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TAN lines

A novel nuclear envelope structure involved in nuclear positioning

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Nuclear position is actively controlled and can be adjusted according to the needs of a cell by nuclear movement. Microtubules mediate the majority of nuclear movements studied to date, although examples of nuclear movements mediated by the actin cytoskeleton have been described. One such actin-dependent nuclear movement occurs during centrosome orientation in fibroblasts polarizing for migration. Here, the centrosome is maintained at the cell center while the nucleus is moved to the cell rear by actin retrograde flow thus positioning the centrosome between the nucleus and the leading edge of the cell. We have explored the molecular mechanism for actin dependent movement of the nucleus during centrosome centration. We found that a novel linear array of nuclear envelope membrane proteins composed of nesprin-2G and SUN2, called transmembrane actin-associated nuclear (TAN) lines, couple the nucleus to moving actin cables resulting in the nucleus being positioned toward the cell rear. TAN lines are anchored by A-type lamins and this allows the forces generated by the actin cytoskeleton to be transmitted across the nuclear envelope to move the nucleus. Here we review the data supporting this mechanism for nuclear movement, discuss questions remaining to be addressed and consider how this new mechanism of nuclear movement may shed light on human disease.

Key words: actin, nesprin, SUN, lamina, centrosome, migration, LINC complex, TAN lines

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Introduction

Contrary to the impression one gets from diagrams and images in cell biology textbooks, the position of nuclei in cells is not static. Nuclei move within the cytoplasm to specific locations in response to the needs of the cell especially during cell polarization and movement associated with tissue reorganization and organ development. Nuclear movements have been best documented during cell division and fertilization, where nuclei are positioned to ensure equal distribution between daughter cells and karyogamy, respectively.[1-3] Recently, nuclear movement has been shown to play important roles during various developmental processes including meiosis, cell migration and differentiation, and the formation of proper tissue architecture.[4-6] How nuclear movement and positioning contributes to these processes remains unclear.

Nuclear movement, like the movement of other organelles, requires forces generated by the cytoskeleton. Forces from microtubule polymerization or motor proteins drive the majority of nuclear movements studied to date (reviewed in refs. 2 and 3). However, a growing list of actin-dependent nuclear movements have been described including the movement of root tip nuclei during root growth in Arabidopsis,[7] axial expansion of nuclei in the *Drosophila melanogastar* syncytial blastoderm,[8] nuclear centration in the *Caenorhabditis elegans* zygote,[9] and rearward movement of nuclei to orient the centrosome for migration in fibroblasts[10] (**Table 1**). Interestingly, there are types of nuclear movement which use forces generated by both microtubules and actin, such as nucleokinesis during neuronal cell migration[11-13] and interkinetic nuclear

Table 1. Actin-dependent nuclear movements

	Nuclear positioning event	System	References
Actin-dependent	Nuclear movement in mature hyphae	*Neurospora crassa*	67
	Nuclear Movement during Root hair development in primary roots	*Arabidopsis thaliana*	7, 68
	Phototropin-dependent positioning of nuclei in leaf cells	*Arabidopsis thaliana*	69
	Pre-mitotic nuclear migration in subsidiary mother cells	*Tradescantia virginiana*	70
	Nuclear centration in the zygote	*Caenorhabditis elegans*	9
	Axial expansion of nuclei in pre-syncytial blastoderm	*Drosophila melanogastar*	71
	Nuclear movement during fibroblast polarization for migration	*Mus musculus*	10, 30
Actin- and microtubule-dependent	Nuclear movement into the bud during mitosis	*Saccharomyces cerevisiae*	72–74
	Post-mitotic nuclear migration	*Micrasterias denticulate*	75, 76
	Nuclear centering	*Spirogyra crassa*	77
	Interkinetic nuclear movement during retinal development	*Danio rerio*, *Oryzias latipes*	78–81
	Nucleokinesis during neuronal migration	*Mus musculus*, *Rattus rattus*	11–13, 82–85
	Interkinetic nuclear movement during neocortex development	*Mus musculus*, *Rattus rattus*	85-91

movement in pseudostratified epithelia[14,15] (**Table 1**). Moreover, the nuclei of the *C. elegans* embryonic hypodermis use both actin and microtubule cytoskeletons but at different times and for different purposes. These nuclei specifically engage microtubule motor proteins to move within the hypoderm while they interact with actin filaments for anchorage to specific subcellular localizations suggesting that regulatory mechanisms dictate when and where these nuclei will interact with a specific cytoskeletal system.[5,6,16] While mechanisms for Microtubule dependent nuclear movement have been proposed,[3,17,18] mechanisms for actin-dependent nuclear movement are unknown and it is unclear how actin forces may be coupled to the nucleus.

