Roles of the nucleus in leukocyte migration

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SUMMARY SENTENCE

Review of morphologies and mechanical properties of leukocyte nuclei, their regulation by nuclear envelope proteins, and their many roles in leukocyte migration.

RUNNING TITLE

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KEYWORDS

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ABBREVIATIONS

3D: Three-dimensional
2D: Two-dimensional
DC: Dendritic cell
CTL: Cytotoxic T lymphocyte
APC: Antigen-presenting cell
EC: Endothelial cell
HIV: Human immunodeficiency virus
PHA: Pelger-Huët anomaly
ECM: Extracellular matrix
LBR: Lamin B receptor
LINC: Linker of nucleoskeleton and cytoskeleton
ONM: Outer nuclear membrane
INM: inner nuclear membrane

ABSTRACT

Leukocytes patrol our bodies in search of pathogens and migrate to sites of injury in response to various stimuli. Rapid and directed leukocyte motility is therefore crucial to our immunity. The nucleus is the largest and stiffest cellular organelle and a mechanical obstacle for migration through constrictions. However, the nucleus is also essential for 3D cell migration. Here, we review the roles of the nucleus in leukocyte migration, focusing on how cells deform their nuclei to aid cell motility and the contributions of the nucleus to cell migration. We discuss the regulation of the nuclear biomechanics by the nuclear lamina and how it, together with the cytoskeleton, modulates the shapes of leukocyte nuclei. We then summarize the functions of nesprins and SUN proteins in leukocytes and discuss how forces are exerted on the nucleus. Finally, we examine the mechanical roles of the nucleus in cell migration, including its roles in regulating the direction of migration and path selection.

1 | INTRODUCTION

Leukocytes, the key components of the immune system, travel in our bodies over very long distances to defend against infections and diseases. The ability to move in complex and heterogeneous environments is critical to their functions. Leukocyte cell migration is fascinating in many ways. First, leukocyte migration is fast. Circulating blood cells are passively moved by the blood flow and their velocities can reach tens of millimeters per second[1]. Active leukocyte migration is much slower, but the velocities can reach tens of microns per minute and are two orders of magnitude higher than those of migrating fibroblasts and many other cell types[2,3]. Second, leukocytes exhibit remarkable deformability and can penetrate and migrate through barriers with extremely small pores. Strikingly, many leukocytes can do so using non-destructive, proteases-independent strategies. Third, leukocytes can undergo multiple modes of cell migration with vast differences in both speed and mechanisms. The cells switch between these modes of cell migration and dynamically and precisely regulate their motility. Lastly, leukocyte migration is regulated by a variety of cell-intrinsic and -extrinsic factors. It is not only cell-type dependent, but also altered by the differentiation/maturation/activation states of the cells and the biophysical and biochemical properties of the extracellular microenvironment. Each of these factors can affect the mode, speed, or even direction of cell migration.

The multiplicity and plasticity of the migration mechanisms rely on the abilities of the cells to drastically change their morphology and their internal organization. The nucleus, the largest and stiffest cellular organelle, is often considered a barrier to cell migration in confined environments. In fact, increased nuclear stiffness impaired 3D migration, and stiff nuclei may be a contributing factor that limits cell migration in leukocyte precursors[4–6]. However, the nucleus is indispensable for 3D migration. Migration in 3D collagen gels is significantly compromised in enucleated cytoplasts from fibroblasts and endothelial cells (EC), compared to cells with nuclei[7]. Enucleated cytoplasts from leukocytes lose their ability to sense the environment for path selection[8]. To resolve the dilemma, fast leukocyte migration is aided by significantly decreased nuclear stiffness and the ability to apply forces to deform the nucleus[5,9].

Why the nucleus is necessary for 3D cell migration is not completely clear. Studies in immune and non-immune cells have revealed multiple roles of the nucleus in cell migration. It can function as a mechanosensor and mediate mechanical signal transductions, as observed in mesenchymal cells[10–12]. It can serve as a force propagator, as demonstrated during leukocyte transmigration[13]. It can also determine the direction of cell migration. In mesenchymal cells on 2D surfaces, the nuclei reside in the trailing ends and, together with the centrosomes, establish proper cell polarity for migration[14]. Nuclei in migrating leukocytes tend to reside closer to the leading edges, and act as a mechanical gauge for path selection[8].

Here, we briefly describe the steps of leukocyte migration before we discuss the mechanical properties of the nucleus and how are they regulated by the nuclear envelope. We then consider forces exerted on the nucleus by the cytoskeleton and how the nucleus mechanically contributes to leukocyte migration.

2 | OVERVIEW OF LEUKOCYTE MIGRATION

Leukocytes shuttle between the vascular system, the lymphatic system, and peripheral tissues. Their tissue locations are the most important determining factors for their migration strategies. For leukocytes circulating in the blood, they are propelled by the blood flow, and their movement is essentially passive. Although leukocytes are shown to have the ability to swim in suspension[15], it is very slow and contributes very little, if any, to their movement in the blood. Circulating leukocytes move in close proximity to the wall of blood vessels, a phenomenon termed margination[16]. Margination enables the leukocytes to have transient interactions with the blood vessel endothelium and prepares the cells for transition to rolling and crawling. Rolling is passive and is mediated by the interactions between E- and P-selectins on the endothelial surface and their ligands, like P-selectin glycoprotein ligand 1 (PSGL1), on the leukocyte membrane (Fig. 1A)[17,18]. These interactions are transient and have high on- and off-rates, so leukocyte movement is significantly slowed down, but not completely arrested. The arrest of leukocyte rolling is rapidly triggered through G-protein coupled receptor signaling by chemokines and chemoattractants[17–19].

Following rolling and arrest, leukocytes start their active migration and crawl on the endothelial surface to search for diapedesis hotspots (Fig. 1B)[19,20]. To crawl in the presence of the blood flow, leukocytes form stable integrin adhesions to resist the shear stress[21,22]. After finding a spot for diapedesis, leukocytes insert protrusions into the endothelium and pass through the EC layer, either through cell (transcellular migration) or cell junctions (paracellular migration), the basement membrane, and the attached pericytes (Fig. 1C)[18]. Because the EC layer is thinner than the size of the leukocytes, these steps can be continuous and overlap[19]. That is, a cell can pass through these layers and start interstitial migration while part of its body is still in the bloodstream (Fig. 1C). These steps can also be separated, as cells may crawl on the other side of the endothelium to search for a preferred spot on the basement membrane (Fig. 1D). Diapedesis is mechanically challenging for the cells and is aided by the high deformability of the leukocytes and the reorganization of the cytoskeleton and cell junctions in the ECs[23–25]. Integrin adhesions are essential for diapedesis[26].

