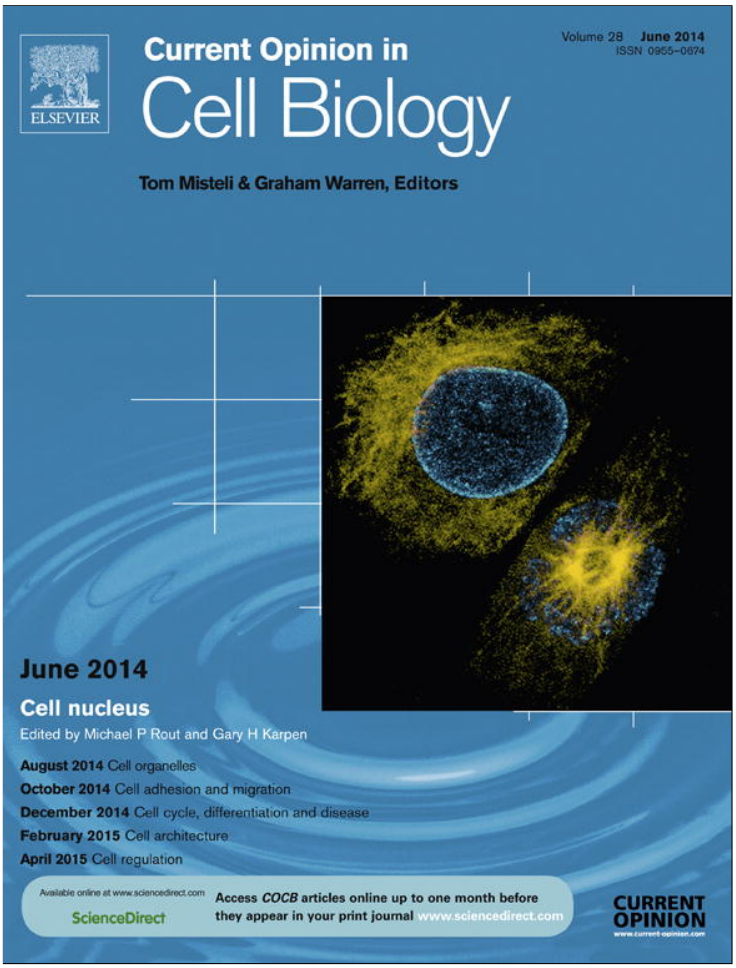

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# KASHing up with the nucleus: novel functional roles of KASH proteins at the cytoplasmic surface of the nucleus

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Nuclear–cytoskeletal connections are central to fundamental cellular processes, including nuclear positioning and chromosome movements in meiosis. The cytoskeleton is coupled to the nucleoskeleton through conserved KASH–SUN bridges, or LINC complexes, that span the nuclear envelope. KASH proteins localize to the outer nuclear membrane where they connect the nucleus to the cytoskeleton. New findings have expanded the functional diversity of KASH proteins, showing that they interact with microtubule motors, actin, intermediate filaments, a nonconventional myosin, RanGAP, and each other. The role of KASH proteins in cellular mechanics is discussed. Genetic mutations in KASH proteins are associated with autism, hearing loss, cancer, muscular dystrophy and other diseases.

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## Introduction

SUN and KASH proteins form a bridge across the nuclear envelope, often referred to as the LINC complex, connecting the nucleoskeleton to the cytoskeleton [1]. KASH proteins are named after the founding members of the family, *Drosophila* Klarsicht, *C. elegans* ANC-1, and mammalian SYNE-1 and SYNE-2 (nesprin-1 and nesprin-2) [2]. All KASH proteins contain a C-terminal trans-membrane domain followed by a short (~10–32 residues), conserved luminal KASH domain that is necessary and sufficient to target the large, unconserved cytoplasmic domains to the outer surface of the nuclear envelope [1,3–5]. KASH proteins are targeted to the outer nuclear membrane through a direct physical interaction between the KASH domain and SUN proteins in the peri-nuclear space of the nuclear envelope. The KASH–SUN interaction was recently described at the structural level [6,7] and thoroughly reviewed [8–11]. There are many excellent comprehensive reviews on KASH and SUN proteins [1,3–5]. Here we focus on recent developments on the diverse array of functions that KASH proteins play at the cytoplasmic surface of the nucleus (Figure 1). KASH proteins function in transmitting mechanical forces from the cytoplasm to the nucleus. During meiosis, KASH proteins transmit forces generated in the cytoplasm that move telomeres inside the nucleus [12]. Given the wide variety of cell and developmental functions KASH proteins play, it is not surprising that defects in KASH proteins have been linked to an ever-growing list of human diseases (Table 1).