To determine the functions of nuclear positioning, a better understanding of the molecular mechanisms for nuclear movement is required. To this end, much progress has been made in the past few years with the identification of the linker of nucleoskeleton and cytoskeleton (LINC) complex, a molecular bridge that allows the transmit of forces generated by bytoplasmic cytoskeletal elements into the nucleoplasm.[5,6,19] The LINC complex consists of the outer nuclear membrane nesprin* proteins and the inner nuclear membrane SUN proteins.[19] Nesprins posses divergent N-termini but contain a conserved, nuclear envelope targeting, KASH domain at their C-termini and are sometimes referred to as "KASH proteins."[6,20] The KASH domain consists of a transmembrane domain and a short tail that projects into the perinuclear space and interacts with the C-terminus of SUN proteins which contains the conserved SUN domain.[6] The divergent N-termini of SUN proteins are found in the nucleoplasm where they interact with the nuclear lamina and/or chromatin binding proteins.[6,20,21] The cytoplasmic N-termini of nesprins differ and this allows for nesprin family members to interact with all three filament systems of the cytoskeleton: actin filaments, microtubules and intermediate filaments.[6,22] The LINC complex has been primarily implicated in microtubule-driven nuclear movement, lending support to the nuclear envelope bridge model of nuclear movement.[5]

A number of years ago, we modified the wounded fibroblast monolayer system to explore the initial polarization of cells in preparation for cell migration.[23,24] NIH3T3 fibroblasts grown to confluence are serum starved for two days and then scratched to create a wound. In the absence of serum, wounding alone does not stimulate polarization or migration into the wound. Subsequent addition of serum or the specific serum factor, lysophosphatidic acid (LPA), triggers rapid polarization of the wound-edge cells. LPA triggers only polarization while serum triggers both polarization and migration.[24,25]

A classic marker for cell polarity in wound edge cells is the reorientation of the centrosome to a position between the nucleus and the leading edge of a cell.[26,27] Centrosome reorientation is thought to be important for directed cell migration due to the fact that the centrosome is intimately associated with the Golgi complex, resulting in the polarization of the secretory system towards the front of the cell.[28] Several years ago we found that centrosome reorientation in wounded monolayers of NIH3T3 fibroblasts did not involve movement of the centrosome, as had been expected, but resulted from movement of the nucleus past a stationary centrosome.[10] Our initial findings indicated that this nuclear movement was required to make protrusive activity at the leading edge productive.[10] Myosin II driven retrograde actin flow was necessary for this nuclear movement, yet it was unclear whether there was a direct connection between actin and the nucleus and if so, what might mediate such an interaction to allow transduction of force to move the nucleus. Below, we describe recent results that have begun to suggest a model for how the linkage between actin filaments and the nuclear envelope is established

***** The original name for mammalian nesprins was Syne, for synaptic nuclei enriched.[22] Lately, the field appears to be adopting the name "nesprin", which we use here.

and how this linkage is anchored by SUN proteins and the nuclear lamina.

TAN Lines: Transmembrane Actin-Associated Nuclear Lines

The data described below are from Luxton et al. 2010.[30] Based on the requirement of actin and myosin II for nuclear movement during centrosome reorientation and the ability of specific mammalian nesprins (nesprin-1 and nesprin-2) to interact with actin filaments,[6,10] we hypothesized that the nucleus might be coupled to moving (retrograde flowing) actin filaments via the LINC complex during centrosome reorientation. To test this hypothesis, we expressed the KASH domain of nesprin-2, which disrupts the nuclear localization of all nesprins by competing for binding to SUN proteins, in serum-starved wound-edge NIH3T3 fibroblasts. Addition of LPA normally triggers nuclear movement, but in KASH expressing cells, nuclear movement failed to occur (**Fig. 1**). This result suggested that nesprins, and by association SUNs, were involved in the rearward movement of the nucleus in LPA stimulated fibroblasts. There are two mammalian nesprins that encode actin binding calponin homology (CH) domains at their N-termini, nesprin-1G and nesprin-2G ("G" refers to the giant splice form; there are smaller splice variants that do not contain CH domains[31]), but we found only nesprin-2G was expressed in NIH3T3 cells. Knockdown of nesprin-2G by siRNA inhibited nuclear movement and re-expression of a green fluorescent protein (GFP)-mini-nesprin-2G chimeric construct comprising the N- and C-termini of nesprin-2G rescued nuclear movement showing the specificity of the nesprin-2G knockdown. As GFP-mini-nesprin-2G lacks most of the spectrin repeats between the N-terminus and C-terminus, this result also suggests that most of the spectrin repeats are dispensable for nuclear movement. Additional experiments revealed that GFP-mini-nesprin-2G lacking CH domains or encoding mutated CH domains was unable to rescue nuclear movement in nesprin-2G depleted cells. Therefore, nesprin-2G and its ability to interact with actin filaments are required for nuclear movement during centrosome reorientation in fibroblasts polarizing for migration.