Leukocytes display massive diversity in strategies of interstitial migration. This is due to not only their differences in functions, but also the biophysical and biochemical

properties of the microenvironment, like stiffness, confinement, topology, pore sizes, matrix composition, chemoattractant gradient, and cell-cell interactions[18,27]. Interstitial migration can be broadly divided into two modes: adhesion-dependent mesenchymal migration and adhesion-independent amoeboid migration. These two modes differ in whether integrin adhesions are formed. Leukocytes show cell-type dependent preferences in using these two modes of migration. Interstitial neutrophil migration is usually adhesion-independent while macrophages prefer mesenchymal migration in dense matrix[3,28]. Although most leukocytes can induce ECM remodeling and migrate efficiently without proteolytic digestion of the ECM, proteolytic digestion is used in some cases. When macrophages (and DCs) encounter barriers, they form 3D podosomes characterized by enriched proteolytic activity[29]. Even neutrophils can use proteolytic digestion to aid their translocation through the basement membrane[30].

Integrins are heterodimeric transmembrane receptors composed of one α and one β subunit. Among the eight β subunits, the β2 subunit (CD18) is the most crucial for leukocytes. Together with 4 α subunits, it forms αLβ2 integrin (LFA-1, or CD11a/CD18), αMβ2 integrin (Mac-1, or CD11b/CD18), αXβ2 integrin (CD11c/CD18), and αDβ2 integrin (CD11d/CD18)[17,19,21]. Both LFA-1 and Mac-1 bind tightly to ICAM-1 on the EC surface. In addition, α4β1 integrin (VLA-4; CD49d/CD29) is also important for leukocytes and binds to endothelial VCAM-1[17,19,21]. To facilitate rapid movement, integrins in leukocytes are diffusely distributed and, unlike fibroblasts, do not form focal adhesions[31].

Rapidly migrating leukocytes do not depend on integrin adhesions and require frictional forces exerted on the substrate[27]. The lack of adhesions makes multiple differences. First, because the formation and disassembly of adhesions are time-consuming, adhesion-independent migration is generally much faster than adhesion-dependent migration. Second, cell shape changes are more dramatic. Weak substrate-cell interaction allows cells to form dynamic and branched pseudopods to explore the microenvironment. Third, the activation of myosin II is spatially distinct. Myosin II activity in the leading end is commonly observed during adhesion-dependent migration. During adhesion-independent migration, myosin II activity is mostly enriched in the uropod[32,27]. Moreover, the magnitude of traction force is reduced in the absence of adhesion, and the force center localizes to the uropod instead of the cell front[32].

Both adhesion-dependent and adhesion-independent cell migration are supported by actin polymerization and, in most cases, require actomyosin contractility. Actin polymerization works by pushing the plasma membrane forward and is sufficient to support cell migration by itself[9,33]. It is driven by formins, which catalyze the elongation of actin filaments, and the Arp2/3 complex, which nucleates new filaments on the sides of existing filaments[34]. A large number of actin-binding proteins are involved in the regulation of actin polymerization and depolymerization. Mutations in many of them lead to immunodeficiency diseases[35,36]. Both the formin-dependent actin polymerization and the Arp2/3-dependent actin nucleation can sustain cell migration. Cells use a combination of both and require both in some cases[37]. Cells may also favor one of them based on the mode of migration[38]. Myosin contractility retracts the cell rear and squeezes the nucleus. It can also cause cell protrusion by increasing hydrostatic pressure and inducing membrane blebbing[39–43]. Bleb-dependent migration can occur both in the presence and absence of integrin adhesions[39–43].

3 | THE MECHANICAL PROPERTIES OF THE NUCLEUS

The mechanical properties of the nucleus are determined by the contents of the nucleoplasm and the nuclear lamina. The nucleoplasm is occupied by nuclear bodies, macromolecules, and chromatin. Among them, chromatin is the most important contributor to nuclear biomechanics[2,44]. Chromatin is viscous and its distribution and movement affect the stiffness of the nuclei. The compaction of chromatin is shown to facilitate nuclear deformation and determine the overall biophysical properties of the nucleus at low deformations[2,44]. Chromatin organization and dynamics are regulated by the nuclear lamina, but some of the effects of chromatin on nuclear mechanics can be independent of lamins[45].

The nuclear lamina, a meshwork underneath the nuclear membrane, structurally supports the integrity of the nuclear membrane. The nuclear lamina is formed by A-type (lamin A and C) and B-type (lamin B1 and B2) lamins (Fig. 2). The meshwork formed by these two types of lamins can be separated and have distinct biophysical properties[46–49]. A-type lamins are key regulators of the mechano-properties of the nucleus and nuclei with higher levels of A-type lamins are less malleable[10]. In Hela, fibroblasts, and ovarian cancer cells, lamin A/C determine the biophysical properties of the nucleus at large deformations[44,50]. Expression of lamin A in HL-60 cells (a promyelocytic neutrophil-like cell line) significantly affects nuclear translocation and reduced cell migration through narrow constrictions[4,6]. Compared to A-type lamins, the contribution of B-type lamins to nuclear stiffness is less characterized. Data from lamin B-deficient mice suggested that B-type lamins do contribute to nuclear stiffness, especially when lamin A/C are lacking[2].

Lamin expression in leukocytes depends on cell types and their differentiation/activation state[51]. Altered expression of lamin isoforms affects hematopoietic lineage differentiation[5,52]. The ratios between A-type and B-type lamins show a correlation to the viscoelasticity of the nuclei and are lower in leukocytes compared to most non-immune cells[5]. This is mostly due to downregulation of A-type lamina during leukocyte differentiation. For example, HL-60 cells express lamin A/C but their levels gradually decrease after cells are induced into neutrophils[53]. Overexpression of either lamin A or lamin C disrupts neutrophil nuclear morphology and impairs chemotaxis and other functions[54]. Lamin A/C level is also affected by the activation state of leukocytes. Expression of lamin A/C is low in lymphoid cell lines[55,56]. Their expression is rapidly induced upon T cell activation and subsequently quickly decreased[57]. This transient expression enhances T cell activation, T cell-antigen presenting cell (APC) interaction, and immune response both *in vitro* and *in vivo*[57]. During HL-60 cell differentiation, the expression of B-type lamins is also changed, but, overall, their expression in leukocytes is more comparable to other cell types[55,56,58–60]. Loss of lamin B in T cells induces lamin A/C expression and apoptosis[61].