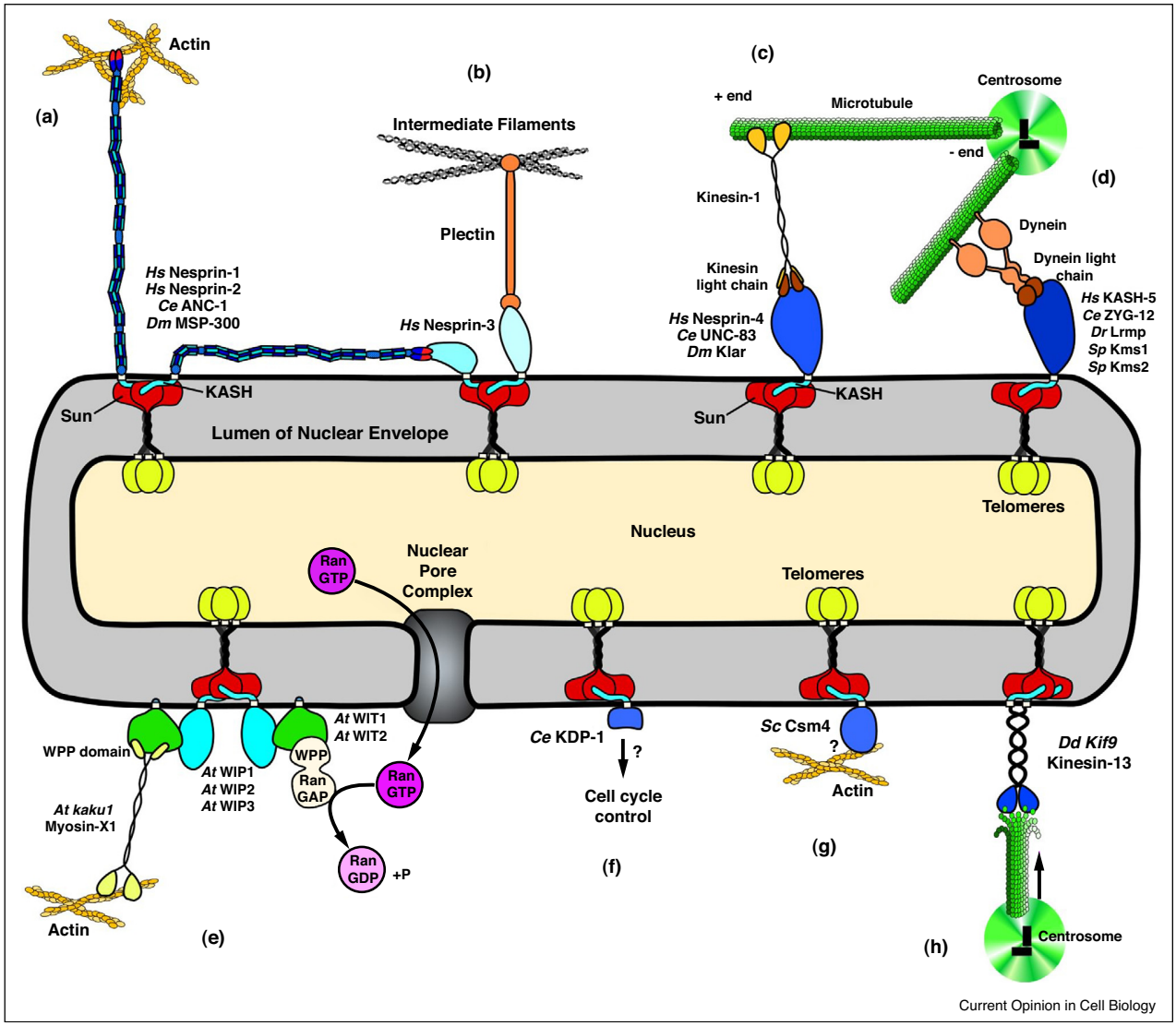
## KASH proteins form a complex nuclear scaffold

