002; Wilhelmsen et al., 2005; Zhen et al., 2002). Interestingly, plectin is ~500 kDa and, when bound to nesprin-3, generates a bridge to IFs similar in size to that which nesprin-1 and nesprin-2 provide for F-actin. This suggests that it is important that there is a significant distance between the nucleus and both the IF and F-actin systems (Fig. 2).

Nuclear functions

In transgenic mice overexpressing the nesprin-1 KASH domain in muscle cells, the nuclei do not aggregate beneath the postsynaptic membrane, because endogenous nesprin-1 is displaced from the NE (Grady et al., 2005). This implies that the number of KASH-domain-binding sites at the NE is limited (as is the case for ANC-1). How nesprin-1 tethers nuclei to the postsynaptic membrane is not known, although an association with actin structures or MuSK is probably required (Apel et al., 2000; Grady et al., 2005). Surprisingly, although the nuclei are not properly localized, the neuromuscular junction functions normally.

Nesprin-1 and nesprin-2 exhibit a variety of isoforms produced through the use of different translational initiation and stop sites and alternative splicing (Cottrell et al., 2004; Mislow
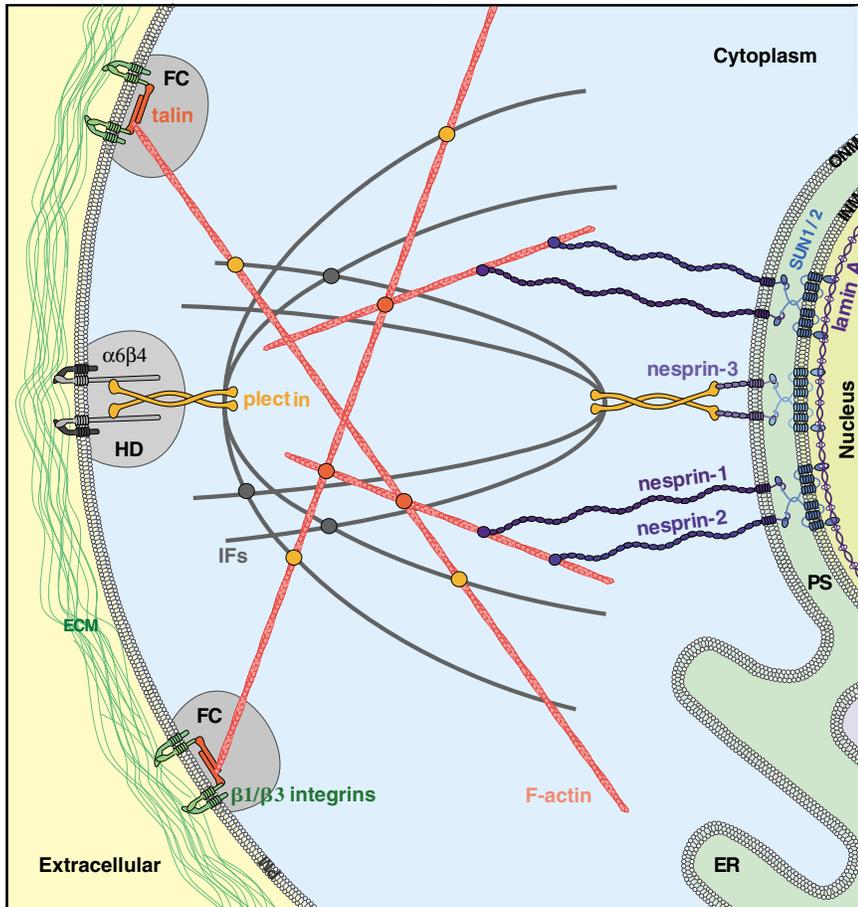
Fig. 5. In mammals, a continuous protein scaffold connects the extracellular matrix to the nuclear lamina via IFs and F-actin. Integrin α6β4, present in hemidesmosomes (HDs), and β1/β3 integrins, present in focal contacts (FCs), connect the extracellular matrix (ECM) to cytoplasmic IFs (via plectin) and F-actin (via talin), respectively. These two cytoskeletal systems are interconnected to each other via the plakins (yellow circles) or to themselves via filamin or α-actinin (red circles) and the plakins (grey circles). F-actin associates with the ABDs of nesprin-1 and nesprin-2; IFs associate with plectin dimers bound to nesprin-3. In turn, the ONM nesprins associate with either SUN1 or SUN2 within the PS. The INM SUN-domain proteins associate with lamin A through their N-termini within the nucleus. ER, endoplasmic reticulum; INM, inner nuclear membrane; ONM, outer nuclear membrane; PM, plasma membrane; PS, periplasmic space.

INM is currently not known, although it is possible that they help to organize the nuclear lamina.