Multiple mechanisms exist to disrupt the nuclear lamina in leukocytes. First, in DCs passing through constrictions, nuclear lamina rupture caused by Arp2/3-driven actin polymerization facilitates nuclear deformation and cell migration[9]. Disassembly of the nuclear lamina can be triggered by lamin phosphorylation. During NETosis, the process of forming the neutrophil extracellular traps[62], disassembly of the lamin B meshwork is induced by PKCα[63]. At the same time, lamin A/C is phosphorylated by cyclin-dependent kinase 4 and 6 (CDK4/6) and disassembles[64]. The nuclear lamina can also be disrupted by autophagy. In immature DCs infected with herpes simplex

virus type 1, lamin A/C, B1, and B2 are degraded by autophagy, allowing the nuclear egress of the virus[65].

Passage through constrictions can cause nuclear ruptures, disruption of the nuclear lamina, and DNA damage[9,66,67]. Loss of the nuclear lamina increases the deformability of the nucleus and facilitates fast cell migration at the cost of more nuclear damage. Nuclear damage, however, is less risky for neutrophils, because they are terminally differentiated and live only shortly (half-life in blood: 19 hours)[68]. Longer-live macrophages and DCs express more lamin A/C, which protect migrating cells from mechanical stress[69]. In the absence of lamin A/C, microtubules may protect the nuclei. In cytotoxic T lymphocytes (CTLs), 3D cell migration is limited by the stiffness of their nuclei. Nocodazole treatment leads to deformed nuclei and improves migration speed and CTL killing efficacy[70].

4 | NUCLEAR MORPHOLOGY AND NUCLEAR LOBULATION

Leukocytes display a great variety of nuclear shapes (Fig. 3A). While lymphocytes have round nuclei, granulocytes (neutrophils, eosinophils, and basophils) are characterized by their multi-lobed nuclei and are therefore also called polymorphonuclear leukocytes. Eosinophil nuclei are typically bi-lobed and basophil nuclei are bi- or tri-lobed. Neutrophil nuclei can contain multiple, usually three to five, lobes. Nuclear lobes in neutrophils and eosinophils are connected by short filamentous regions of nucleoplasm. How such a fine and energetically unfavorable structure is stabilized is unknown. During neutrophil transmigration, the nucleus loses the lobed morphology but reforms afterwards[71]. Monocyte nuclei are also bilobed, but the two lobes are not well-separated[72].

The shapes of leukocyte nuclei are so characteristic that nuclear morphology has been an important criterion for cell type identification and diagnosis of diseases[73]. For example, the presence of multinucleated, B cell-derived Reed-Sternberg cells is representative of the Hodgkin's lymphoma (Fig. 3B)[74]. Other abnormal nuclear morphology includes lobed nuclei, cerebriform nuclei and cup-like nuclei in non-granulocytes and reduced number of nuclear lobes in neutrophils (termed Pelger-Huët anomaly, PHA, Fig. 3C)[73,75,76]. PHA is usually caused by heterozygous mutations in *LBR* encoding the lamin B receptor. Interestingly, heterozygous *LBR* mutations are benign and neutrophil functions are normal[77]. Homozygous *LBR* mutations are rare and may cause embryonic lethal diseases, like the Greenberg skeletal dysplasia[78,79].

Nuclear lobulation gradually occurs during granulocyte differentiation and is accomplished by substantial changes in the composition of the nuclear envelope[72]. The most notable changes are the increase of *LBR* and the decrease of lamin A/C. The number of nuclear lobes is correlated with the expression of *LBR*. Loss of *LBR* causes the loss of polymorphonuclear phenotypes in granulocytes[76,80,81], and hyperlobulation is associated with an increased copy number of the *LBR* gene (Fig. 3C)[82]. Defective *LBR* mRNA splicing and abnormal LBR protein folding also lead to PHA[83,84]. Down-regulation of lamin A/C is necessary because overexpression of either lamin isoform greatly reduces the number of multi-lobed nuclei[54].

How LBR mediates nuclear lobulation is unknown. LBR contains an N-terminal nucleoplasmic domain that binds to lamin B, histones, DNA and HP1, and a C-terminal eight-transmembrane domain that functions as a sterol reductase (Fig. 2)[85]. Many of the mutations associating with PHA are found in the sterol reductase

domain and overexpression of the sterol reductase domain alone is able to, but only limitedly, rescue nuclear morphology, suggesting that cholesterol biosynthesis is important for lobulation[76,86]. However, in patients with homozygous *LBR* mutations that abolish its enzymatic activity, neutrophil nuclear lobulation is normal[78,79], arguing against the role of the sterol reductase in lobulation. Recent studies suggested that LBR's interaction with lamin B may be involved in lobulation. Lamin B1 is involved in granulocyte differentiation and may thus contribute to changes in nuclear morphology[87]. Lamin B1 is farnesylated and protein prenylation was shown to be required for remodeling the nucleus and the PHA phenotype in patients with myeloid neoplasms is caused by the loss of lamin B1[88,89]. Moreover, chromatin binds to LBR and inhibition of DNA synthesis leads to increased lobulation[90]. Therefore, a role of chromatin in nuclear lobulation has been suggested[2]. Finally, lamin A/C expression can be induced by LBR-deficiency[81] and may thus inhibit nuclear lobulation.

Nuclear lobulation requires ample force and very likely relies on the cytoskeleton. Microtubules seem necessary for nuclear lobulation, as the formation of nuclear lobes is inhibited in nocodazole-treated HL-60 cells[91]. Nocodazole also inhibits nuclear hyperlobulation induced by *H. pylori*[92]. In addition, dynein becomes enriched on the nuclei and is involved in lobulation[92]. Depletion of α-dystrobrevin, a microtubule- and kinesin-binding protein, also leads to rounded nuclei[93]. Interestingly, preexisting nuclear lobes are not affected by the loss of microtubules[89,92], suggesting that microtubules are only involved in the formation, but not the maintenance of the nuclear lobes.

