KASH 'n Karry: the KASH domain family of cargo-specific cytoskeletal adaptor proteins

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Summary
A diverse family of proteins has been discovered with a small C-terminal KASH domain in common. KASH domain proteins are localized uniquely to the outer nuclear envelope, enabling their cytoplasmic extensions to tether the nucleus to actin filaments or microtubules. KASH domains are targeted to the outer nuclear envelope by SUN domains of inner nuclear envelope proteins. Several KASH protein genes were discovered as mutant alleles in model organisms with defects in developmentally regulated nuclear positioning. Recently, KASH-less isoforms have been found that connect the cytoskeleton to organelles other than the nucleus. A widened view of these proteins is now emerging, where KASH proteins and their KASH-less counterparts are cargo-specific adaptors that not only link organelles to the cytoskeleton but also regulate developmentally specific organelle movements. *BioEssays* 27:1136–1146, 2005.
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Introduction

How cytoskeletal forces are connected to specific cargos and organelles remains largely mysterious. A recent flurry of papers has uncovered an exciting new mechanism for a class of organelle-specific adaptors that connect their cargo to the cytoskeleton. Most of these studies focus on how the nuclear membrane interacts with the cytoskeleton to position nuclei within the cell. As the nucleus is probably the easiest cargo to visualize and follow in real time, nuclear positioning makes an excellent model to study cargo adaptors. Also, nuclear migration through the cytoplasm and nuclear anchorage within a polarized cell are essential processes for the development of nearly all eukaryotes (for review see Refs. 1–3). Our story begins with a group of *Drosophila* and *C. elegans* mutants with mispositioned nuclei (Fig. 1). As described below, molecular characterization of these mutants has identified a class of outer nuclear membrane components called KASH proteins. Recent evidence has shown that certain isoforms of these proteins adapt cargos other than the nucleus. Therefore, these proteins likely coordinate the interaction of a variety of cargos with the cytoskeleton.

The importance of KASH domains was first speculated by a number of different groups working on a protein called Klarsicht and the ANC-1/MSP-300/Syne family. They noticed that *C. elegans* ANC-1, *Drosophila* MSP-300, and mammalian Syne-1 and Syne-2 all contained a short C-terminal region highly conserved with the C terminus of the *Drosophila* Klarsicht protein (Fig. 2).[4–7] This region has been termed the KASH domain for Klarsicht, ANC-1, and Syne homology. (The KASH domain has also been called the KLS or Klarsicht domain.) More recently, KASH domains have been identified in *C. elegans* UNC-83 and human C14orf49.[8] We also note that, based on low levels of similarity and proposed common functions, there are divergent KASH domains in *C. elegans* ZYG-12 and *S. pombe* Kms1p (Fig. 2). It is likely that other KASH domains have escaped identification due to low levels of amino acid sequence conservation.

KASH domains consist of a predicted transmembrane region followed by fewer than 35 residues at the very C terminus of the protein. C-tail-anchored integral membrane proteins are usually targeted to the ER membrane post-translationally through Sec61-independent mechanisms.[9] In all tested cases, the KASH domain is sufficient for targeting the protein to the nuclear envelope. The large N-terminal domains of KASH proteins perform a variety of functions. These functions, including centrosome attachment to the nucleus, nuclear migration, lipid droplet movement and tethering nuclei to the actin cytoskeleton, are detailed below. The genes

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Abbreviations: KASH, Klarsicht, ANC-1, Syne homology; SUN, Sad1p, UNC-84 homology; LD, Lipid Droplet.

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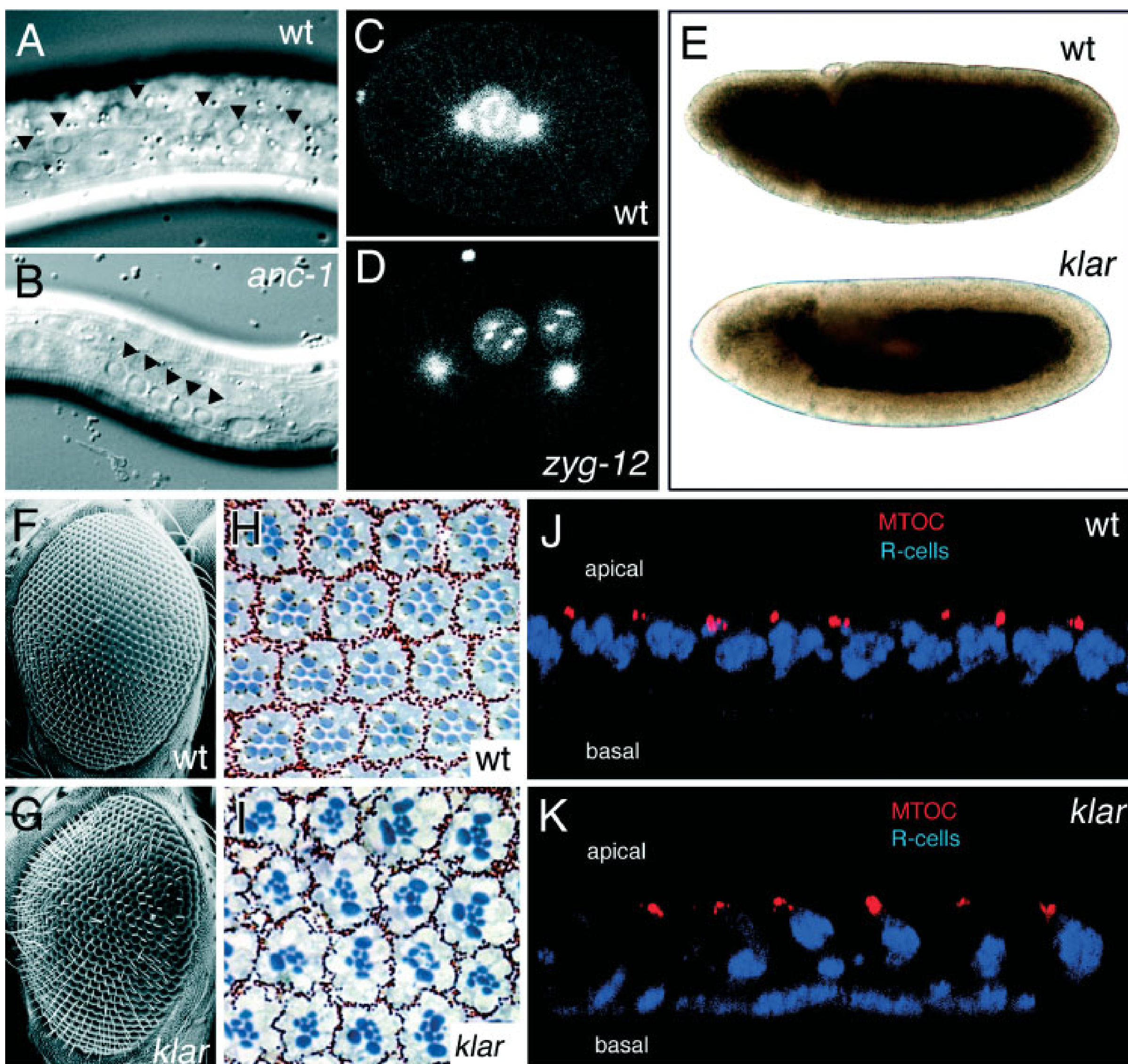