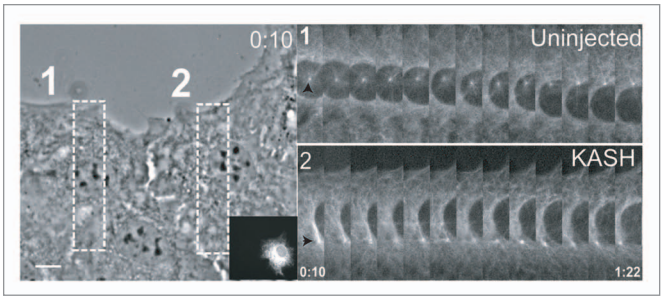

Figure 1. Nuclear movement during centrosome orientation involves nesprins. Nuclear movement in RFP-KASH-expressing (insert), GFP-α-tubulin NIH3T3 fibroblasts. The left panel shows a phase contrast image from the begining of the movie. Boxes indicate the regions used to generate the GFP-α-tubulin fluorescence kymographs on the right. Arrowheads indicate centrosome position. Time is in h:min. Bar: 10 μm. From Luxton et al. Science 2010; 329:956.

In cells rescued with GFP-mini-nesprin-2G, we were surprised to find that GFP-mini-nesprin-2G accumulated in linear arrays, mostly parallel to the leading edge and on the dorsal surface of nuclei (**Fig. 2A**). Endogenous nesprin-2G also formed linear arrays as revealed by immunofluorescence with a nesprin-2G specific antibody. These linear arrays of GFP-mini-nesprin-2G co-localized with actin cables near the dorsal surface, were sensitive to drugs that perturbed actin or myosin-II and required the actin binding CH domains of GFP-mini-nesprin-2G (**Fig. 2A**). Fluorescence recovery after photobleaching demonstrated that GFP-mini-nesprin-2G within the linear arrays was immobilized relative to bulk GFP-mini-nesprin-2G in the nuclear membrane. Endogenous SUN2, but not SUN1 nor a number of other nuclear envelope proteins, co-localized with these nesprin-2G arrays, indicating the arrays had a specific molecular composition and were not the result of nuclear envelope deformations (**Fig. 2B**). Depletion of SUN2 also prevented nuclear movement in response to LPA. Based on these findings, we concluded that the linear arrays of nesprin-2G and SUN2 represent a novel structure on the nuclear envelope and named them trans-membrane actin-associated nuclear (TAN) lines. The co-localization of TAN lines with dorsal actin cables suggested a possible mechanism for moving the nucleus rearward during centrosome orientation. Previous studies in the 1980s identified "transverse arcs" of actin filaments beneath the dorsal surface of migrating fibroblasts (and other cells) that moved rearward toward the nucleus.[32,33] These transverse arcs resemble the dorsal actin cables in our study, although the actin cables in NIH3T3 fibroblasts are not typically curved, perhaps because of constraints to cell spreading imposed by adjacent cells in the wounded monolayer.

To test whether the dorsal actin cables moved rearward, we used Lifeact-mCherry, which detects filamentous actin,[34] to monitor the dynamics of the dorsal actin cables during nuclear movement. These studies showed that several dorsal actin cables were associated with the nucleus during its movement and moved at the same rate as the nucleus. Ventral stress fibers, generally oriented perpendicular to the dorsal cables, did not move with the nucleus. In LPA stimulated cells, actin initially formed from an isotropic meshwork of filaments that resolved into dorsal actin cables and began moving rearward just before nuclear movement was initiated. Depletion of nesprin-2G did not alter the formation of dorsal actin cables nor their rearward movement. These results strongly suggested that the dorsal actin cables were responsible for moving the nucleus rearward. Questions that remain to be addressed are how the isotropic actin meshwork forms dorsal actin cables with near uniform orientation parallel to the leading edge and what forces drive their retrograde movement. Recent work from the Lappalainen lab has identified stress fibers that project from the ventral towards the dorsal surface of