Although early studies suggested that actin is not required for nuclear lobulation[91], abnormal regulation of actin dynamics leads to multilobulated nuclei. Wdr1 is a cofactor of the F-actin severing protein cofilin and its deficiency is associated with immunodeficiencies[35]. Wdr1-deficient neutrophils exhibited nuclear herniations caused by increased F-actin[35]. Zebrafish larval neutrophils are normally not multilobulated, but the loss of Wdr1 results in the appearance of nuclear lobes[94]. Interestingly, myosin II may play a role in maintaining the integrity of the nuclear envelope and inhibiting nuclear lobulation, as inactivation of myosin II by inhibiting Rho kinase also leads to the formation of nuclear lobes[94]. Inhibition of calmodulin, a calcium-sensing protein that binds to and activates several unconventional myosins and various calcium-sensitive proteins, induces nuclear lobulation in T lymphocytes within 30 minutes[95].

The functional importance of nuclear lobes is not clear. The development and chemotaxis of neutrophils from LBR-deficient mice are impaired[96]. However, neutrophils with heterozygous *LBR* mutations are functional and, although HL-60 cells lacking LBR have spherical nuclei, they migrate normally in transwell migration assay[4,77]. Moreover, leukocytes with unlobed nuclei penetrate the endothelium and the basement membrane efficiently. Furthermore, melanoma cells overexpressing LBR have highly folded nuclei and exhibit reduced pore transmigration[97]. Taken together, these results suggest that nuclear lobulation does not necessarily correlate with nuclear deformation and cell's ability to pass through constrictions.

5 | THE LINC COMPLEXES IN LEUKOCYTES

For both nuclear lobulation and nuclear deformation, forces need to be applied to the nucleus. Although pushing force from the cytoskeleton and the environment can directly act on the nucleus, pulling force must be mediated by the linker of

nucleoskeleton and cytoskeleton (LINC) complexes that mechanically couple the nucleus to the cytoskeleton (Fig. 2)[98]. The LINC complex is composed of KASH-motif-containing nesprins on the outer nuclear membrane (ONM) and SUN-domain proteins on the inner nuclear membrane (INM)[98]. The cytoplasmic domains of nesprins (nesprin-1 to 4 in mammalian somatic cells) are diverse and bind to various cytoskeletal elements, like actin filament, kinesin, dynein, and plectin. The nucleoplasmic termini of SUN proteins (SUN1 and SUN2) bind to the nuclear lamina. The luminal domains of nesprins and SUN proteins interact with each other, thus connecting the cytoskeleton to the nuclear lamina[98].

Although the roles of the LINC complexes in nuclear mechanosignaling and movement are well characterized in various cell types, much less is known about their functions in leukocytes. A systematic analysis of the expression of LINC complex proteins and their many isoforms in leukocytes is also lacking[99,100]. Studies in HL-60 cells revealed that they express both SUN1 and SUN2 and some isoforms of nesprins in a differentiation-dependent manner[60,101]. Proteomic studies have demonstrated the presence of SUN1/2, nesprin1/2, and related nuclear envelope proteins (emerin, Lap1, Lap2, Man1, Samp1, Luma, torsin-A, *etc*. See Fig. 2) in peripheral blood mononuclear cells[102]. The expression of nesprin-2G, the giant isoform of nesprin-2, is high in the spleen, lymph nodes, and peripheral blood leukocytes[103]. In addition, nesprin-3 and cytoskeletal interactor of nesprins, like fascin, FHOD1, plectin, and AKAP9 are also expressed in immune cells[104–108].

Several functions of the LINC complex have been demonstrated in leukocytes. During T cell activation, lamin A/C are expressed and modulate actin polymerization, centrosome translocation, and T cell-APC interaction[57]. Overexpression of dominant-negative KASH or SUN domain reduces actin polymerization and impairs T-cell activation, suggesting a role of the LINC complexes in T cell functions[57]. Similarly, in resting B lymphocytes, the centrosome is linked to the nucleus through the LINC complexes and actin filaments nucleated by Arp2/3[109]. Upon activation, centrosome separation is required for its polarization and immune synapse formation. KASH overexpression increases nucleus-centrosome distance and rescues centrosome separation defects caused by increased centrosomal F-actin[109]. Silencing either nesprin-1 or SUN1 inhibits changes in nuclear morphology and position during this process and impairs cell polarization[110].In addition, both SUN1 and SUN2 have been observed to work together with lamin A/C to regulate HIV infection, but the underlying mechanism is unclear due to conflicting results[111–116]. Whether nesprins are involved in HIV infection is also undetermined. Various studies have demonstrated that SUN1/2's effects are LINC-independent[113–116]. But a recent study suggested that the nesprin-SUN interaction is required and proposed that SUN proteins reduce HIV infection by constraining nuclear rotation and chromatin movements[117]. Moreover, depletion of α-dystrobrevin in HL-60 cells inhibits nuclear lobulation and reduces the expression of nesprin-1 and lamin A/C[93]. Whether nesprin-1 is involved in nuclear lobulation, however, is unknown. Furthermore, in DCs migrating through micrometric pores, loss of SUN1 reduces the persistency of migration[9]. Loss of either SUN1 or SUN2 reduces the nuclear passage time, suggesting the involvement of the LINC complexes in DC migration[9]. Finally, the expression of a BICD2-nesprin3 fusion protein causes the nucleus to be moved together with the centrosome in CTLs, demonstrating that the LINC-lamin machinery is functional in leukocytes[118].

Given the importance of nucleus mechanics during leukocyte migration, and the

complicated interactions between the nucleus and the cytoskeleton, the functional importance of the LINC complexes merits further investigation.

6 | FORCES ON THE NUCLEUS

Theoretically, forces exerted on the nuclear envelope can be generated inside or outside of the nucleus. Forces from the inside, however, are inadequately studied and their contributions to cell migration are unknown. There is clear evidence that filamentous actin and multiple myosin isoforms are present in the nucleus[119,120]. The nuclear envelope protein emerin can bind to the slow-growing minus-ends of actin filaments and may thus tether them to the nuclear membrane[121]. Formin-mediated actin polymerization originated from the nuclear envelope was observed in fibroblasts upon the increase of intracellular Ca^{2+}[122,123]. A role of nuclear actin in remodeling the nucleus has been demonstrated in starfish oocytes and fibroblasts (Fig. 4A). During oocyte meiosis, Arp2/3 mediates actin polymerization underneath the nuclear envelope, leading to the insertion of actin filaments into the membrane, nuclear rupture and nuclear envelope breakdown[124,125]. Such a phenomenon has not been demonstrated in somatic cells. In fibroblasts, nuclear actin filaments are induced by cell spreading and DNA replication stress[126,127]. Replication stress-induced nuclear F-actin depends on Arp2/3 and functions to promote stress repair and counteract the changes in nuclear architecture[127].