Figure 1. Mutant phenotypes for some KASH protein genes. **A,B:** Nomarski images of the lateral syncitial hypodermis of wild-type (wt) and *anc-1* L2 *C. elegans* hermaphrodites.[22] Nuclei (indicated by arrows) are not held in place in *anc-1* mutants. **C,D:** Wild-type and *zyg-12 C. elegans* one-cell embryos.[26] Tubulin (centrosomes) and histones (DNA) are labeled with GFP. Pronuclei (only one is visible in the focal plane of C) are mispositioned with respect to the centrosome in *zyg-12* mutants. **E:** Transmitted light micrographs of gastrulating *Drosophila* embryos from wild-type and *klarsicht* (*klar*) mothers. *klarsicht* embryos appear clear at the periphery because while lipid droplets spread peripherally (apically) in wild-type embryos, they remain central (basal) in *klarsicht* mutants.[34,58] **F,G:** Scanning electron micrographs of *Drosophila* compound eyes. The external eye surface appears smooth in wild-type and rough in *klarsicht* mutants.[4,28] **H,I:** Light micrographs of tangential sections of *Drosophila* compound eyes. The blue circles are photoreceptor rhabdomeres, which are malformed in *klarsicht* mutants.[4,28] **J,K:** Confocal images (Z-sections) of developing larval eye discs. In wild-type, photoreceptor (R-cell) nuclei are apical and the centrosomes (MTOC) lie just above them. In *klarsicht* mutants, most of the R-cell nuclei lose their connections to centrosomes and are basal.[27]

encoding KASH proteins also encode isoforms lacking KASH domains that do not target to the nuclear envelope, suggesting that the roles of these loci have been identified only partially.

Here we discuss the roles of KASH proteins involved in tethering the nucleus to actin filaments, centrosomes and Golgi. We then describe a model for how KASH proteins might be targeted to the outer nuclear envelope through interactions

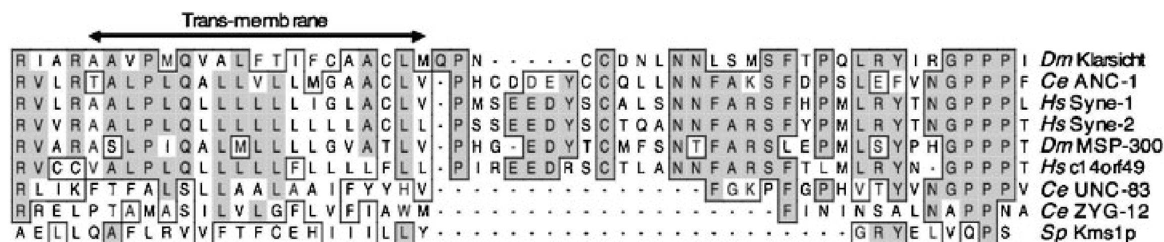

Figure 2. KASH domains. A ClustalW alignment of the C-terminal amino acid sequences of predicted KASH domains is shown. Similar residues are boxed and identical residues are boxed and shaded. The residues shown for each protein are of the predicted trans-membrane regions through the C-terminus. As the sizes of the KASH domains vary in length, dashes indicate gaps in the alignment. ZYG-12 and Kms1 are included, despite low levels of similarity, based on genetic interactions with SUN proteins.

with SUN proteins. Finally we describe the emerging roles of the KASH-less isoforms.

ANC-1, MSP-300 and Syne proteins anchor nuclei to the actin cytoskeleton

C. elegans ANC-1, *Drosophila* MSP-300, and mammalian Syne-1 and Syne-2 (also known as Nesprin-1 and Nesprin-2, Myne, NUANCE and Enaptin; we use Syne because of its precedence in the literature) form a family of proteins that connect the actin cytoskeleton to the nuclear envelope (Fig. 3A). This ANC-1/MSP-300/Syne family has the following features in common. (1) They are huge; the longest isoform of Syne-2 is over 6800 amino acids and full-length Syne-1, MSP-300 and ANC-1 are over 8200 amino acids long, on the order of 1 Mda.[6,7,10,11] (2) The bulk of MSP-300 and the Syne proteins consist of spectrin repeats,[5,10–12] which align end-to-end to form long rope-like structures.[13] ANC-1 is predicted to be highly helical with interspersed regions of coiled-coil stretches; the bulk of ANC-1 likely has a similar structure to spectrin.[6] (3) At the N termini of these proteins are two calponin-like domains.[6,10–12] When paired, calponin domains usually bind filamentous actin.[14] The N termini of ANC-1, MSP-300, Syne-1 and Syne-2 bind actin in vitro.[6,10,11,15] Furthermore, antibodies to the N terminus of MSP-300 or Syne-2 associate with actin structures as do tagged versions of the N termini of ANC-1, Syne-1 and Syne-2.[6,10,11,15,16] *(iv)* The C termini of this protein class all contain KASH domains that, in all cases tested, are sufficient for nuclear envelope localization.[6,17]