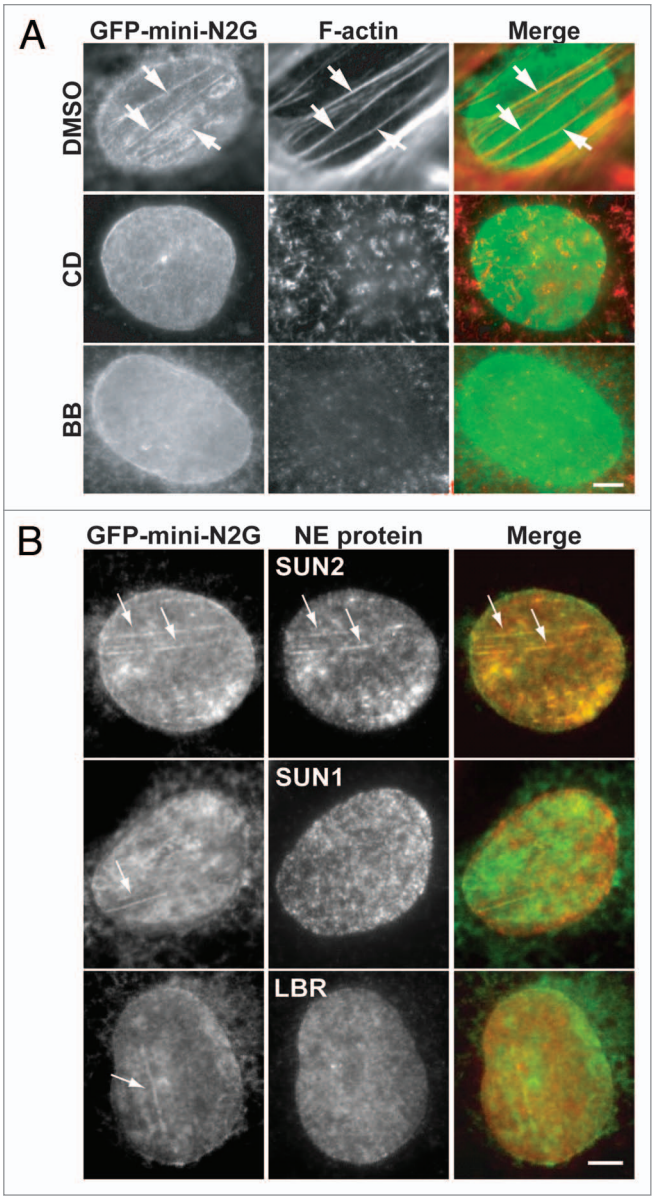

Figure 2. TAN lines are actomyosin-dependent structures composed of nesprin-2G and SUN2. (A) Fluorescence images of nuclei in nesprin-2G-depleted cells expressing GFP-mini-nesprin-2G (GFP-mini-N2G). Staining: GFP antibody (GFP-mini-N2G) and rhodamine-phalloidin (F-actin). Cells were treated with vehicle (DMSO), 0.5 μM cytochalasin D (CD) or 50 μM blebbistatin (BB) for 1 hr before and during LPA treatment. The wound edge is towards the top left in all parts. (B) Fluorescence images of nuclei in nesprin-2G-depleted cells expressing GFP-mini-nesprin-2G (GFP-mini-N2G). Staining: SUN1, SUN2, LBR and GFP (GFP-mini-N2G) antibodies. Arrows indicate GFP-mini-nesprin-2G colocalizing with SUN2, but not SUN1 or LBR. The wound edge is towards the top (SUN2, SUN1) and toward the right (LBR). All images are of the dorsal nuclear surface. Bars in (A and B): 5 μm. From Luxton et al. Science 2010; 329:956.

the cell.[35] It is possible that the contraction of these fibers provides the motive force for the retrograde movement of the dorsal actin cables.

There was also a strong correlation between the rate of movement of the nucleus and the rate of movement of the TAN lines. Direct imaging of TAN lines on individual moving nuclei showed that the TAN lines moved at the same rate as the leading or lagging edge of the nucleus. To confirm that all three structures (TAN lines, actin cables and nuclei) moved coordinately, we prepared live cell recordings of cells expressing both GFP-mini-nesprin-2G and Lifeact-mCherry. TAN lines were observed to form on a subset of the dorsal actin cables above the nucleus and then moved coincidently with the nucleus. These results provide strong evidence for the direct coupling of the nucleus through TAN lines to moving dorsal actin cables.