The canonical KASH proteins are mammalian nesprin-1 and nesprin-2, and their worm and fly orthologs ANC-1 and MSP-300 (Figure 1A). These giant (800–1000 kDa) proteins tether nuclei to the actin cytoskeleton, and play important roles in muscle and neuronal development [1]. They consist of N-terminal actin-binding domains and C-terminal KASH domains separated by a long rod consisting of over 70 spectrin repeats in the case of nesprin-1 [13]. Adding to the difficulties of studying nesprin-1 and nesprin-2 is the abundance of isoforms; 16 5′ start sites and 14 3′UTR in the nesprin-1 locus alone encode countless isoforms, many of which are tissue specific [14•]. It was recently shown that the N-termini of nesprin-1 and nesprin-2 interact with the cytoplasmic domains of other KASH proteins, including the intermediate filament-associated KASH protein nesprin-3 [15] (Figure 1A,B). Together, these data suggest a model where various isoforms of nesprin-1 and nesprin-2 form a scaffold around the nucleus [15,16]. Furthermore, this nuclear scaffold likely plays a role in regulating the size of the nucleus [15].

## Hearing and KASH protein-mediated recruitment of kinesin to the nuclear envelope

Another class of KASH proteins including mammalian nesprin-4, *C. elegans* UNC-83, and *Drosophila* Klarsicht, interact with a kinesin light chain to target kinesin-1 to the surface of the nucleus [17,18] (Figure 1C). Recently, nesprin-4 was found to function in ear development and hearing. Human geneticists identified a family with a mutation in nesprin-4 that caused hearing loss, and nesprin-4 knockout mice were shown to be normal except for loss of hearing [19••]. Major structural defects were observed in the cochlea of nesprin-4, or the SUN partner sun-1, knockout mice. By postnatal day 30, the outer hair cells in these mice exhibited degenerated stereocilia and

**Figure 1**




Functions of KASH proteins at the cytoplasmic face of the nucleus. SUN proteins form trimers in the inner nuclear membrane with their conserved SUN domains (red) and coiled domains in the lumen of the nuclear envelope and nucleoplasmic domains (yellow). SUN domains interact with the KASH domain (light blue) in the lumen of the nuclear envelope. The cytoplasmic domains of KASH proteins (various shades of blue) are on the outer surface of the nucleus. SUN and KASH proteins are thought to interact in a three-to-three ratio. Only one or two KASH proteins are shown for each complex for simplicity. **(A)** Giant KASH proteins made of spectrin repeats tether the nucleus to actin networks. (A–B) Giant KASH proteins also interact with nesprin-3 to form a cage around the nucleus. **(B)** Nesprin-3 interacts with intermediate filaments through plectin. **(C)** KASH proteins recruit kinesin-1 to move nuclei. **(D)** KASH proteins recruit dynein to move nuclei, telomeres (or pairing centers in worms) or to connect centrosomes. **(E)** The plant KASH proteins WIP1, 2, and 3 interact with WITs (green) to recruit a myosin-XI to move nuclei, and a RanGAP to catalyze the hydrolysis of GTP in Ran (pink) as it exits the nucleus. **(F)** The worm KASH protein KDP-1 regulates the cell cycle through unknown mechanisms. **(G)** Yeast Csm4 fits the definition of a KASH protein used to move telomeres along actin. **(H)** A novel kinesin-13 is a KASH protein that functions to attach the centrosome to nuclei. The names of KASH proteins from various systems, including humans (*Hs*), roundworms (*Ce*), fruit flies (*Dm*), zebrafish (*Dr*), fission yeast (*Sp*), budding yeast (*Sc*), slime molds (*Dd*), and angiosperms (*At*) are indicated.

nuclei that were mispositioned to the apical cellular surface [19••].