The prevailing model is that the largest isoforms of the ANC-1/MSP-300/Syne proteins tether nuclei in the cytoplasm by linking the outer nuclear membrane directly to the actin cytoskeleton (Fig. 3A).[1] The N termini of ANC-1/MSP-300/Syne proteins could extend 300–500 nm into the cytoplasm based on the 5 nm size of a spectrin repeat.[13] In this model, the C-terminal KASH domain binds to the outer nuclear envelope. In support of the tethering model, an engineered form of Syne-2 with the N-terminal actin-binding domain directly connected to the KASH domain, localizes to the nuclear envelope and recruits actin to the periphery of the nucleus.[10]

The tethering model is strikingly similar to the idea for how dystrophin and its associated proteins connect the actin cytoskeleton to the plasma membrane.[18] Dystrophin and ANC-1/MSP-300/Syne have homologous N-terminal actin-binding domains and long spectrin repeats. Thus, ANC-1/MSP-300/Syne are similar to dystrophin, except for the addition of a KASH domain. Mutations in dystrophin and associated proteins cause damage to the muscle plasma membrane, leading to Duchenne's muscular dystrophy.[19] Also, mutations in certain nuclear envelope components that may interact with KASH domain-containing proteins (see below) lead through unknown mechanisms to Emery-Dreifuss muscular dystrophy.[20,21] The role of ANC-1/MSP-300/Syne proteins in the progression of muscular dystrophies remains to be characterized.

The best functional data for the role of the ANC-1/Syne/MSP-300 family of proteins and the tethering model comes from the null mutant phenotypes in *C. elegans*. Normally nuclei are anchored in place. They might move slightly within the cell, but return quickly to their original positions.[1] Null mutations in *anc-1* or the SUN gene *unc-84* disrupt the anchorage of nuclei in a variety of *C. elegans* tissues, allowing nuclei to float freely through the cytoplasm, often forming large clusters in syncytial cells (Fig. 1).[6,22,23] UNC-84 recruits ANC-1 to the nuclear envelope[6] and the double mutation is equally as severe,[23] suggesting that they work in the same pathway.

Additional information about ANC-1 and Syne-1 functions comes from dominant negative phenotypes in *C. elegans* tissues and mouse skeletal muscle. The *anc-1(null)* phenotype was copied completely upon overexpression of the C-terminal KASH domain of ANC-1 in an otherwise wild-type background.[6] It was proposed that overexpression of the KASH domain titrates the available nuclear envelope docking sites (presumably UNC-84; see below) blocking endogenous