Previous studies in developmental systems (*C. elegans* and mouse) have implicated nesprin and SUN proteins in cell migration,[36,37] raising the possibility that nuclear position was important for cell migration. Yet, these studies were unable to assess whether the effects of interfering with nesprins or SUNs were cell autonomous or whether alteration of nesprins or SUNs in these systems significantly altered actin organization. In the fibroblast system, we could directly ask whether nuclear positioning was important for cell migration since our studies showed that interfering with nesprin-2G or SUN2 blocked TAN line formation and nuclear movement, but not actin filament organization or movement. Importantly, we found that expression of the KASH domain or siRNA-mediated depletion of either nesprin-2G or SUN2 inhibited efficient migration into the wound. Combined with the earlier results from model organisms, these data strengthen the idea that nuclear position within a migrating cell is an important component of cell migration.

TAN Lines are Anchored by SUN2 and the Nuclear Lamina

The data described below are from Folker, et al. 2011.[38] The TAN lines we have described are important for coupling the nucleus to moving actin filaments. Yet, to move the nucleus productively, TAN lines must be anchored to a nuclear structure capable of resisting the force exerted on the nucleus by moving actin cables. We hypothesized that the nuclear lamina that lies just under the inner nuclear membrane may provide such an anchoring site. The nuclear lamina is a fibrillar network composed of type V intermediate filaments that are either A-type (lamin A and lamin C) or B-type (lamin B1 and lamin B2).[39] Lamin A/C has been shown to interact directly with SUN proteins in *C. elegans* and in mammalian cells.[19,40,41] A type lamins have been implicated in

nuclear-cytoplasmic interactions because lamin A/C deficient fibroblasts fail to orient their centrosome toward the leading edge, although it was unclear whether this was due to an effect on the centrosome or on the nucleus (or both).[42,43]

To explore the role of lamins in nuclear movement, we first tested whether lamins might be found in TAN lines and did not detect accumulation of either endogenous lamin A/C or lamin B1 in TAN lines. Nonetheless, in fibroblasts derived from lamin A/C knockout mice or NIH3T3 fibroblasts acutely depleted of lamin A/C, we observed that the lack of centrosome orientation was due to a defect in nuclear movement. Similar to cells depleted of nesprin-2G, the overall organization of actin filaments and their retrograde movement was unperturbed in the absence of lamin A/C. These results suggested that the absence of lamin A/C might affect either the formation or dynamics of TAN lines.

To probe for TAN lines, we expressed GFP-mini-nesprin-2G in cells lacking lamin A/C. TAN lines composed of nesprin-2G and SUN2 still formed yet they appeared discontinuous and their stability was reduced compared to lamin A/C expressing cells: only 30–40% of the TAN lines persisted for 20 min in cells lacking lamin A/C compared to 70% of them in wild type controls. In movies of TAN lines and nuclei, nuclei did not move in the lamin A/C deficient cells and yet the TAN lines still moved, showing that the TAN lines slipped over the nucleus rather than coupling to the actin cables for movement. A similar TAN line "slippage" phenotype was observed in cells depleted of SUN2. Therefore, nesprin-2G TAN lines form in the absence of either SUN2 or lamin A/C, but they are less stable and are unable to couple the nuclear envelope to the actin cytoskeleton in order to move the nucleus. Together, these data suggest that lamin A/C and thus the nuclear lamina functions to anchor TAN lines for productive force transmission across the nuclear envelope during nuclear movement.

There are two possible scenarios leading to TAN line assembly following LPA-stimulated actin cable formation on the dorsal nuclear surface. In the first scenario, actin cables recruit freely diffusing nesprin-2G molecules in the outer nuclear envelope along the length of the cable. The high, local concentration of nesprin-2G along the actin cable then acts to recruit SUN2 in the inner nuclear membrane. In the second scenario, actin cables recruit pre-assembled nesprin-2G/SUN2 LINC complexes along the length of the actin cable. Consistent with the first scenario, nesprin-2G accumulates along a perinuclear actin cable in the absence of SUN2. Additional experiments, such as simultaneous imaging of nesprin-2G and SUN2 during TAN line assembly, are necessary to differentiate between these two models.