Membrane trafficking and organization

Nesprin-1 might also be involved in protein trafficking and it has been detected at the Golgi (Gough et al., 2003). Moreover, expression of certain SR fragments of nesprin-1 in cells results in the collapse of the Golgi complex, prevents the recycling of protein disulfide isomerase and alters the localization of both the KDEL ER-retrieval receptor and β-COP [part of the COPI coat complex involved in ER-Golgi transport and NE breakdown (Liu et al., 2003; Nickel et al., 2002)] (Gough and Beck, 2004). The nesprin-1 splice variant Golgi-localized SR-containing protein 56 (GSRP-56) contains two SRs present in the central region of the full-length protein and lacks both the ABD and the KASH domain. GSRP-56 is localized at the Golgi complex and, interestingly, its overexpression results in expansion of the Golgi complex (Kobayashi et al., 2006). The brain-specific splice variant CPG2 is also derived from the SR-rich region (Cottrell et al., 2004; Padmakumar et al., 2004). Detected in a screen for plasticity-related genes upregulated after neuronal excitation in rat brains (Nedivi et al., 1993), it is localized at the postsynaptic endocytic zones of excitatory synapses in neurons. CPG2 regulates the constitutive internalization of glutamate receptors and the activity-induced internalization of α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid (AMPA) receptors (Cottrell et al., 2004), and is thereby proposed to modify synaptic strength. Variants of nesprin-1 might thus function in protein trafficking, control of Golgi morphology and, possibly, NE breakdown.

et al., 2002b; Padmakumar et al., 2004; Zhang et al., 2001; Zhang et al., 2005). Interestingly, some of these do not contain an ABD and/or a KASH domain. Several isoforms are localized and function at areas of the cell other than the ONM, such as the INM. For example, nesprin-1α might localize at the INM, because it can associate with the INM protein emerin and lamin-A/C (Mislow et al., 2002a; Mislow et al., 2002b). Nesprin-1 is also found within the nucleus, where it is associated with heterochromatin (Zhang et al., 2001). Additionally, expression of lamin A/C can influence the localization of certain nesprin-2 isoforms at the NE, and in vitro experiments suggested that these bind directly to lamin A/C and emerin within the nucleus. Indeed, ultrastructural analysis suggested that they are present at the INM (Libotte et al., 2005; Zhang et al., 2005). Notably, no splice variants of nesprin-3 have been detected at the INM (Wilhelmsen et al., 2005). The function of the nesprins at the

Other functions

Several other roles of nesprin-1 isoforms have been described. For instance, nesprin-1α can recruit muscle A-kinase anchoring protein (mAKAP) to the NE in striated myocytes through an association between their SRs (Pare et al., 2005). mAKAP is a scaffold protein that forms signaling complexes containing protein kinase A (PKA), the ryanodine receptor (RyR) Ca^{2+}-release channel, protein phosphatase 2A and phosphodiesterase type 4D3 (Kapiloff et al., 2001). Phosphorylation of the RyR by PKA augments the release of Ca^{2+} from the sarcoplasmic reticulum and other luminal areas associated with the NE (Bers and Perez-Reyes, 1999). Nesprin-1α might thus serve as a receptor on the nucleus.

Nesprin-1 has also been shown to be involved in cytokinesis. A non-SR central fragment of nesprin-1 interacts with the KIF3B subunit of the kinesin II motor. Overexpression of this

fragment or the C-terminus of KIF3B prevents cytokinesis. Furthermore, the proteins are colocalized at the central spindle and midbody during cytokinesis. Their association might allow nesprin-1 to attach vesicles to the kinesin motor protein, bringing extra membrane to the cleavage site to increase the surface area available for the formation of the two daughter cells (Fan and Beck, 2004).

Finally, two splice variants of nesprin-3 have been characterized: nesprin-3α and nesprin-3β. Interestingly, nesprin-3β is unable to associate with the plakin family members (Wilhelmsen et al., 2005). This suggests that nesprin-3, like the smaller isoforms of nesprin-1 and nesprin-2, has functions in the cell other than tethering the nucleus to the cytoskeleton.

Links with microtubules

Like the *Drosophila* proteins dHk and dHkRP, the mammalian Hook and Hook-related (HkRP) family members share regions of similarity with ZYG-12, but lack a conserved KASH domain (Malone et al., 2003; Simpson et al., 2005; Walenta et al., 2001) and do not appear to be responsible for the association of the centrosome with the nucleus. Nesprin-3, however, has been shown to associate with the ABDs of the plakin family members MACF and BPAG1 (Wilhelmsen et al., 2005). These proteins interact with MTs (Leung et al., 2002) and, thus, nesprin-3 might provide the necessary link between the nucleus and this cytoskeletal system. However, an interaction between these proteins has not yet been demonstrated in cells.

Mammalian SUN-domain proteins

Two mammalian UNC-84 orthologues were discovered through an EST database search for sequences that share similarity with *unc-84*: SUN1 (UNC84A) and SUN2 (UNC84B) (Malone et al., 1999). SUN1 was independently discovered in a proteomic analysis of neuronal NE components (Dreger et al., 2001). Both SUN1 and SUN2 are INM components. Two other mammalian proteins containing a SUN domain have been identified, SUN3 and SPAG4, but they are instead primarily detected at the ER membranes (Crisp et al., 2006; Hasan et al., 2006). In common with matefin/SUN-1, but not UNC-84, SUN1 does not require lamin proteins for its localization at the INM, although it does bind specifically to lamin A (Crisp et al., 2006; Haque et al., 2006; Hasan et al., 2006; Padmakumar et al., 2005). Lamin A is, however, partially responsible for the localization of SUN2. Interestingly, both SUN1 and SUN2 preferentially bind to the unprocessed (i.e. non-cleaved) form of lamin A in vitro and in vivo (Crisp et al., 2006), which suggests that they play a role in the recruitment and organization of these proteins at the nuclear lamina.