Outside of the nucleus, the actin cytoskeleton can exert both pushing and pulling forces on the nucleus through several mechanisms. First, during adhesion-dependent migration, actin and myosin at the front of the cells can pull on this nucleus (Fig. 4B). This occurs during lobopodia-dependent 3D migration. In fibroblasts and cancer cells, myosin contractility pulls the nuclei forward through nesprin-3[128,129]. In mammary gland carcinoma cells, myosin IIB is involved in nuclear translocation[130]. Myosin IIB is enriched in the cell rear, suggesting that it may squeeze the nucleus. However, its dorsal perinuclear actin cable localization and the involvement of nesprin-2 indicate that Myosin IIB may also pull on the nucleus[130]. A role of the LINC complexes in leukocyte nuclear migration has only been observed in DCs, but LINC complexes in lymphocytes can also transmit force to the nucleus for its rotation[9,117].

Second, myosin II behind the nucleus can squeeze it forward (Fig. 4C). In effector T cells, FMNL1 localizes in the posterior perinuclear region to modulate actin polymerization, which promotes nuclear translocation during diapedesis into inflamed tissues[131]. It is unknown whether this is driven solely by actin polymerization or also by actomyosin contractility, but myosin IIA was shown to localize to the uropod and squeeze the nucleus during T cell diapedesis[132]. Similarly, myosin contractility in the uropod facilitates rapid cell migration by squeezing the nuclei during adhesion-independent mature DC migration[33]. Nuclear squeezing can be dynamically regulated by the nucleus. Nuclear deformation activates $cPLA_2$, which activates myosin II contractility at the cell cortex through the production of arachidonic acid[97,133]. Increased myosin II contractility squeezes the cells (and their nuclei) out of the confined environment and allows the cells to switch to a bleb-based migration mode[97,133]. Nuclear squeezing by myosin contractility is common in adhesion-independent migration but can be dispensable under some conditions[9,33,134].

Third, actin polymerization at the constriction can deform the nucleus without myosin contractility (Fig. 4D). In immature DCs expressing lamin A/C, inhibiting Arp2/3 or F-

actin, but not microtubule, myosin II, or mDia1, impairs efficient nuclear passage through constriction[9]. Both actin and Arp2/3 are enriched at the beginning of the constriction during nuclear passage to generate lateral pushing force to drive nuclear deformation. The requirement of Arp2/3 for nuclear passage is relieved in lamin A/C depleted DCs, suggesting that the primary function of Arp2/3 is to facilitate the deformation of the nucleus[9]. Accumulation of F-actin at the constriction was not observed in neutrophils, which don't express lamin A/C and have soft nuclei[9].

Lastly, through an unknown mechanism, myosin 1F in neutrophils aids the deformation of the nucleus. Myosin 1F is enriched in the uropod, the leading edge, and to a less extent, at the constriction. The lack of myosin 1F compromises the dynamic deformation of the nucleus and transmigration[135].

7 | THE MECHANICAL ROLES OF THE NUCLEUS IN CELL MIGRATION

The nucleus plays several crucial mechanical roles during cell migration[10,11]. First, it can serve as a force propagator. During diapedesis, the insertion of a leukocyte nuclear lobe into the vascular endothelium exerts a force on the ECs to induce the disassembly of actin filaments, which opens a pore to enable the passage of the cell[13]. Pore size is not determined by the ECs, but by the size of leukocytes, highlighting the crosstalk between leukocytes and ECs during diapedesis[136]. In a confined 3D environment, migrating human fibroblasts and cancer cells form lobopodia at their leading edge[128,129]. Lobopodia are bleb-like protrusions that form under high localized pressure. Actomyosin contractility is high in the leading edge of these cells and pulls the nucleus forward[128]. Nuclear squeezing leads to the compartmentalization of the cytoplasm and significant differences in the anterior and posterior pressures. This anterior high pressure contributes to the formation of bleb protrusions. Force generated by myosins was transmitted to the nuclei through LINC complex protein nesprin-3 and intermediate filament protein vimentin[128]. Lobopodial migration is adhesion-dependent and has only been observed in mesenchymal cells.

Second, the nucleus can work as a mechanosensor[10,11]. For example, nuclei in mesenchymal stem cells and fibroblasts show a strong preference for positioning on a concave surface[137]. This phenomenon, termed curvotaxis, results in relaxed nuclei due to reduced force applied by dorsal actin cables. Curvotaxis is regulated by the nucleus and requires lamin A/C and the LINC complexes[137]. It should be noted that curvotaxis is not universal and macrophages do not react to surface topography[137]. The mechanosensing functions of the nucleus are less studied, but exist, in leukocytes. In T cells, integrin signals regulate epigenetic changes and nuclear properties in a lamin B1-dependent manner[138]. On the molecular level, the nucleus senses mechanosignals in part by regulating the translocation of mechanosensitive transcription factors, like the serum response factor (SRF), into the nucleus. Nuclear import/export of megakaryoblastic leukemia 1 (MKL1), which activates SRF, in fibroblasts is regulated by lamin A/C[139]. MKL1 regulates the expression of actin regulators and adhesion proteins and its mutations lead to reduced neutrophil motility and immunodeficiency[140,141]. Mechanical force can also stretch the nuclear membrane, leading to membrane dilation. Membrane dilation relaxes the nuclear pore complexes, allowing the nuclear import of YAP in fibroblasts and mammary MCF10A epithelial cells[142]. YAP and its homolog TAZ are transcription factors of the Hippo pathway and key mechanosensors and mechanotransducers[143]. YAP/TAZ regulates T cell differentiation and functions[144]. Membrane dilation is also sensed by $cPLA_2$, which associates with stretched membrane to initiate the eicosanoid

signaling[145]. cPLA$_2$ senses membrane dilation resulting from spatial confinement and upregulates myosin contractility on demand in both cancer cells and leukocytes[97,133]. Such a response is absent in enucleated cells[97].

The nucleus can act as a mechanical gauge for path selection[8]. Migrating cells preferentially enter larger over smaller pores. When mature DCs, T cells, and neutrophils encounter multiple pores, pseudopods protrude into all these pores. In addition, when the nucleus reaches the branching point, nuclear protrusions form and enter several of these pores simultaneously. The nuclear protrusions in smaller pores quickly retract and much of the nucleoplasm enters one of the larger pores[8]. Subsequently, the centrosome enters the same pore, and membrane protrusions in other pores retract. These observations show that cells use the nucleus, whose size is the rate-limiting factor, as a gauge for path selection[8].