TAN Lines and Laminopathies

Mutations in *LMNA* are associated with a variety of tissue-specific diseases, commonly referred to as laminopathies, which include diseases of striated muscle (Emery-Dreifuss muscular dystrophy [EDMD]), dilated cardiomyopathy [DCM] and limb girdle muscular dystrophy 1B, adipose tissue (Dunnigan-type familial partial lipodystrophy [FPLD]), peripheral nerve (Charcot-Marie-Tooth type 2B1), as well as progeroid syndromes characterized by symptoms of premature aging.[44,45] Currently, there are two favored hypotheses used to explain the pathogenesis of these diseases. The mechanical stress hypothesis posits that the nuclear lamina is important for nuclear integrity, especially in tissues subjected to high levels of stress such as striated muscle. This is supported by the findings that A-type lamin deficiency disrupts nuclear integrity in model systems.[46,47] The gene expression hypothesis posits that since the lamina interacts with chromatin, alterations in the nuclear lamina may result in aberrant gene expression. This is supported by the finding that lamin A is necessary to repress inhibitors of MyoD expression.[48] A third possibility is that compromised interactions between the nucleus and cytoskeleton may lead to nuclear positioning defects that contribute to the pathogenesis of laminopathies. This third hypothesis is supported by the recent identification of mutations in nesprin-1 and nesprin-2 which are associated with EDMD[49] as well as the finding that nesprin-2G appears to protect *LMNA* mutant cells associated with progeria from developing dysmorphic nuclei.[50] In addition, skeletal muscle nuclei in EDMD and other muscle disorders including Duchenne muscular dystrophy and central nuclear myopathy are observed in the center of the myofiber, rather than at their normal location at the periphery.[51,52] Although the presence of central nuclei is indicative of a damaged myofiber undergoing a repair response,[53] it is possible that the abundance of central nuclei in muscle disorders reflects an inability of nuclei to move towards the periphery of the myofiber due to defects in nuclear-cytoskeletal connections. Such a defect in proper nuclear positioning could compromise proper sarcomere alignment, which in turn could contribute to disease.

Based upon these considerations, we explored whether the wounded fibroblast monolayer system could be used to determine if disease-associated variants of *LMNA* were defective in nuclear movement or centrosome centration. Given that the lamin A/C null phenotype was defective nuclear movement, this analysis would also reveal whether individual disease variants resulted in a loss of lamin A/C function. We screened a panel of lamin A variants by expressing them in NIH3T3 fibroblasts and then stimulating nuclear movement and centrosome orientation with LPA. The panel contained variants associated with striated muscle diseases including EDMD and DCM as well as variants associated with lipodystrophy including FPLD. While expression of wild type lamin A had no effect on centrosome orientation, expression of almost all of the disease associated variants inhibited centrosome reorientation. Interestingly, there was a segregation of phenotypes based on whether the variant caused disease affecting striated muscle or adipose tissue. Nuclear movement was inhibited by almost all of the striated muscle disease variants (11/12), while centrosome centration, but not nuclear movement, was inhibited by the adipose tissue disease variants (3/4). Similar results were observed following expression of the disease variants in fibroblasts lacking lamin A/C suggesting that the

