Squish and squeeze – the nucleus as a physical barrier during migration in confining environments

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Abstract

From embryonic development to cancer metastasis, cell migration plays a central role in health and disease. It is increasingly becoming apparent that cells migrating in three-dimensional (3-D) environments exhibit some striking differences compared with their well-established 2-D counterparts. One key finding is the significant role the nucleus plays during 3-D migration: when cells move in confined spaces, the cell body and nucleus must deform to squeeze through available spaces, and the deformability of the large and relatively rigid nucleus can become rate-limiting. In this review, we highlight recent findings regarding the role of nuclear mechanics in 3-D migration, including factors that govern nuclear deformability, and emerging mechanisms by which cells apply cytoskeletal forces to the nucleus to facilitate nuclear translocation. Intriguingly, the 'physical barrier' imposed by the nucleus also impacts cytoplasmic dynamics that affect cell migration and signaling, and changes in nuclear structure resulting from the mechanical forces acting on the nucleus during 3-D migration could further alter cellular function. These findings have broad relevance to the migration of both normal and cancerous cells inside living tissues, and motivate further research into the molecular details by which cells move their nuclei, as well as the consequences of the mechanical stress on the nucleus.

Introduction

In multicellular organisms, cell migration is essential in the development, maintenance and repair of various tissues [1]; it also enables immune cells to survey tissues and to respond to local challenges [2]. At the same time, cell migration drives the tissue invasion and metastasis of cancer cells, which is responsible for the vast majority of cancer deaths [3]. While much of our current knowledge regarding the molecular and biophysical principles of

cell migration stems from studying cells moving on 2-D substrates [4], it is now becoming evident that cells migrating in 3-D environments encounter distinct physical challenges. During *in vivo* migration/invasion, cells must navigate many microstructural obstacles, including extracellular matrix (ECM) networks and neighboring cells. The pore sizes encountered in the interstitial space range from 0.1 to 30 μm in diameter, i.e., comparable to or significantly smaller than the size of the migrating cell [5–7]. Cells have two strategies to penetrate such confining environments: *(i)* expanding the openings via physical remodeling and/or proteolytic degradation of the ECM [8], or *(ii)* contorting their shape to accommodate the available spaces [9]. The cell membrane and cytoplasm are able to quickly deform and remodel to penetrate openings less than 1 μm in diameter [10]. In contrast, deformation of the nucleus, the largest and stiffest organelle, presents a more formidable challenge. Here we discuss emerging insights into the intracellular biomechanics and molecular processes involved in translocating the nucleus through tight spaces, including implications on migration efficiency and other biological functions.

The size and rigidity of the nucleus: a physical barrier for cell migration

The nucleus is the largest organelle in the cell, with a diameter between 3–15 μm [11,12], making it substantially larger than many pores encountered during migration in physiological tissues. Furthermore, biophysical measurements of isolated nuclei and intact cells reveal that the nucleus is typically 2- to 10-times stiffer than the surrounding cytoplasm [11]. This combination of large size and relative rigidity of the nucleus led to the hypothesis that the nucleus can impact the cells' ability to migrate [13]. Early support for this hypothesis came from work on tumor cells migrating through microfabricated channels with precisely defined constrictions [14–16] (see Box 1 for more information on such devices). While moderate confinement results in increased migration speed by allowing cells to employ faster migration modes (e.g., 'amoeboid migration' and 'chimneying') than during 2-D migration [17], constrictions below approximately 5 μm in diameter require substantial nuclear deformation and result in reduced migration speeds [14–16,18–20]. A seminal study by Friedl, Wolf, and colleagues using a range of cell types demonstrated that nuclear deformability presents a physical limit for the migration through collagen matrices with varying pore sizes [10]. When inhibiting matrix metalloprotease (MMP) activity required to degrade ECM, migration speed declined with decreasing pore size as nuclei had to undergo increasing deformation [10]. At pore sizes smaller than 10% of the non-deformed cross-section of the nucleus, cells reached a 'nuclear deformation limit' resulting in complete migration arrest, despite continued protrusion of the cytoplasm [10]. Subsequent studies using a variety of cell lines and experimental assays ranging from microfluidic devices, membranes with defined pores, ECM matrices, and *in vivo* xenografts have painted a similar picture, in which the deformability of the nucleus limits the cell's ability to pass through tight spaces, reducing or even stalling migration as the pore size decreases below the cross-section of the nucleus [18–26]. Assessing the role of specific physical factors on cell migration in confined environments, Lautscham and colleagues [20] found that increased nuclear (but not cytoplasmic) volume, increased nuclear stiffness, reduced cell adhesion and lower cell contractility impaired migration through microfluidic constrictions. While the above findings prove common to a large variety of cell lines, including neutrophils,

fibroblasts, and tumor cells, the exact degree of confinement necessary to elicit such effects, and the magnitude of the effect, varies with cell type. These differences indicate that variation in nuclear deformability, or the cytoskeletal forces applied to the nucleus, may be important modulators of the 'nuclear barrier' effect.