8 | THE NUCLEUS IN REGULATING THE DIRECTION OF MIGRATION

Although directional persistence is important for speedy movement, maintaining a persistent direction of cell migration is not always desirable. Many leukocytes, including T cells and neutrophils, show a cell-intrinsic weaving behavior that is increased by the topology of the ECM[146,147]. Cells have evolved mechanisms to increase random migration. For example, myosin 1G in T cells was shown to associate transiently with the deformed plasma membrane in turning cells and modulate membrane tension. Its loss results in increased cell speed and persistency and decreased random migration[147]. When migrating in 3D, cells encounter barriers and intersections and need to make directional decisions. Leukocytes select paths by simultaneously protruding pseudopods into multiple paths. By integrating the environmental cues, like pore sizes and chemoattractant gradients, one of the protrusions is selectively stabilized and the cell repolarizes. Expectedly, many actin regulatory proteins are involved in cell polarization and chemotaxis, like mDia1, Arp2/3, and WAVE[34,148].

Directional cell migration relies on proper cell polarity. The centrosome position relative to the nucleus has long been considered a good indication of migration direction[14]. In mesenchymal cells migrating on 2D surfaces, they actively move their nuclei rearwards to establish a nucleus-centrosome axis pointing to the direction of migration[149]. Migrating immune cells, however, have their nuclei located in front of their centrosome[14]. Cells in 1D and 3D migration also have front nuclei[150]. It should be noted that migrating cells need to dynamically adjust their direction and may frequently reposition both their nuclei and centrosomes[151,152]. Thus, the relative nucleus-centrosome position is not absolute. In addition, the *in vivo* orientation of these two organelles is not completely clear. For example, in motile neutrophils in zebrafish tissues, the centrosome is positioned between the leading edge and the nucleus[153].

This dynamic yet consistent positioning of the nucleus and the centrosome suggests that their positions may contribute to cell migration. In fact, in myoblasts, depletion of nesprin-2G inhibits rearward nuclear movement and significantly impairs persistent cell migration[154]. Similarly, depletion of SUN1 interferes with persistent DC migration[9]. The centrosome and microtubules are crucial for the regulation of cell polarity necessary for directional and persistent cell migration. Stabilizing microtubules with taxol suppresses polarization and cell migration[155]. Depolymerizing microtubules with drugs in neutrophils induces cell polarization associated with

increased random cell migration[153,155,156]. Microtubule depolymerization in T cells also leads to increased random cell migration[157].

The nucleus and the centrosome not only maintain the direction of migration, but also work together to select the path of migration. In mature DCs migrating in 3D, microtubules nucleated from the centrosome grow into all leading pseudopods and the trailing tail[8]. Once the centrosome follows the nucleus and enters a protrusion (winner), due to the geometric restriction and the rigidity of microtubules, growing microtubules increase in the winner protrusion and decrease in the loser protrusions. Loss of microtubules releases GEF-H1 to destabilize the loser protrusions and the tail, resulting in their retraction and cell repolarization[8,158,159]. The behavior of the nucleus is particularly interesting during this process. At the branching point, multiple nuclear protrusions form to enter and probe the branches. The mechanical signals from these protrusions are integrated and the loser protrusions, usually the ones in smaller branches, retract and the whole nucleus enters the winner's branch[8].

The nuclear gauge model highlights the dynamic nature of the nucleus and raises several outstanding questions: 1) How are nuclear protrusions formed? Myosin contractility can squeeze the nucleus to cause nuclear protrusions. However, path selection is unaffected by myosin inhibition[8], suggesting that myosin contractility is not required for nuclear protrusion. The morphologies of the nuclei during this process suggest that they are under force[8], but the shapes vary greatly and both pushing and pulling forces can be interpreted. It is also unknown whether this is driven by actin, microtubule, or their motors. 2) Are lamins and the LINC complexes involved in nuclear protrusion? Pulling on the nucleus by both actin filaments and microtubules requires the LINC complex and the nuclear lamina. However, a stiff lamina will reduce the deformability of the nucleus[8]. The function and regulation of the nuclear lamina during this process are therefore of great interest. 3) What are the mechanical signals and how are they integrated? 4) How is the retraction of nuclear protrusion triggered and driven? One intriguing but untested possibility is that nuclear protrusions in small pores lead to nuclear membrane dilation or have higher membrane curvatures, which can be sensed by $cPLA_2$[97,133,160]. $cPLA_2$ triggers local myosin contractility to push the nuclear protrusions out of the corresponding branches. In agreement with this, inhibition of myosin significantly impairs the retraction of nuclear protrusions[8].

9 | CONCLUDING REMARKS

The importance of the mechanical properties of cells and their microenvironment has been increasingly appreciated. The nucleus contributes greatly to the biomechanics of the cells and is a major sensor, propagator, and integrator of mechanical signals. Its importance in mediating cell mechanics is further increased by its interaction with the cytoskeleton through the LINC complexes. Leukocytes are great model systems to study nuclear architecture and mechanosensing because of the great variety in both their nuclear shapes and the composition of their nuclear envelope. Their migration is also a wonderful process to study the dynamic remodeling of the nucleus. Previous studies have revealed many novel functions and regulation mechanisms related to the nucleus, including those that modulate cell migration. More nuclear functions will be uncovered and provide further insights into not only their roles in cell migration but also our understanding of immunodeficiency disorders and the development of cancer immunotherapies[35,36161].