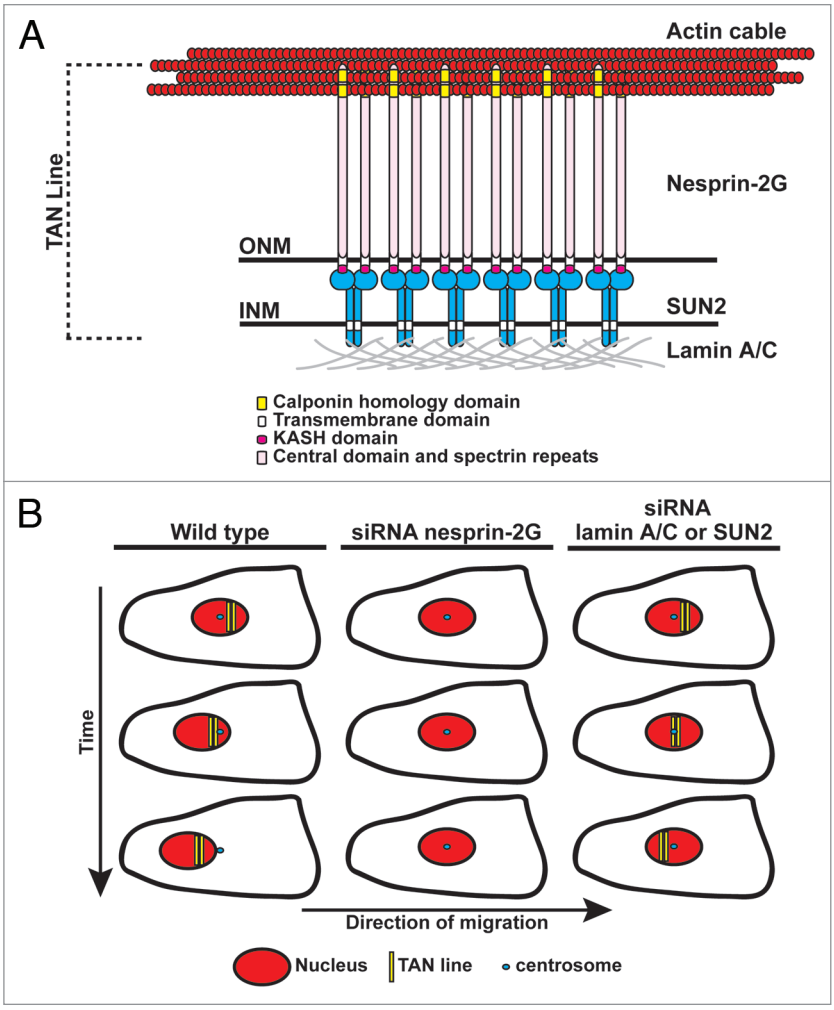

Figure 3. Working model of TAN line structure and function. (A) Illustration of the molecular composition of a TAN line. INM, inner nuclear membrane; ONM, outer nuclear membrane. (B) Depiction of TAN line behavior during centrosome orientation under different conditions. TAN lines in wild type cells (left) form on the dorsal surface of the nucleus and function to harness the forces generated by the actin cytoskeleton to move the nucleus rearward while the centrosome remains stationary resulting in centrosome orientation towards the leading edge. In nesprin-2G-depleted cells (middle) TAN lines do not form resulting in the lack of nuclear movement and centrosome orientation. In lamin A/C- or SUN2-depleted cells (right), nesprin-2G TAN lines form but are unanchored causing them to slip over the surface of the nucleus with the rearward moving actin cables. Neither nuclear movement nor centrosome orientation occurs in this situation.

defects observed were intrinsic properties of the variants. Fibroblasts from a patient with EDMD also showed a defect in nuclear movement. Consistent with their affects on nuclear movement, expression of striated muscle disease variants in lamin A/C knockout cells expressing GFP-mini-nesprin-2G resulted in TAN line "slippage" while adipose tissue disease variants did not. These results point to the possiblity that defective nuclear movement may be contributing factor in the pathology of muscle diseases caused by lamin A variants.

A Model for TAN Lines

We have described an actin-dependent mechanism for nuclear movement during centrosome reorientation in fibroblasts polarizing for migration. This mechanism involves the coupling of the nucleus to actin cables through a novel structure within the nuclear envelope, which we refer to as TAN lines (**Fig. 3A**). Composed of linear arrays of nesprin-2G/SUN2, TAN lines form on the dorsal surface of the nuclear envelope along actin cables that move towards the rear of the cell through the action of non-muscle myosin II. This coupling of the nuclear envelope to the actin cytoskeleton results in nuclear movement because TAN lines are anchored in the nuclear lamina by lamin A/C and this anchoring allows force generated by the actin cytoskeleton to be transferred across the nuclear envelope and into the nuclear lamina. Interfering with nesprin-2G, SUN2 or lamin A/C all block nuclear movement, but with distinct TAN line phenotypes (**Fig. 3B**). The identification of TAN lines and their role in nuclear movement raises several new questions that we consider below.

Questions for the Future

Our studies highlight a number of questions about the proper positioning of the nucleus in migrating cells and how this positioning impacts overall cellular function. Perhaps the most intriguing question is how nuclear positioning affects cell migration. Does the position of the nucleus affect cytoskeletal organization or dynamics? Or could it affect gene expression? We did not observe striking alterations in actin filament arrays when nuclear positioning was disrupted, but perhaps more subtle effects or changes in dynamics result from improper nuclear position. Alternatively, perhaps positioning the nucleus toward the back of a migrating cell simply gets it out of the way so that the cell can create a thin leading edge and lamella that concentrate molecules necessary for efficient migration?