## Dynein–KASH protein interactions

There is a large class of KASH proteins that recruit dynein to the surface of the nuclear envelope (Figure 1D). Mouse Sun1 functions in homologous chromosome pairing in meiosis to attach telomeres to the inner surface of the nuclear envelope [20]. KASH5 was recently identified as a meiosis-specific protein with a conserved KASH domain that localizes to the nuclear envelope in a Sun1-dependent manner [21,22••]. Ectopic expression of KASH5

**Table 1**

**Human diseases associated with genetic mutations in KASH proteins.**

| KASH protein | Disease | Reference |
|---|---|---|
| Nesprin-1 | Emery-Dreifuss muscular dystrophy | [70] |
| | Myogenic autosomal recessive arthrogyroposis | [71] |
| | Autosomal recessive cerebellar ataxia | [72,73] |
| | Colorectal cancer | [74] |
| | Ovarian cancer | [75] |
| | Bipolar disorder | [76,77] |
| | Recurrent major depression | [77] |
| | Familial autism spectrum disorders | [78,79] |
| Nesprin-2 | Emery-Dreifuss muscular dystrophy | [70] |
| | Breast cancer | [74] |
| | Gastrointesitnal stromal tumors | [80] |
| Nesprin-4 | High-frequency hearing loss | [19••] |

recruits dynein to the nuclear envelope, KASH5 co-immunoprecipitates with dynein and dynactin, and KASH5 co-localizes with SUN proteins and telomeres during meiosis [22••]. Furthermore, KASH5 knockout mice are infertile, have a severe meiotic chromosome pairing defect, fail to repair double-strand breaks, and fail to recruit dynein to telomere attachment sites [22••]. The KASH5 homolog in zebrafish, Lrmp, functions in the zygote to attach centrosomes to the male pronucleus and is required for female pronuclear migration [23]. In *C. elegans*, ZYG-12 and SUN-1 recruit dynein to subtelomeric regions (pairing centers in worms) that serve as attachment sites in meiosis. Dynein then is required for the intranuclear movements of chromosomes, which aid in homolog pairing and synapsis [24]. ZYG-12 also mediates the nuclear envelope localization of dynein during pro-nuclear migration [25], making ZYG-12 a likely functional ortholog of KASH5 and Lrmp. During meiosis in *S. pombe*, the KASH proteins Kms1 and Kms2 recruit dynein to specific spots on the nuclear envelope that associate with telomeres attached to the inside of the nuclear envelope. The γ-TuRC complex is then recruited to these spots resulting in the formation of mini microtubule organizing centers, or telocentrosomes, which move the telomeres toward the spindle pole body [26•]. The KASH proteins UNC-83, Klarsicht, nesprin-1 and nesprin-2 also function, in part, to recruit dynein to the nuclear envelope [27–30]. Therefore, dynein is recruited to the surface of nuclei by SUN–KASH bridges. At the surface of the nucleus, dynein movements function to move nuclei, connect microtubules or centrosomes to nuclei, and to move chromosomes inside the nucleus during meiotic homolog pairing.