Lamins determine nuclear deformability and migration through confined environments

The deformability of the nucleus is largely determined by two components, the nuclear lamin network and chromatin [27,28]. Lamins are type V nuclear intermediate filaments that can be divided into two sub-types, A-type (A, C, C2) and B-type (B1-3) lamins [29–36]. The different lamin subtypes form separate but interdigitating fibrillar networks at the nuclear periphery [37,38]. In addition to regulating nuclear shape and stiffness [27,39–42], they play important roles in chromatin organization, DNA damage repair, and transcriptional regulation [37,43,44]. Cell-stretching and micropipette aspiration experiments indicate that A-type lamins have a larger impact on nuclear stiffness than B-type lamins—nuclear stiffness strongly scales with expression of lamins A/C [39–42], although increased expression of lamin B1 can also increase nuclear rigidity [45]. Consistent with the 'nuclear barrier' hypothesis, recent studies found that cells with reduced levels of lamins A/C have more deformable nuclei and migrate faster through tight spaces than control cells with normal lamin A/C levels [18,46]. Conversely, increased expression of lamin A, or expression of a mutant lamin (progerin) that increases nuclear stiffness, impaired transit through narrow constrictions [21,25]. Loss of lamin A/C promotes cell migration through small constrictions by allowing larger nuclear deformation, rather than increased nuclear compression, as the nuclear volume does not decrease during nuclear translocation [19]. These findings have direct physiological and clinical relevance, since downregulation of lamins A/C during granulopoiesis is critical for the ability of neutrophils to pass through micron-sized constrictions [21], and misregulation of lamins is common to many cancers [47] (see Box 2 for more information). Less is known about the role of B-type lamins in 3-D migration. Loss of B-type lamins impairs migration of neurons, which lack A-type lamins, in the developing brain, and this effect is thought to be caused by defects connecting the nuclear interior and cytoplasm [48,49]. Given recent reports that lamin A/C levels and organization can vary in response to substrate stiffness and cytoskeletal tension [41,50,51], it is intriguing to speculate that cells could also dynamically adjust their nuclear stiffness during migration.

The role of chromatin in nuclear deformability and migration

Chromatin, consisting of DNA wrapped around histone octamers, occupies most of the nuclear interior and contributes to the viscoelastic response of nuclear deformation [28,52]. Chromatin exists in two configurations: *(i)* open 'euchromatin', which is typically transcriptionally active, and *(ii)* closed, more compact 'heterochromatin', which is associated with inactive genes [53]. Promoting euchromatin over heterochromatin organization, for example by treatment with the deacetylase inhibitor trichostatin A (TSA), results in softer and more deformable nuclei [52]. Interestingly, treatment with 5′-deoxy-5′-methylthioadenosine (MTA), a methyltransferase inhibitor that cause de-condensation of chromatin, impairs the migration of the cells through microchannels [15]. It remains unclear whether this counterintuitive effect was due to the increase in nuclear size resulting from chromatin de-condensation, which may counteract reduced nuclear stiffness [20], or due to

altered transcriptional regulation, motivating future research on the role of chromatin organization in cell migration through confining constrictions.

Cytoskeletal forces pulling or pushing on the nucleus

Recent studies have shed light onto the molecular components physically connecting the nucleus and cytoskeleton (Fig. 1A), revealing that the linker of nucleoskeleton and cytoskeleton (LINC) complex is the 'clutch' that enables transmission of mechanical force across the nuclear envelope [54]. We refer the reader to excellent recent reviews [55,56] and the article by Burridge *et al.* in this issue for more details on LINC complex function. However, the mechanism by which cells move the nucleus through confining constrictions, including whether cells predominantly pull or push on the nucleus (Fig. 1B), remains incompletely understood. It is likely that cells apply a varying combination of both mechanisms, depending on the specific conditions.

Pulling the nucleus forward

In most migrating cells, the nucleus is positioned rearward, i.e., in the back of the cell [55]. Recent studies indicate that actomyosin contractility, possibly in combination with intermediate filaments [57,58], physically pull the nucleus forward during 2-D and 3-D migration [10,59]. In 3-D lamellipodial-based migration, actomyosin contractility and integrin-mediated traction at the leading edge are required to translocate the nucleus through narrow constrictions [10]. During 3-D lobopodial-based migration, non-muscle myosin IIA (NMIIA)-containing actomyosin bundles work with vimentin filaments to pull the nucleus forward by binding to nesprin-3α of the LINC complex via plectin [58,60]. Whereas microtubule associated motors are dispensable for nuclear translocation in 2-D migration [59], the microtubule-associated motors dynein/kinesin, which can directly bind to LINC complex proteins [56], are essential for interkinetic nuclear migration in neurons [61]. Dynein and kinesis are also required for nuclear positioning in multinucleated myotubes, where nuclei are squeezed, rotated and translocated to form proper myotube structure [62]. Thus, it is possible that microtubule-associated motors also participate in nuclear translocation during 3D cell migration.