SUN1 and SUN2 each contain a conserved SUN domain that extends into the PS and interacts with the KASH domains of nesprin-1, nesprin-2 and nesprin-3 (Crisp et al., 2006; Haque et al., 2006; Padmakumar et al., 2005) (M.K. and A.S., unpublished data). However, a region just upstream of the SUN domain may mediate high-affinity binding to the KASH domain (Padmakumar et al., 2005). The importance of this interaction is highlighted in transgenic mice overexpressing the KASH domain of nesprin-1, which prevents the localization of endogenous nesprin-1 at the ONM in muscle cell nuclei at the neuromuscular junction (Grady et al., 2005). Additionally,

RNAi-induced depletion of SUN1 and SUN2 proteins in HeLa cells results in the mislocalization of nesprin-2γ away from the ONM. Importantly, this study also showed that interactions between nesprins and the SUN-domain proteins are crucial for maintenance of the NE because the absence of SUN1 and SUN2 leads to an expansion of the PS (Crisp et al., 2006). The evolutionarily conserved interactions that take place between the conserved SUN and KASH domains within the PS are thus crucial not only for associations between the nucleus and cytoplasmic proteins but also for the integrity of the NE in mammalian cells.

Conclusions and perspectives

It is now clear that SUN-domain proteins of the INM retain the KASH-domain proteins at the ONM and these proteins probably directly interact within the PS. This generates a continuous protein scaffold that physically links the nucleoskeleton to the cytoskeleton: the so-called LINC complex (Crisp et al., 2006) (Figs 2, 5). However, other proteins present in the PS might influence these interactions.

It is intriguing that the ONM proteins in *C. elegans*, although unrelated to those of *Drosophila* and mammals, except for their KASH domains, have evolved similar mechanisms for nuclear anchorage and migration. ANC-1 has a function analogous to that of Msp-300/nesprin and the mammalian nesprins in nuclear anchorage, whereas ZYG-12 has a function analogous to that of Klarsicht in the attachment of the centrosome to the nucleus. Although no proteins related to UNC-83 have been identified, its role in migration is likely to be similarly conserved in higher organisms.

For several reasons, few studies have examined the importance of the mammalian nesprins in nuclear positioning. First, the genes and their mRNAs are very large (Zhang et al., 2002), which makes molecular, biochemical and cellular studies difficult. Second, there are many splice variants, some of which are cell-type specific, which complicates the interpretation of the results. Third, the three nesprins may be able to compensate for one another if one is absent or dysfunctional. Studies in which the function of all three nesprins is disrupted may ultimately prove their importance in nuclear positioning, but such studies are complicated by the fact that the nesprins have other crucial functions.

The only study to demonstrate that the mammalian nesprins are essential in nuclear positioning depended on the overexpression of the nesprin-1 KASH domain in muscle cells (Grady et al., 2005) and was based on similar experiments using the *C. elegans* ANC-1 KASH domain (Starr and Han, 2002). Both studies suggest that the number of SUN-domain-binding sites in the PS is limited. However, overexpressed KASH domains might displace other KASH-domain proteins from the ONM, such as nesprin-3 in mammals, which is also expressed in muscle cells (Wilhelmsen et al., 2005). Furthermore, overexpression of the Klarsicht and Msp-300/nesprin KASH domains in *Drosophila* does not affect nuclear positioning, or produce other phenotypic defects, in photoreceptors or the egg chamber, respectively (Fischer et al., 2004; Yu et al., 2006). Therefore one should be cautious when interpreting results from studies using KASH domain overexpression in higher organisms.

An intriguing theory called cellular tensegrity proposes that eukaryotic cells are pre-stressed through the concerted action

of all three cytoskeletons (Ingber, 2003a; Ingber, 2003b). Importantly, it would explain how simultaneous changes in cytoskeletal, nuclear and other cellular structures can occur in response to localized force in the absence of any biochemical changes (Maniotis et al., 1997). We have known for a relatively long time that integrins can mediate cross-talk between the ECM and the different cytoskeletal systems (Geiger et al., 2001), and that integral INM proteins can mediate the interactions of the nuclear lamina with heterochromatin (Gruenbaum et al., 2005). The well-studied NPCs are known to bridge the nucleoskeleton and the cytoskeleton and have been suggested to mediate the mechanotransduction of signals from the cytoskeleton to the nuclear environment (Ingber, 2006). KASH-domain proteins may provide an alternative mechanism to direct forces into the nucleus (Fig. 5). There is no doubt that future studies on the architecture and positioning of the nucleus and cellular mechanotransduction will reveal many new, exciting functions for this family of proteins.

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