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Nucleocytoplasmic connections and deafness

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The linker of nucleoskeleton and cytoskeleton (LINC) complex connects the nuclear lamina to the cytoskeleton, in part to aid in nuclear positioning. Mutations in genes encoding LINC complex and lamina components cause a range of human diseases. In this issue of the JCI, Horn et al. report that mutations in the gene SYNE4 encoding the LINC complex protein nesprin-4 lead to progressive high-frequency hearing loss. Further, in mice deficient in nesprin-4 and Sun1, another LINC complex component, outer hair cells of the cochlea form normally during development, but die in the early postnatal weeks. These results link improper nuclear positioning specifically to the death of outer hair cells in the organ of Corti and ultimately to deafness.

The nuclear envelope is composed of the nuclear membranes (inner and outer), nuclear pore complexes, and nuclear lamina. It separates the nucleoplasm from the cytoplasm of eukaryotic cells, and the transport of proteins, nucleic acids, and other molecules among these compartments in interphase is restricted to the pore complexes. The nuclear envelope has been a growing focus of

clinical investigation, as in the past 15 years a wide range of inherited diseases have been linked to mutations in genes encoding proteins of this subcellular structure (1, 2).

Nuclear envelope and nucleocytoplasmic connections

Recent research has shown that the nuclear envelope not only separates the nucleus from the cytoplasm, but also connects the structural networks of these subcellular compartments (Figure 1). The linker of nucleoskeleton and cytoskeleton (LINC) complex mediates this con-

nection (3). The core of the LINC complex forms from the interaction of SUN (Sad1, UNC-84) domain proteins with KASH (Klarsicht, ANC-1, Syne homology) domain proteins called nesprins (4, 5). This creates a protein bridge spanning the inner and outer nuclear membranes. The SUN domains, attached to a trimeric coiled coil of Sun proteins, bind three KASH domains, with a disulfide bond between cysteines in SUN and KASH further covalently linking them (6). Suns are integral proteins of the inner nuclear membrane that also bind to A-type lamins of the nuclear lamina, a meshwork of intermediate filament proteins providing structural support to the nucleus. The SUN-KASH binding within the perinuclear space retains the nesprin, which also contains a transmembrane segment, in the outer nuclear membrane. At the other end of this transnuclear membrane bridge, different nesprins interact with unique cytoskeletal components. For example, nesprin-1 and nesprin-2 isoforms bind directly to actin, nesprin-3 via plectin to cytoplasmic intermediate

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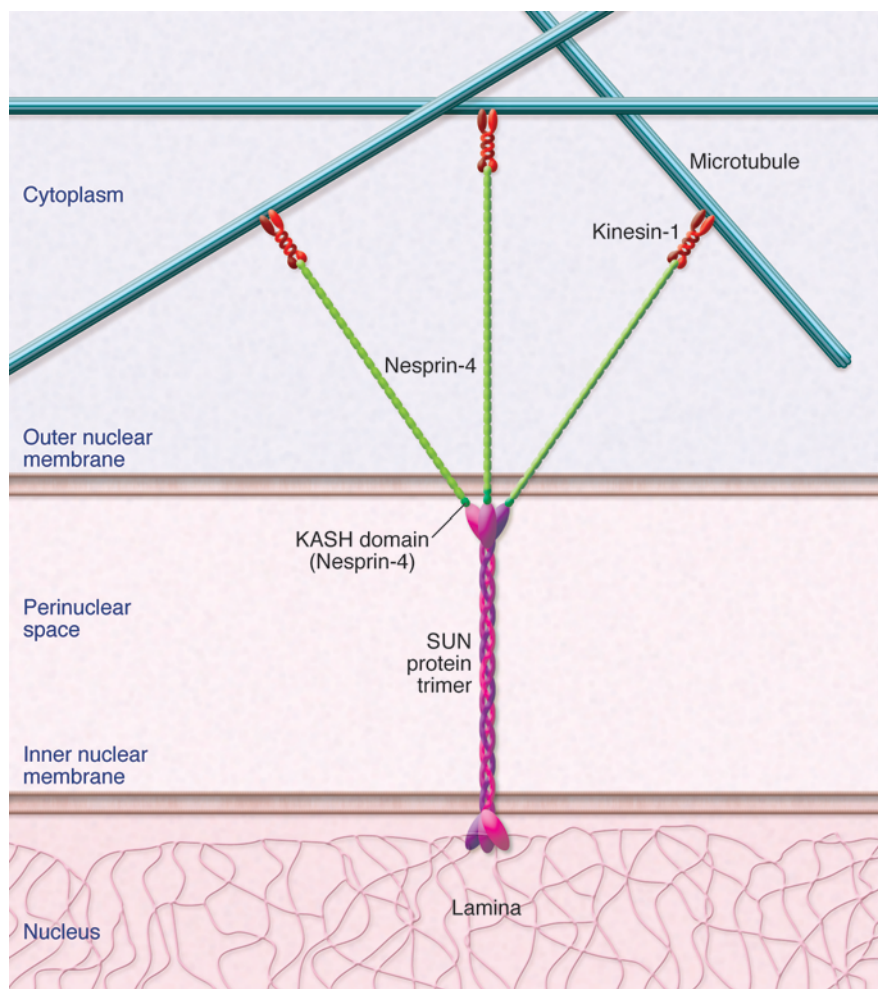