## KASH proteins in plants

SUN and KASH proteins were recently described in plants [31,32••,33] and have been hypothesized to mediate actin-dependent nuclear migrations [34]. Three *Arabidopsis* WIP (for WPP domain-Interacting Proteins; Figure 1E) proteins are the first identified KASH proteins in plants [32••,35]. WIP proteins interact with WIT1 and 2 (WPP-Interacting Tail-anchored proteins) in the outer nuclear membrane [36]. AtWIP1, 2, and 3 fail to localize to the nuclear envelope in SUN double mutants and all three co-immunoprecipitate with AtSUN1 and AtSUN2 in a KASH-domain-dependent manner [32••]. *Arabidopsis* mutants lacking the SUN, WIP, or WIT proteins lead to abnormally round nuclei, suggesting that nuclei are no longer attached to the actin cytoskeleton [32••,37•]. Forward genetic screens for similar mutants identified the *Arabidopsis* gene *kaku1*, which encodes a nonconventional myosin, myosin-XI [37•]. Myosin-XI contains a WPP domain that interacts with WIT2 and then the WIP/SUN bridges to recruit it to the outer nuclear membrane to stretch and move nuclei [37•] (Figure 1E). WIP and WIT proteins also recruit RanGAP1 to the cytoplasmic surface of the nuclear envelope [32••,35,36,38]. In animal cells, RanGAP interacts with the cytoplasmic filaments of nuclear pore complexes to induce the hydrolysis of RanGTP to RanGDP as it exits the nucleoplasm [39]. Alternatively, in plants, RanGAP has a novel WPP domain that interacts with WITs at the outer nuclear membrane [32••,36,38] (Figure 1E). Other plant KASH proteins likely remain unidentified since SUN proteins appear to play a similar role in maize meiosis as they do in yeast and animals [31], but their KASH partners remain unknown.

## Novel KASH proteins and their functions

Novel functions for new KASH proteins continue to be found. *C. elegans* KDP-1 is a KASH protein that regulates progression of the cell cycle through unknown mechanisms [40] (Figure 1F). Genetic phenotypes, the presence of a tail-anchored domain, and the interaction with the SUN protein Mps3 [41,42] are consistent with Csm4 being a KASH protein that connects actin to meiotic telomeres in *S. cerevisiae* (Figure 1G). Kif9, a *Dictyostelium* kinesin-13, is a KASH protein that functions to connect nuclei to centrosomes [43••,44]. Kif9 localizes to the nuclear envelope near the centrosome with Sun1; once at the nuclear envelope, it is thought that Kif9 depolymerizes microtubules, pulling the centrosome toward the nucleus [43••] (Figure 1H). It is likely that additional KASH proteins remain to be discovered.

## KASH proteins and cellular mechanics

Mechanical stimuli have been known for sometime to be communicated into the nuclear interior through the cytoskeleton during various fundamental cellular processes such as cell adhesion, migration, and differentiation [45–47]. However, the molecular mechanism responsible for this communication remained unclear until recently. KASH proteins, through their participation in LINC complexes, have been hypothesized to be responsible for this force transmission [48,49].

Four lines of experimental evidence where the overexpression of dominant negative KASH or SUN protein constructs was used to disrupt the formation of endogenous LINC complexes have supported this hypothesis. First, the lack of functional LINC complexes was shown to cause a loss of cytoskeletal mechanical stiffness in fibroblasts [50]. Second, cells containing disrupted LINC complexes had defective nuclear–cytoskeletal coupling as measured by the deformation of the nucleus caused by a microneedle pulling on the cytoskeleton [51]. These cells also displayed an altered organization of the actin and intermediate filaments. Similar results were obtained in experiments where shear forces transmitted through magnetic beads adhered onto the plasma membrane of cells [52]. Third, the actin-dependent rotation of nuclei observed in cells exposed to cyclic stretch requires intact LINC complexes [53]. Fourth, the rigidity dependence of nuclear height was disrupted in cells lacking functional LINC complexes [54]. While collectively these results implicate LINC complexes in intracellular force transmission, they do not identify specific KASH or SUN proteins.