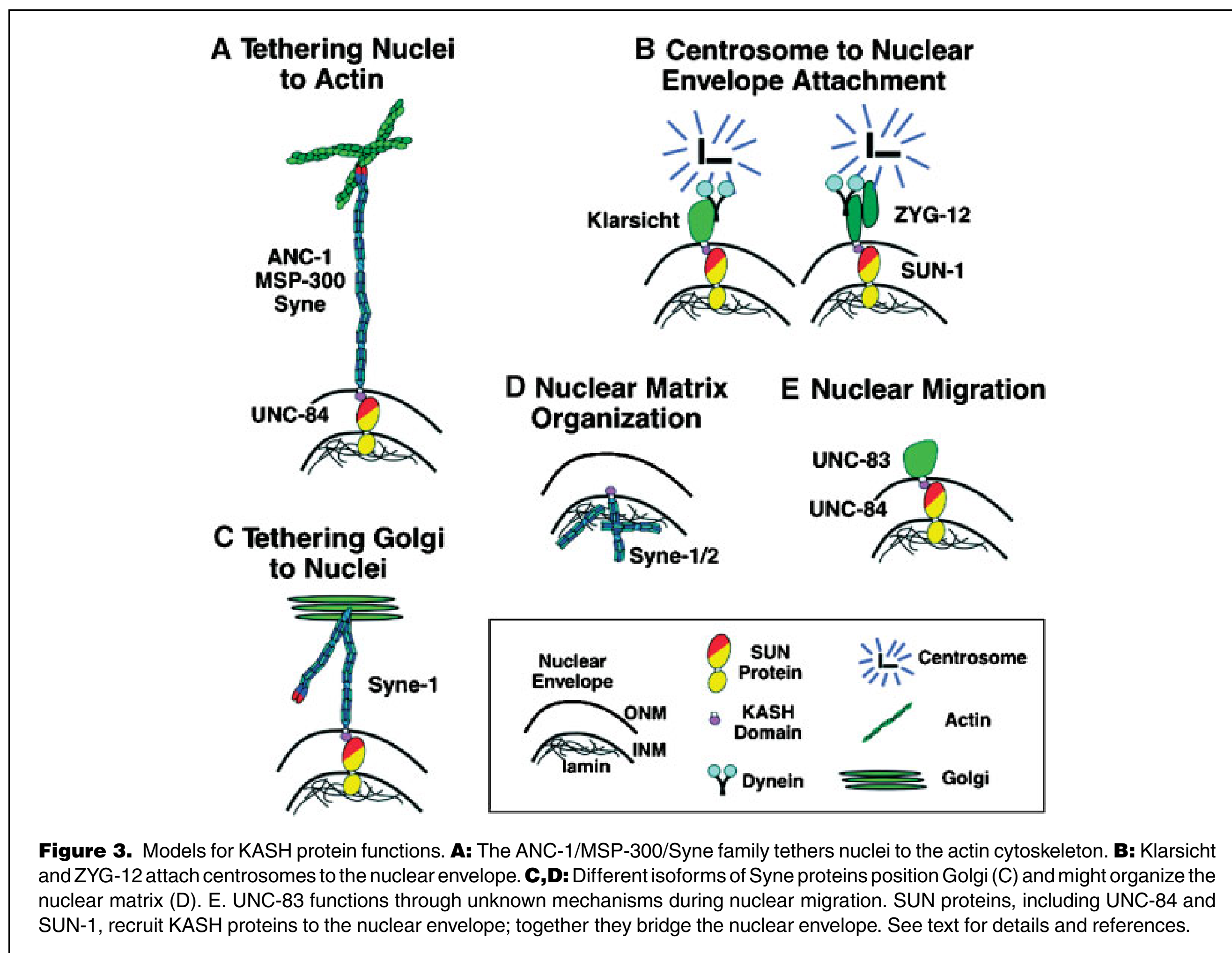

Figure 3. Models for KASH protein functions. **A:** The ANC-1/MSP-300/Syne family tethers nuclei to the actin cytoskeleton. **B:** Klarsicht and ZYG-12 attach centrosomes to the nuclear envelope. **C,D:** Different isoforms of Syne proteins position Golgi (C) and might organize the nuclear matrix (D). E. UNC-83 functions through unknown mechanisms during nuclear migration. SUN proteins, including UNC-84 and SUN-1, recruit KASH proteins to the nuclear envelope; together they bridge the nuclear envelope. See text for details and references.

ANC-1 from the nuclear envelope. A similar dominant negative approach was undertaken in mice, where a transgenic line expressing the C terminus of Syne-1 in skeletal muscles was generated.[24] The Syne-1 protein fragment was targeted to the nuclear envelope efficiently, and blocked most of the endogenous Syne-1 from accumulating at the nuclear envelope. Transgenic mice had 66% fewer myonuclei at the neuromuscular junction, suggesting that Syne-1 anchors muscle nuclei there.[24,25]

Klarsicht and ZYG-12 link the nucleus to the centrosomes

Two different KASH proteins, *C. elegans* ZYG-12 and *Drosophila* Klarsicht, are the first proteins discovered to link the nucleus to centrosomes.[26,27]

Klarsicht (also known as *marbles*) is required in the developing compound eye for apical nuclear migrations in differentiating cells (Fig. 1).[4,28] Failure of apical nuclear migration affects cell shape and thereby overall compound eye morphology. Like *anc-1* and its orthologs described above, *klarsicht* is a large and complex gene, with at least two promoters and alternately spliced messages that give rise to different protein isoforms (see below and Fig. 4). *klarsicht* alleles with mutations in the KASH domain result in nuclear migration defects in the eye, suggesting that the KASH domain is required for nuclear migration. In addition, a 2266 amino acid KASH form of Klarsicht (isoform α Fig. 4) expressed by a transgene partially restores nuclear migration.[4,27] It remains to be determined if the partial rescue is due to failure of the transgene to express early enough during eye development or if other forms of Klarsicht are necessary. The amino acid sequence N-terminal to the KASH domain of Klarsicht bears little resemblance to other proteins.[4]

A variety of observations point to a model for Klarsicht function analogous to the model for ANC-1, where Klarsicht acts a bridge between the nucleus and cytoskeleton, in this case, likely microtubules. The C-terminal KASH domain targets Klarsicht to the outer nuclear envelope while the N