AUTHORSHIP

Y.L., M.C. and W.C. wrote and edited the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

FIGURES AND LEGENDS

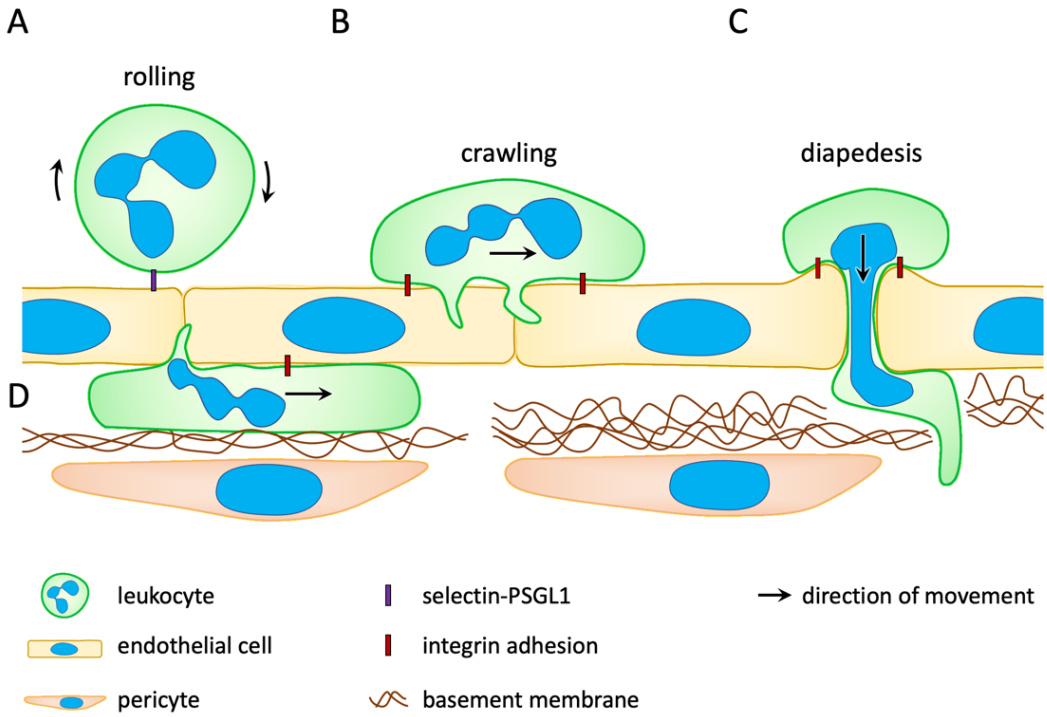

Figure 1. Steps of leukocyte transmigration. (A) Transient interactions between selectins and PSGL1 generate frictional force and, together with shear force, cause the rolling of leukocytes. (B) Integrin adhesions anchor leukocytes to the endothelium, allowing forces generated by actin polymerization and myosin contractility to drive cell crawling on the endothelial surface. (C) During diapedesis, leukocytes insert protrusions into the endothelium to cause its reorganization and pore opening. Actin and myosin then squeeze the cell body through the pore. During this process, neutrophil nuclei lose their lobed morphology. Nuclei lobes reform after transmigration. (D) If a transmigration spot is not found on the adjacent basement membrane, leukocytes crawl between the endothelial cells and the basement membrane to search for one.

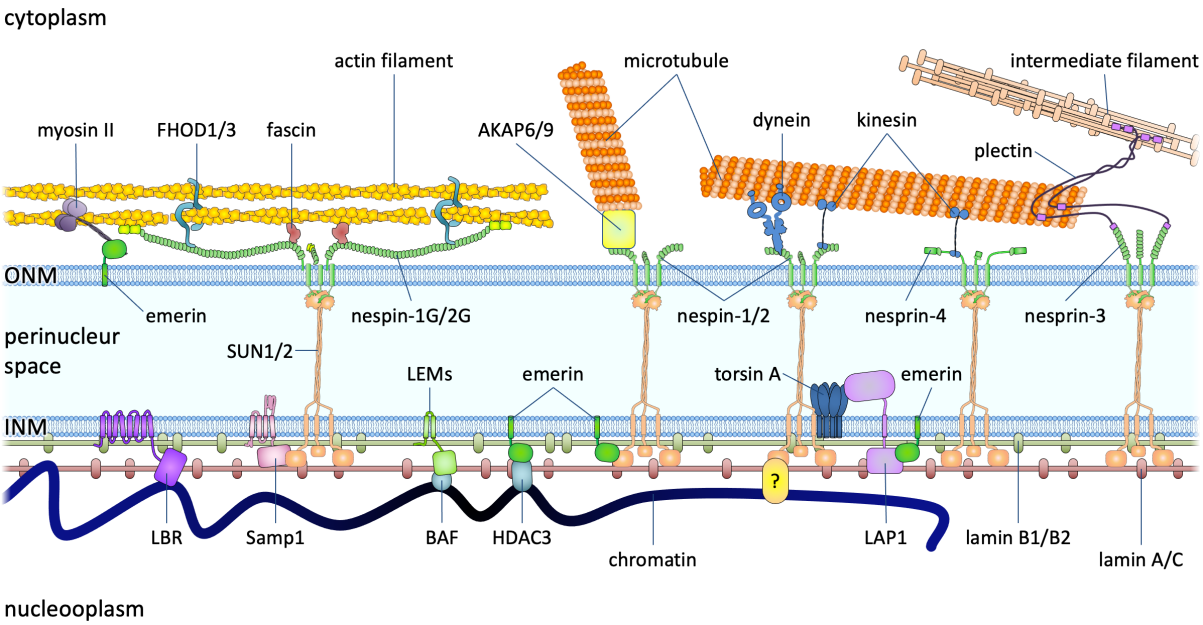

Figure 2. The LINC complexes connect the cytoskeleton to the nuclear lamina and chromatin in somatic cells. The LINC complexes are composed of nesprins on the ONM and SUN proteins on the INM. The cytoplasmic domains of nesprins interact with the cytoskeleton. The giant isoforms of nesprin-1 and -2 contain actin binding domains and interact with actin filaments. Such an interaction is regulated by emerin through its interaction with myosin II and is strengthened by fascin and formin proteins FHOD1 and FHOD3. Nesprin-1α interacts with AKAP6 and AKAP9 to facilitate microtubule nucleation. Both the long and short isoforms of nesprin-1 and -2 contain a kinesin-1- and dynein- binding region and associate with microtubules through the motors. Nesprin-4, which may not be expressed in leukocytes, can also bind to kinesin-1. Nesprin-3 binds to plectin (and the related BPAG1 and MCAF), which can interact with both microtubules and intermediate filaments. In the perinuclear space, trimeric SUN proteins bind to nesprins and torsin A. In the nucleoplasm, SUN proteins bind to lamins for anchorage and interact with Samp1 and emerin. Many of these proteins, like lamin A, lamins B (through LBR), SUN proteins (through an unknown adaptor), and LEM domain proteins (Man1, LAP2, emerin, and LEMD2, through BAF and HDAC3) directly or indirectly bind to chromatin.