Specific KASH proteins are necessary for three examples of nuclear–cytoskeletal force transmission. First, nesprin-1 is required for the reorientation of endothelial cells in response to applied uniaxial cyclic strain perpendicular to the direction of mechanical strain, which is important during proper angiogenesis [55]. Nesprin-1-depletion also resulted in an increase in focal adhesion assembly, cell traction, and nuclear height. Since a similar change in nuclear height was observed in cells where myosin-II activity was inhibited, it was suggested that the actomyosin tension is balanced in part by the nucleus. However, in the absence of nesprin-1, actomyosin tension was balanced by the increase in focal adhesions [55]. In a separate study, nesprin-1-depleted endothelial cells subjected to uniaxial stretching exhibited increased nuclear strain, which is an indicator of force transmission to the nucleus [56]. Second, nesprin-3 was found to be required for the ability of endothelial cells to polarize in response to shear stress such that their centrosomes are positioned on the side of the nucleus facing the source of stress [57]. Third, the ability of shear flow-stimulated myoblasts to assemble a specialized subset of actin cables above their nuclei was shown to require nesprin-3 and to a lesser extent, nesprin-2G [58•]. The formation of these cables, which have been referred to as the PeriNuclear Actin Cap (PNAC), occurs in response to sheer stresses that are 50-fold lower than those required to form the actin cables found underneath the nucleus. PNAC cables terminate in a specialized subset of focal adhesions that are important for the mechano-sensing of matrix stiffness [59]. Interestingly, the focal adhesion protein zyxin was specifically required for the fast assembly of the PNAC, suggesting that extracellular mechanical stimuli can be sensed by the PNAC and quickly relayed to the LINC complex and into the nucleus [58]. Taken together, these results are consistent with a model where the LINC complex mediates nuclear–cytoskeletal force transmission.

## A comparison between the PNAC and TAN lines

The PNAC is a subset of highly organized and dynamic actin cables that form over the apical surface of interphase nuclei in adherent cells [59–61]. In addition to mechanosensation, several functions have been attributed to the PNAC including maintenance of nuclear shape, control of cellular differentiation, and three-dimensional cell migration [62•,63,64]. A similar structure to the PNAC, known as transmembrane actin-associated nuclear (TAN) lines, has also been identified and demonstrated to be required for nuclear movement in migrating fibroblasts [65,66]. TAN lines are linear arrays of nesprin-2G/Sun2 LINC complexes that form on the dorsal nuclear surface of fibroblasts that are preparing to migrate. Through their anchorage by A-type lamins, TAN lines are able to harness the forces generated by retrograde flow of the perinuclear actin cables, resulting in the movement of the nucleus to the rear of the cell, which is required for efficient cell migration [65–67]. The inner nuclear membrane protein Samp1 is also a TAN line component [68]. Samp1 associates with both SUN2 and A-type lamins, but requires the later for its localization to the nuclear envelope, suggesting that Samp1 assists in anchoring TAN lines. While linear arrays of LINC complexes have not been visualized in the PNAC, it is possible that these structures are related to one another. For instance, TAN lines may only form during active movement of the nucleus in migrating cells. It will be important to further understand the similarities and differences between the PNAC and TAN lines.

## Conclusions

Our understanding of the cellular, developmental, and disease-related roles of KASH proteins is growing at an accelerating rate. It has become clear that KASH proteins, as part of LINC complexes, are critical mediators of nuclear–cytoskeletal force transmission events and cellular mechanics. KASH proteins also function in other events including cell cycle regulation and nuclear transport. The ability of KASH proteins to participate in such a wide variety of cellular functions stems from the ever-growing list of KASH proteins and their functions at the surface of the nucleus. Unsurprisingly, genetic mutations in KASH proteins are associated with various human diseases including Emery-Dreifuss muscular dystrophy, mental disorders, several cancers, and hearing loss [3,69] (Table 1 and references within). Much remains to be studied about the regulation of KASH proteins. It is not know when KASH proteins are assembled onto SUN proteins, or how KASH proteins are regulated during important developmental switches such as between nuclear migration and anchorage. Most importantly,

how mutations in KASH proteins contribute to the pathologies of various human diseases remains to be determined.

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