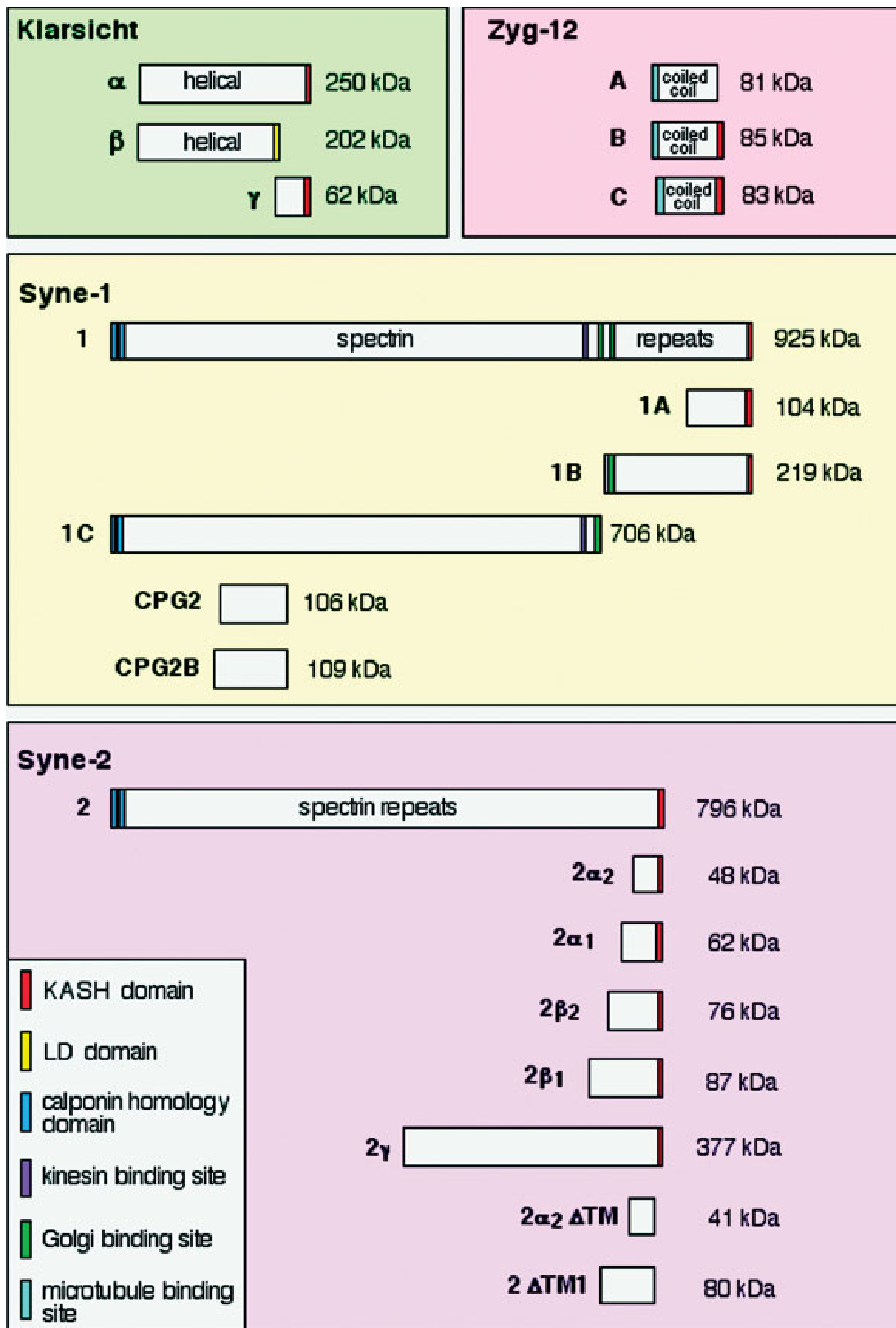

Figure 4. KASH protein genes generate multiple protein isoforms. Shown are the four examples where multiple protein isoforms generated by KASH protein genes are well-characterized. See text for details and references.

terminus attaches to microtubules, probably via dynein. First, an epitope-tagged Klarsicht α expressed in the eye by a transgene localizes to the nuclear envelope and to microtubules apical to the nucleus.[27,29] An identical Klarsicht protein missing its KASH domain localizes to microtubules only, while a complementary protein fragment containing the KASH domain localizes only to apical microtubules.[29] In addition, the *Drosophila* gene encoding B-type Lamin

(Lam_{Dm0}) interacts genetically with *klarsicht, Lam_{Dm0}* mutants have a *klarsicht*-like eye phenotype, and Lam_{Dm0} eye discs lack nuclear envelope localization of Klarsicht.[27] Also, while centrosomes form normally in *klarsicht* or Lam_{Dm0} mutant eye discs, they lose their association with the nuclei; the nuclei are positioned randomly, but the centrosomes are all apical.[27] Finally, flies with mutations in the genes for the dynein regulators dynactin, Lis1 or BicD, have nuclear migration defects in the eye similar to those in *klarsicht* and Lam_{Dm0} mutants[30–32] and in the context of its role in lipid droplet migration (see below), Klarsicht is thought to regulate dynein.[33,34]

Although ZYG-12 performs a function similar to Klarsicht, ZYG-12 differs from Klarsicht in many respects.[26] While Klarsicht functions in post-mitotic cells, ZYG-12 is required for nucleus/centrosome attachment during pronuclear migration in one-cell embryos and also for many subsequent cell cycles. *zyg-12* mutants die as embryos due to cellular aneuploidy. In addition, ZYG-12 protein isoforms are small. There are three ZYG-12 isoforms (Fig. 4), two that contain KASH domains (B and C) and one that does not (A). Also, N-terminal to its KASH domain, ZYG-12 is similar to another protein called Hook. Hook was identified originally in *Drosophila* and has vertebrate homologs.[35–38] Remarkably, *Drosophila* and vertebrate Hook proteins do not have KASH domains. Like isoforms of Syne-1 that may be KASH-less (see below), Hook links Golgi and endosomes to microtubules.

The results of many experiments support a model where the centrosome is linked to the nucleus through dimerization of ZYG-12 B or ZYG-12 C anchored at the nuclear envelope with ZYG-12 A linked to the centrosome.[26] First, experiments with anti-ZYG-12 antibodies and GFP-tagged transgenes show that ZYG-12 is present at the nuclear envelope and the centrosome. While GFP-tagged B or C isoforms localize to both the nuclear envelope and the centrosome, GFP-tagged A is detected only at the centrosome. In addition, the coiled-coil domains dimerize, and dimerization was shown to be required for ZYG-12 function genetically. Finally, the KASH-less isoform A and a KASH form are both required in transgene rescue experiments in order to complement *zyg-12* mutations. ZYG-12 also interacts with a subunit of dynein, so like Klarsicht, ZYG-12 might also link nuclei to centrosomes via dynein.[26]

UNC-83, a third *C. elegans* nuclear envelope KASH protein, is essential for certain nuclear migration events. Null mutations in *unc-83* block nuclear migration in two groups of hypodermal cells in the late embryo and first larval stage and in the developing intestine.[39,40] Unlike ZYG-12 and Klarsicht, which connect the centrosome to the nucleus, UNC-83 functions through a different, unknown mechanism (Fig. 3); centrosomes remain attached in nuclei that fail to migrate in *unc-83* mutants.[40] Like Klarsicht, UNC-83 has no detectable conserved domains outside of the KASH domain. It appears that ZYG-12 and UNC-83 function in complementary tissues to control nuclear migration in *C. elegans*. An otherwise novel KASH protein, c14orf49, has also been identified in humans (Fig. 2).[8] Nothing is known about c14orf49 other than it is a likely nuclear envelope protein.[41] As they all contain KASH domains and the bulk of their amino acid sequences are predicted to be helical, we speculate that Klarsicht, UNC-83 and c14orf49 might be homologs. As UNC-83 is not essential for the connection between centrosomes and nuclei, the functions of these proteins may have diverged to some extent.