Figure 1

The LINC complex and its connection to the nuclear lamina and cytoskeletal components. The carboxyl termini of Suns, integral proteins of the inner nuclear membrane, bind within the perinuclear space to the carboxyl terminal KASH domain of nesprins, which are transmembrane proteins localized to the outer nuclear membrane. The amino termini of Suns bind to the nuclear lamina, an intermediate filament meshwork of lamins on the inner aspect of the inner nuclear membrane. Different nesprins have variable cytoplasmic amino terminal domains that interact with different cytoskeletal elements. Nesprin-4, shown in this diagram, binds to kinesin, which in turn can associate with microtubules.

filaments, and nesprin-4 to kinesin (4, 5). As a result of these series of interactions, the nucleoskeleton is connected to the cytoskeleton.

The nucleocytoplasmic interactions established by the LINC complex play an important role in the transmission of mechanical forces across the nuclear envelope and in nuclear movement and positioning. In migrating fibroblasts, linear arrays of nesprin-2-G and Sun2 mediate the retrograde actin-dependent movement of nuclei (7). In mammalian neurons, Sun1/2 and nesprin-1/2 function in microtubule-dependent nuclear migration during neurogenesis via their interactions with dynein and kinesin-1 (8). Nesprin-4 binds to kinesin, and its expression in cultured cells is associated with changes in the locations of the centrosome and Golgi apparatus relative to the nucleus, suggesting a role in microtubule-dependent nuclear positioning (9). The relevance of these nuclear position-

ing processes to health and disease are only beginning to be understood.

Linking the LINC complex to deafness

In this issue of the *JCI*, Horn et al. report that mutations in the gene *SYNE4*, encoding nesprin-4, cause autosomal recessive, progressive high frequency hearing loss in two families of Iraqi Jewish ancestry (10). Nesprin-4 was previously shown to be present in the nuclear envelope in cells of the salivary gland, exocrine pancreas, bulbourethral gland, mammary gland, and in some kidney cells (9), among others. In the current work, Horn et al. show its expression in hair cells of the cochlea, specifically the three rows of outer hair cells and one row of inner hair cells in the organ of Corti. Further, Horn et al. demonstrated that nesprin-4-deficient mice — as well as mice lacking Sun1 — have progressive hearing loss. In these mice, outer hair

cells are formed but appear to degenerate as hearing matures, while the inner hair cells remain intact. Nuclei of the outer hair cells fail to maintain a normal basal localization. The authors conclude that the nucleocytoplasmic connections established by the LINC complex are essential for viability of the outer hair cells and propose that proper nuclear positioning in these cells is critical to the maintenance of normal hearing (10).