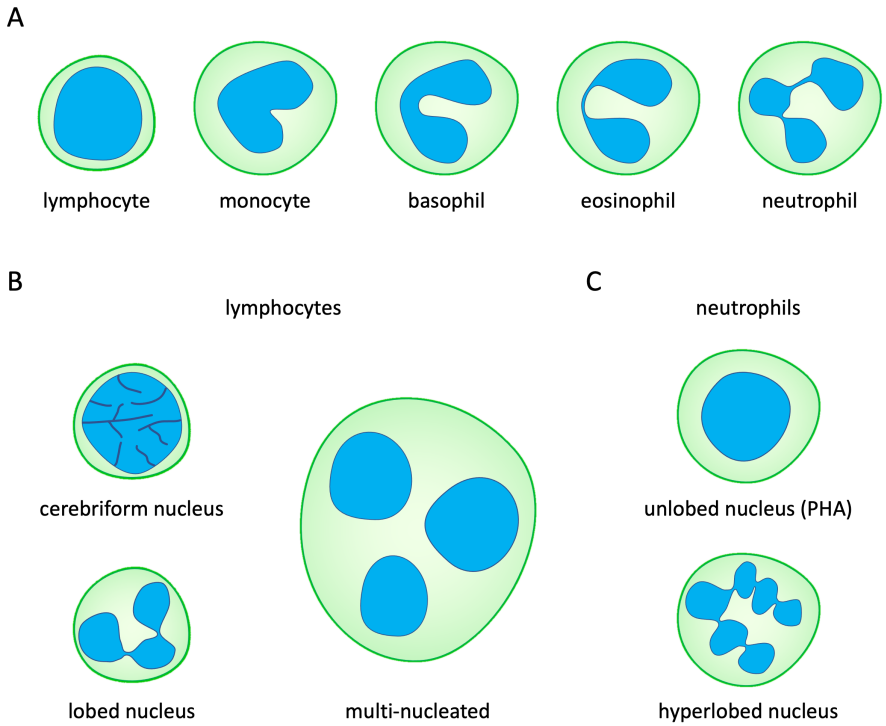

Figure 3. Nuclear shapes of leukocytes and in diseases. (A) Leukocyte nuclei exhibit diverse shapes. From left to right, lymphocytes have rounded nuclei; monocyte nuclei have invaginations; Basophil nuclei have two or three lobes; Eosinophil nuclei have two well-separated lobes; Neutrophil nuclei usually contain 3-5 lobes. (B) Abnormal nuclear shapes are associated with immune diseases. Top, highly convoluted cerebriform nuclei are found in lymphocytes from patients with Sézary syndrome or T-cell Prolymphocytic Leukemia. Bottom, lobed nuclei can be found in lymphoblasts with acute lymphoblastic leukemia. Right, the presence of large, multinucleated Reed-Sternberg cells is a feature of the Hodgkin's lymphoma. (C) Nuclear lobulation in neutrophils is primarily regulated by LBR. Reduced expression of LBR leads to unlobed/hypolobulated nuclei (PHA, top) while increased LBR causes hyperlobulation (bottom). Hypolobulation caused by most heterozygous *LBR* mutations is benign.

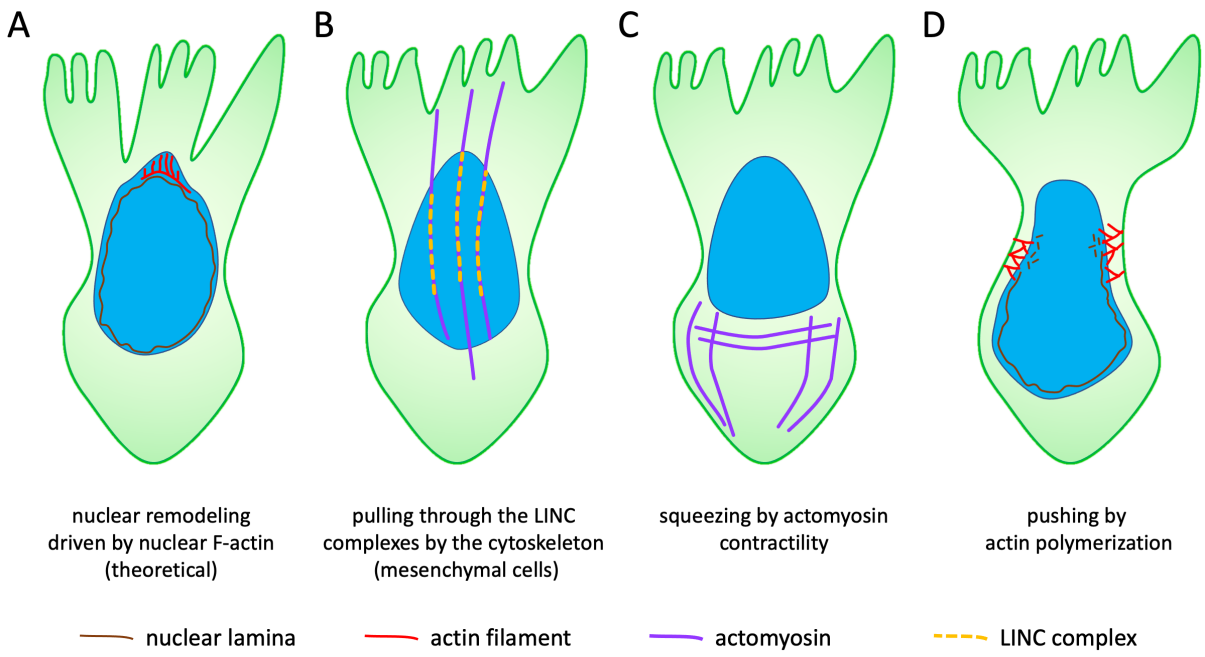

Figure 4. Nuclear movement and deformation by the cytoskeleton. (A) Theoretically, nuclear membrane remodeling can be driven by actin polymerization (red lines), similar to the protrusion of the plasma membrane. However, although actin filaments have been observed to grow from the nuclear envelope, their role in causing nuclear membrane protrusion has not been demonstrated. (B) During adhesion-dependent migration, actomyosin contractility (purple lines) at the front of the cell pulls the nucleus and mediates its translocation. Actomyosin is coupled to the nucleus by the LINC complexes (orange dashes) on the nuclear membrane. Microtubule and its motors can also move the nucleus by pulling. (C) During both adhesion-dependent and -independent modes of migration, myosin contractility behind the nucleus squeezes it through constriction. (D) During adhesion-independent migration, branched actin filaments (red lines, nucleated by Arp2/3) at the constriction push on the nucleus to cause its deformation and the disruption of the nuclear lamina, allowing the softened and deformed nucleus to enter the constriction.

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