Syne-1 links nuclei to Golgi and signaling complexes

In skeletal muscles, Syne-1 may also link the nucleus to Golgi. Supporting this model, large Syne-1 isoforms have two Golgi-binding sites (Fig. 4), Syne-1 is found at the Golgi, and overexpressed fragments of Syne-1 containing the Golgi binding sites disrupt Golgi structure in cultured cells.[42] Remarkably, Golgi are perinuclear in muscle cells and their distance from the nucleus, 150–200 nm,[43] is the predicted distance between the Golgi binding sites and the KASH domain of Syne-1.[42] Thus, in addition to anchoring the nucleus to actin, Syne-1 also tethers the nucleus to Golgi in muscle cells.[44] By keeping the Golgi closely associated with the nuclear envelope, Syne-1 could mediate retrograde transport from the Golgi to the ER in skeletal muscles.[45]

In heart muscle cells, Syne-1 anchors a scaffold protein called mAKAP (muscle A-kinase anchoring protein) to the nuclear envelope.[46] mAKAP maintains a complex consisting of multiple signal transduction molecules and interacts with a small Syne-1 isoform (Fig. 4) through spectrin repeats on both proteins.[46] Along these lines, Syne-1 was isolated initially as an interactor of MuSK, the kinase atop a MAP kinase cascade at the neuromuscular junction of skeletal muscles.[5] Thus in addition to connecting organelles to each other and the cytoskeleton, KASH proteins localize protein complexes to the nuclear envelope.

Are all KASH proteins in the outer nuclear membrane?

The models presented above for nuclear anchorage and centrosome attachment postulate that KASH proteins are components of the outer nuclear membrane (Fig. 3). There is overwhelming, albeit indirect, evidence that large KASH isoforms reside in the outer, as opposed to the inner, nuclear envelope. Recent data, however, also provide evidence for the presence of KASH proteins in the inner nuclear envelope.

What do immunolocalization studies reveal? At the light microscope level, it is not possible to differentiate between antibody localization to the inner or outer nuclear membrane. Digitonin experiments and electron microscopic analyses have led to every possible conclusion about KASH proteins: that they are only in the outer nuclear envelope, only in the inner envelope, or in both membranes.[10,16,17,47,48] What

makes all of these studies inconclusive is the lack of negative controls—in no case were results with wild-type cells compared with protein nulls.

What do genetic and molecular analyses suggest? Models for ANC-1/MSP-300/Syne and Klarsicht and ZYG-12 function require that KASH protein isoforms are lodged in the nuclear envelope and in contact with cytoplasmic proteins simultaneously. As described above, these models are well-supported by genetic, molecular and biochemical data.

Nevertheless, biochemical data also suggests that a subset of Syne proteins reside in the inner nuclear membrane, closely associated with the nuclear matrix (Fig. 3D). Syne-1 and Syne-2 interact directly with lamin and emerin, two inner nuclear membrane proteins.[16,47,49] Furthermore, overexpression of a C-terminal domain of Syne-1 disrupts the localization of emerin, suggesting that at least the C terminus of Syne-1 is involved in organization of the nuclear matrix.[47,50] It seems unlikely that large KASH isoforms would be present in the inner nuclear envelope, as transmembrane proteins >60 kDa are generally excluded from diffusing from the outer envelope through the nuclear pore.[51] Small KASH isoforms of Syne-1 and Klarsicht are known to exist (Fig. 4). One way that the data could be reconciled is if the small forms reside in the inner envelope, while the large forms are present in the outer nuclear envelope. It remains to be determined what the roles of the small KASH forms would be.

SUN proteins target KASH proteins to the outer nuclear envelope

As discussed above, KASH domains are sufficient for nuclear envelope localization and connect the nuclear envelope to cytoplasmic structures. Here we address how KASH domains are targeted to the nuclear envelope. This process is mediated by a class of inner nuclear membrane components called SUN domain proteins (Figs. 3, 5). SUN proteins were first identified in *S. pombe* (Sad1p) and *C. elegans* (UNC-84)[23–52] and have since been found across all eukaryotes, even in the basal eukaryote *Giardia* (GenBank #EAA41593). Fungi appear to have a single SUN protein, while invertebrates have two, and mammals have four (Fig. 5). SUN proteins contain a 115–175 residue conserved domain at their C terminus, at least one predicted *trans*-membrane domain, and a less conserved, mostly helical N-terminal domain. In all examined cases, SUN proteins are components of the nuclear envelope.[23,26,52–56] Recent data suggest that human Sun2 is an inner nuclear membrane component, with its SUN domain in the intermembrane space, and its N-terminal domain in the nucleoplasm, interacting with lamin.[57]

The best evidence that the SUN proteins interact with KASH proteins comes from three different studies in *C. elegans* where it is clear that SUN proteins are required to target KASH proteins to the nuclear envelope. In the early embryo, SUN-1 (also known as MTF-1) is required to recruit ZYG-12 to the nuclear envelope.[26] It is because of this interaction that we call the C terminus of ZYG-12 a KASH domain, despite its low level of similarity to other KASH domains (Fig. 2). Later in development, UNC-84 is required to recruit both ANC-1 and UNC-83 to the nuclear envelope.[6,40] Moreover, missense mutations in the SUN domain of UNC-84 do not disrupt UNC-84 localization to the nuclear envelope,[23,53] but disrupt the targeting of ANC-1 and UNC-83 to the nuclear envelope.[6,40] Outside of *C. elegans*, the exact KASH/SUN domain pairs are uncharacterized; possible KASH/SUN pairs are in Figure 5. Although we conclude from these experiments that SUN domains are required for KASH localization, it is not yet known if this occurs through a direct physical interaction. We hypothesize an additional role for UNC-84 as a switch between nuclear migration and anchorage. In support of this model, *unc-84* mutants display both nuclear migration and anchorage phenotypes, and *unc-84* interacts genetically with both *unc-83* and *anc-1*.[6,23,40]