Perspective and remaining questions

Sensory hair cells in the mammalian inner ear are extremely sensitive to environmental stress and undergo apoptosis in response. While hair cells in nonmammalian vertebrates are able to regenerate from the surrounding supporting cells, mammalian hair cells do not, so their degeneration leads to permanent hearing loss (11). The basal-to-apical gradient of cell death Horn et al. observed in mice with defects in the LINC complex is



common to many forms of cochlear damage, including acoustic trauma and exposure to aminoglycoside antibiotics and chemotherapy agents (12). The underlying causes of this gradient of sensitivity are poorly understood but have been hypothesized to result from a gradient in stress-induced sensitivity to reactive oxygen species or apoptotic signaling. It will therefore be interesting to test whether therapies that mitigate the normal sensitivity to stress in these cells, such as treatment with free radical scavengers, also mitigate the precocious outer hair cell degeneration in the nesprin-4-deficient and Sun1-deficient mice.

Nesprin-4 and Sun1 are present in the nuclear envelopes of both inner and outer hair cells of the organ of Corti; however, only outer hair cells appear to be severely affected by deletion of their genes. Horn et al. hypothesize that this is the result of extreme mechanical stress placed on these cells because of their well-known power of movement in response to sound as part of the cochlear amplifier (10). However, depletion of Suns and nesprin-2 has been shown to lead to a very similar degeneration of postmitotic photoreceptors in the mouse eye (13). This suggests that other processes may be at work, since photoreceptors do not harbor the same properties of electromotility as outer hair cells. It is therefore important to directly determine whether the electromotility of outer hair cells is indeed affected in nesprin-4-deficient and Sun1-deficient mice, as hypothesized, and which apoptotic pathways may be activated as a result of any observed abnormality. In addition to sparing of the inner hair cells, it is also unclear why organs other than the inner ear containing cells that express nesprin-4 and Sun1 and may be subjected to various stressful forces are apparently not affected in the knockout mice.

Thus, while the effects of LINC complex defects on the unique electromechanical properties of outer hair cells of the inner ear is a parsimonious hypothesis, alternative mechanisms need to be ruled out. Alterations in nesprins and Sun proteins could potentially affect cellular functions in ways that do not result from the altered transmission of mechan-

ical forces across the nuclear envelope or abnormal nuclear positioning. For instance, Sun proteins interact directly and indirectly with several elements of the nuclear interior, including chromatin (14). Hence, mutations in their genes could lead to changes in cell type-specific gene expression that are detrimental to physiological function and result from abnormalities in nuclear functions separate from those involving connecting the nucleoskeleton to the cytoskeleton.

Finally, mutations in genes encoding proteins of the LINC complex and associated structures cause many disorders other than hearing loss. Mutations in *SYNE1*, encoding nesprin-1, cause an autosomal recessive cerebellar ataxia (15). They have also been reported to cause an autosomal recessive form of arthrogryposis multiplex congenita characterized by decreased fetal movements, delay in motor milestones, and progressive motor decline after the first decade (16). *SYNE1* and *SYNE2* mutations have further been implicated in Emery-Dreifuss muscular dystrophy-like phenotypes (17, 18). Various mutations in the lamin A/C gene (*LMNA*) cause several different tissue-selective diseases (1, 2), but an effect on nuclear movement has only been shown for a subset of *LMNA* mutations that cause cardiomyopathy and muscular dystrophy and only in a model fibroblast system (19). These fascinating results point to the need for a better understanding of the tissue- and cell-selective expression of the protein components of the LINC complex and associated structures. In addition, a better understanding of the functional roles of each of these proteins in force transmission across the nuclear envelope and in nuclear movement and positioning is required to explain the divergent phenotypes of diseases caused by alterations in nucleocytoplasmic connections.

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