Additional questions raised by our work concern how TAN lines are formed. It will be important to determine the steps in the assembly of TAN lines. GFP-mini-nesprin-2G has relatively high diffusional mobility in the outer nuclear membrane suggesting that it may randomly encounter actin cables that closely approach the nucleus.[56] However, it is not yet clear whether the full length endogenous nesprin-2G might have to be activated in some way to interact with actin cables. SUN2 also accumulates in TAN lines above its concentration in the bulk nuclear membrane. How this occurs is somewhat of a puzzle, because it requires that SUN2 differentiate between nesprin-2G that is engaged by actin cables from

the remainder of nesprin-2G in the nuclear envelope that is not. Given that the latter is more abundant, it is possible that there is a recognition signal in the actin-engaged nesprin-2G. Perhaps, as in focal adhesions,[54,55] it will turn out that there is "outside-in" signaling (a change in nesprin-2G conformation or recruitment of other proteins) that may help the SUN2 recognize actin-associated nesprin-2G from unengaged molecules. Additionally, it remains unclear why SUN2 is recruited to TAN lines while SUN1 is not. Both molecules interact with the KASH domain of nesprin-2G with nearly equal affinities,[56] yet only SUN2 appears in TAN lines. There is increasing evidence that SUN1 and SUN2 associate with the nuclear envelope through different mechanisms. SUN1 appears more closely associated with the nuclear lamina than SUN2 as measured by fluorescence resonance energy transfer experiments[56] and SUN1 interacts with the nuclear pore complex.[57] Furthermore, nesprin-2G mobility in the nuclear envelope appears to depend on SUN2 not SUN1.[56] Further studies of TAN lines may lead to insights into how specific LINC complexes are established.

To better understand how TAN lines form, it would be helpful to define more precisely the structure of a TAN line. An obvious first step would be to determine the ultrastructure of TAN lines using either electron microscopy or super-resolution light microscopy. Recent work has beautifully demonstrated the utility of super-resolution microscopy with another actin-dependent structure, the focal adhesion.[58] Given the similarities of TAN lines to focal adhesions (both are actin-dependent, trans-membrane and force transmitting structures required for cell migration), it will be interesting to see if nanoscale architectural features of TAN lines resemble those of focal adhesions.

Another important question is whether there are other proteins that accumulate with nesprin-2G and SUN2 in TAN lines. By analogy to focal adhesions, which are composed of dozens of cytoplasmic proteins important for linking transmembrane integrins to the actin cytoskeleton and signaling pathways,[59] it is likely that TAN lines are composed of additional cytoplasmic and nucleoplasmic proteins. The identification of additional TAN line proteins will not only extend our knowledge of how TAN lines form and transmit force across the nuclear envelope, but may also shed light on other functions TAN lines perform in the cell. For instance, work in the fission yeast, *Schizosaccharomyces pombe*, has elegantly shown that focal accumulations of LINC complexes in association with heterochromatin harness the forces of microtubule polymerization to position the nucleus in the cell center.[60] The inner nuclear membrane protein, Ima1, interacts with centromeric heterochromatin and is thought to help distribute the forces generated by microtubules through the LINC complex throughout the nuclear envelope allowing for productive force transduction. It will be interesting to see if the mammalian homologue of Ima1 (NET-5/Samp1),[61] or other heterochromatin interacting proteins are important for TAN line formation and function.

A final question that needs to be addressed is how widespread the use of TAN lines is during nuclear positioning events. Are TAN lines restricted to fibroblasts polarizing for 2D migration or do they occur in other cellular contexts, such as 3D migration where the movement of the nucleus through narrow channels in the extracellular matrix can be rate-limiting?[62] Exploring the possibility that TAN lines mediate other processes dependent on LINC complexes such as meiosis (particularly in budding yeast, given its dependence on actin[63]), nuclear movement during neuronal migration as well as movement and anchorage of nuclei in myotubes would be informative.

LINC complexes are becoming increasingly implicated in developmental processes in such tissues as the retina, brain, muscle, skin and testes.[6] It would be interesting to examine these processes more carefully to see whether TAN line-like structures are involved. How defective TAN line structure and function might contribute to disease pathogenesis is an avenue of research, which is sure to be informative and may present new avenues for disease treatment. An increasing number of human diseases are being linked to mutated and dysfunctional nesprins including EDMD,[49] autosomal recessive cerebellar ataxia type 1,[64] as well as colorectal and breast cancer.[65,66] The use of the simple wounded fibroblast monolayer system to explore the mechanism of nuclear movement and its deficiency in disease has opened a new line of research that promises to uncover exciting new biology as well as increase our understanding of disease pathogenesis.

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