Functions for KASH-less isoforms

Many KASH domain-containing proteins also exist as KASH-less isoforms whose functions are poorly understood. The best understood KASH-less isoform is Klarsicht β (Fig. 4), which is formed by alternate splicing that replaces the KASH domain with a domain called LD that binds lipid droplets.[58] In addition to its role in eye development, *klarsicht* is required for developmentally regulated movements of lipid droplets in early embryos.[34] One role of Klarsicht in this process is to link, through dynein, lipid droplets with the microtubule skeleton. Many results support the idea that the KASH form of Klarsicht (α) and the LD form (β) have separate functions. Mutant *klarsicht* alleles with lesions that disrupt the KASH domain but not the LD domain affect eye development but not lipid droplet migration.[58] Also, anti-Klarsicht antibodies and RFP-tagged LD domains associate with lipid droplets.[58]

Biophysical and microscopic analysis of lipid droplet motions in *Drosophila* embryos suggest that the N terminus of Klarsicht β does not simply link lipid droplets to dynein, but actually regulates motor proteins.[33,34,59–61] The model is that Klarsicht may control the activities of kinesins and dyneins bound simultaneously to a lipid droplet, so that the net movement of lipid droplets is towards the minus ends of microtubules. As Klarsicht β and Klarsicht α have a common N terminus and both mediate minus-end-directed movement, Klarsicht may also regulate dynein in its role in nuclear migration in the eye.

Small, KASH-less isoforms of Syne-1 are essential for postsynaptic receptor endocytosis.[62] Two brain-specific isoforms, called CPG2 and CPG2b (Candidate Plasticity Gene 2), are required in the postsynaptic endocytic zone of excitatory synapses for endocytosis of glutamate receptors. CPG2 is found at the postsynapse in cultured glutamatergic

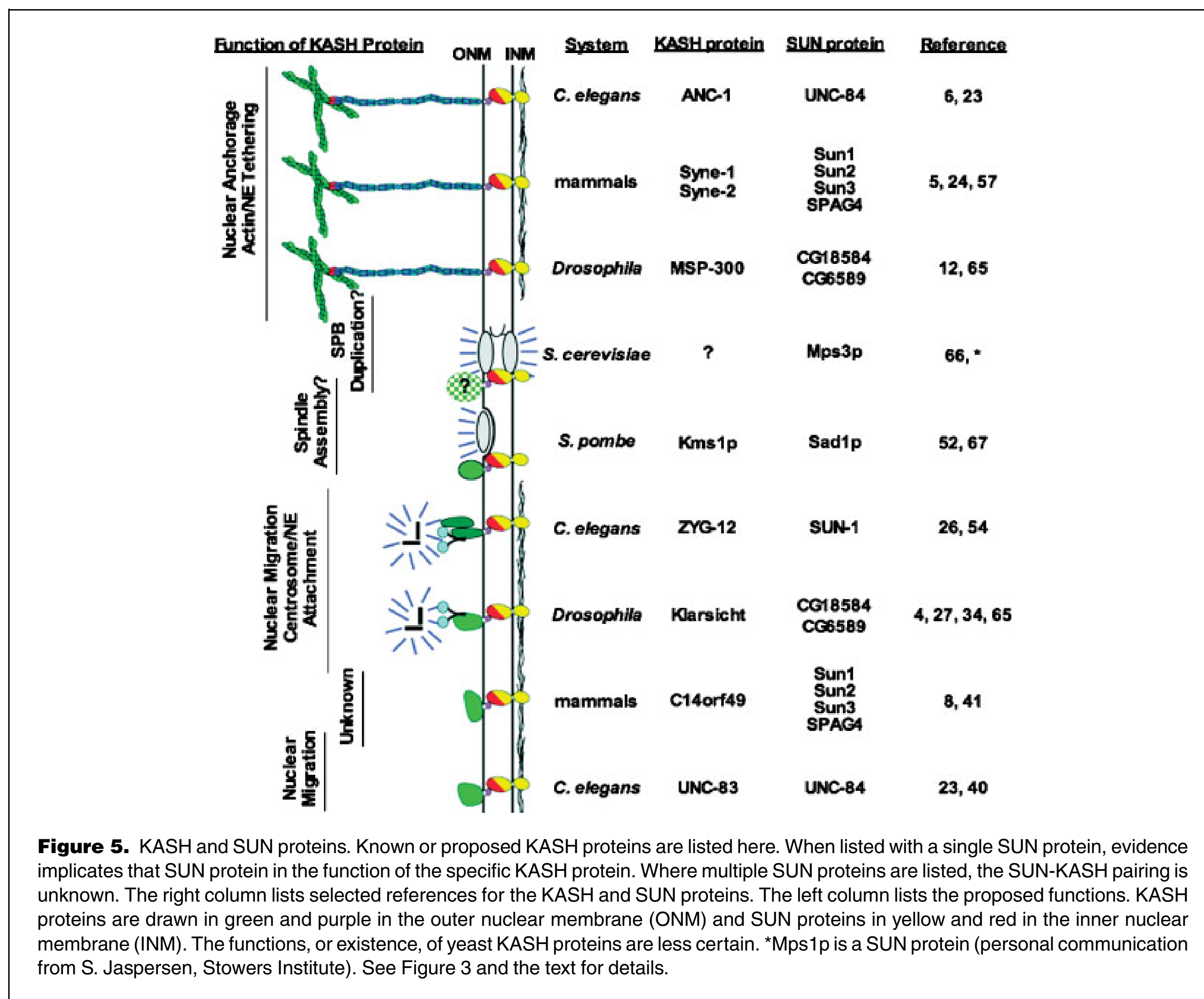

Figure 5. KASH and SUN proteins. Known or proposed KASH proteins are listed here. When listed with a single SUN protein, evidence implicates that SUN protein in the function of the specific KASH protein. Where multiple SUN proteins are listed, the SUN-KASH pairing is unknown. The right column lists selected references for the KASH and SUN proteins. The left column lists the proposed functions. KASH proteins are drawn in green and purple in the outer nuclear membrane (ONM) and SUN proteins in yellow and red in the inner nuclear membrane (INM). The functions, or existence, of yeast KASH proteins are less certain. *Mps1p is a SUN protein (personal communication from S. Jaspersen, Stowers Institute). See Figure 3 and the text for details.

neurons. In neurons where CPG2 levels are lowered by RNAi, clathrin-coated vesicles containing glutamate receptors accumulate and internalization is disrupted.[62] This suggests that CPG2 is required to clear internalized vesicles from synapses. Exactly how CPG2 functions is not known.

Other functions that appear not to involve the nucleus have been described for the KASH family proteins. In these cases, it would stand to reason that KASH-less isoforms are involved, but this has not been demonstrated. For example, a function for Klarsicht has been described in polarized delivery of membrane during salivary gland development.[63] It is not clear if this function involves the KASH-less Klarsicht β or Klarsicht α. Mutant *klarsicht* alleles that disrupt both the LD and KASH domains result in decreased apical area of the salivary gland lumen, while overexpression of Klarsicht α results in the opposite phenotype. As the minus ends of microtubules are apical in this tissue, Klarsicht is thought to function similarly here as it does in the eye and embryo, but here its cargo is membrane required for growth.

Another example is *Drosophila* MSP-300.[12,15] A muscle-specific phenotype has been described for a single point mutation in the *MSP-300* gene; mutant flies die during embryogenesis with defective somatic muscle morphology. *MSP-300* interacts genetically with *laminin A* (an extracellular basement membrane component) and an anti-MSP-300 antibody colocalizes with actin at plasma membrane sites where muscle cells attach to each other or to ectoderm.[12] However, nuclear positioning was not assayed in *MSP-300* mutant muscles and the antibody was generated to amino acids encoded by an N-terminal exon that may not be part of a processed transcript with the C-terminal KASH-encoding exon. Thus, the role of KASH-containing isoforms of MSP-300 in nuclear positioning and muscle development remains to be characterized. Generation of targeted mutations that delete

specific exons of *MSP-300* will enable a more complete picture of the function of this protein.

KASH-less isoforms of Syne proteins and ANC-1 may also have critical functions. As described above, Syne-1 connects the nucleus and Golgi and it also has a role in maintaining Golgi structural integrity.[42] Syne-1 also has a kinesin (KIF3B)-binding site, overexpression of which blocks cytokinesis in cultured cells.[64] While its role there is unclear, most of the Syne-1 in dividing cultured cells is found at a central portion of the mitotic spindle. There is a KASH-less Syne-1 isoform with both Golgi- and Kinesin-binding sites (Fig. 4), which may play some role in these processes. Also, the roles of KASH-less isoforms of Syne proteins in the nuclear matrix remain to be characterized.[16,47] Another example is the role of ANC-1 in mitochondrial integrity; mutations in *anc-1* severely disrupt the shape and anchorage of mitochondria.[6,22] The portions of ANC-1 necessary for mitochondrial anchorage and the roles of ANC-1 in positioning of other organelles require further studies to define. Finally, high levels of Syne-2 have been detected at the sarcoplasmic reticulum,[16] suggesting that Syne-2 may also help organize this specialized ER.

One observation to emerge from all of these preliminary studies is that the world is not so simple as first thought. It is not that one class of proteins (Klarsicht and ZYG-12) connects cargos to microtubules, and another class (ANC-1/MSP-300/Syne) connects cargos to actin filaments. The many isoforms of these proteins are involved in a wide variety of cellular functions.

Conclusions

In the past few years, an exciting class of proteins with conserved C-terminal KASH domains has been identified. These "KASH proteins" have little or nothing in common with one another outside of their KASH domains. A strong model is emerging that KASH domains are necessary and sufficient for nuclear envelope targeting. We hypothesize that KASH domains are likely targeted to the nuclear membrane through interactions with SUN proteins in the intermembrane space of the nuclear envelope. Based on functional data described above, KASH domain-containing proteins appear to localize to the outer nuclear membrane. Once attached to the nuclear membrane, with the bulk of the protein likely facing the cytoplasm, KASH proteins play a variety of different functions. The clearest functions are in nuclear migration and anchorage by tethering nuclei to either the actin or microtubule cytoskeleton. KASH proteins have also been proposed to coordinate microtubule motors, to organize the nuclear matrix, to localize protein complexes at the nuclear envelope, and to position Golgi and mitochondria.

Just as the models for KASH protein functions at the nucleus have become clear, more complexity has emerged. Many, if not all, of the KASH-containing proteins also exist as KASH-less isoforms that are targeted to a variety of intracellular locations. Some of these KASH-less isoforms connect organelles other than the nucleus to the cytoskeleton.

From a developmental biology point of view, many interesting questions remain to be addressed. For example, in the fly eye, it remains unknown how nuclear migration is coordinated with cell determination. Could a regulatory event be specific expression or modification of Klarsicht itself or a SUN protein required for Klarsicht function? Also, the specificity of both Klarsicht and ZYG-12 is puzzling. Klarsicht is not required for nuclear migrations in *Drosophila* oocytes or embryos. Likewise, ZYG-12 is required only for early embryonic cell divisions in *C. elegans*. What alternative mechanisms exist for other developmentally regulated nuclear movements—and do they involve other KASH proteins? KASH proteins are also involved in an important developmental switch between nuclear anchorage and migration. It is an exciting prospect that KASH-less isoforms might play a variety of similar roles in developmental switches in the behavior of multiple organelles.

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