Pushing the nucleus forward

Actomyosin contraction at the back of the cell is required for squeezing the leukocyte nucleus through narrow pores [63]. In breast and brain cancer cells, non-muscle myosin IIB (NMIIB) is recruited to the perinuclear cytoskeleton and posterior of the cell, possibly via nesprin-2, where it applies pushing forces to the nucleus to facilitate nuclear translocation through confining 3-D environments [64,65]. Depletion of NMIIB, but not NMIIA, impairs migration of breast cancer cells through microfluidic constrictions and dense collagen matrices [64], suggesting a specific role of perinuclear NMIIB actin networks in squeezing the nucleus through constrictions. Nonetheless, further studies are required to determine whether NMIIB-dependent actomyosin contraction constitutes a general mechanism for nuclear translocation, whether it also involves nesprin-3α and vimentin filaments, and whether increased pulling forces can compensate for impaired pushing forces and vice versa.

Physical compartmentalization by the nucleus

During migration through confined spaces, the nucleus takes up most of the space within the constriction, thus leaving little or no room for cytoplasmic transport around the nucleus and effectively dividing the cytoplasm into "front" and "back" compartments (Fig. 1B). This intracellular compartmentalization plays an important role in two mechanisms: osmotic pressure difference caused by water permeation, and intracellular pressure generated by the nucleus as an active 'piston' [60,66]. Polarized distribution of Na^+/H^+ pumps and aquaporins on the plasma membrane at the front and back of the cell alters water permeation and can drive migration and nuclear translocation, even when actin polymerization is inhibited [66]. However, since this osmotic pressure-based mechanism has only been described in tube-like microchannels that restrict fluid flow to the front and back of the cells, its relevance in more physiological environments still needs to be examined. Work by the Yamada group [60] reveals that during lobopodial-based migration, actomyosin-mediated pulling of the nucleus compresses the front of the cell, similar to a piston in a cylinder, building up higher intracellular pressure within this compartment and driving the formation of new lobopodial protrusions at the leading edge. The nucleus may similarly serve as a counterbalance for the directional protrusion of invadopodia, causing visible indentation of the basal nuclear surface [67]. Taken together, these studies illustrate how cells can take advantage of the large and rigid nucleus to promote cell migration in 3-D environments.

Biological consequences of nuclear deformation during 3-D cell migration

The nucleus is not only a passive mechanical element, but also houses the cell's genomic information and is the site of DNA replication, transcription, and RNA processing. Thus, the substantial mechanical forces and deformation incurred during migration through tight spaces may have severe biological consequences that impact cellular function and viability.

Influences on cell survival and genomic stability

Recent studies have produced conflicting results on the effect of cell migration through tight spaces on cell viability. Whereas one study found increased apoptosis after migration through small (3 μm) pores, particularly in lamin A/C-deficient cells [46], others reported no noticeable increase in cell death during confinement [17–19,21]. These apparent differences may reflect differences in cell type or mechanical confinement (e.g., porous membranes *vs.* microfluidic devices and collagen matrices). Interestingly, pharmacological inhibition of the heat shock protein 90 (HSP90), which facilitates DNA damage repair [68], results in increased cell death after passage through 3-μm diameter pores, suggesting that cells may have suffered DNA damage during migration that causes apoptosis when not adequately repaired [46]. Mechanical stress alone can induce DNA damage [69], but it remains to be seen whether the mechanical stress incurred during 3-D cell migration is sufficient to cause DNA damage.

Influences on mechanotransduction signaling and gene expression

Mechanical stress on the nucleus during migration may also trigger non-lethal changes, which could further affect cell migration and cellular function. Recent studies in which

isolated nuclei were subjected to mechanical stress suggest that the nucleus itself can act as a mechanosensitive element. Force application via the LINC complex induces rapid phosphorylation of the inner nuclear membrane protein emerin, leading to recruitment of lamin A/C to the nuclear envelope and nuclear stiffening [70]. Shearing isolated nuclei causes partial unfolding of lamin A/C, exposing cryptic binding sites that could initiate mechanotransduction events [41]. Furthermore, changes in the mechanical microenvironment and force application to intact cells can induce chromatin remodeling [71,72] and dissociation of nuclear protein complexes [73], which could affect both nuclear deformability and gene expression (see [74] and [75] for a detailed discussion of nuclear mechanotransduction). While these reports suggest that nuclear deformation during 3-D migration could impact nuclear organization, chromatin remodeling, and gene expression, direct experimental evidence for this hypothesis is still missing.

Conclusions and Outlook

The nucleus has long been recognized as a central hub for genomic information and its processing. Work published over the last few years makes it clear that one has to also consider the physical impact of the nucleus on cellular function, particularly during migration in 3-D environments. The nucleus, with its large volume and relative rigidity, acts as physical barrier when cells encounter pore sizes smaller than the nuclear diameter, resulting in reduced migration efficiency or even complete migration arrest. The extent of this nuclear barrier effect is largely driven by the nuclear size and stiffness, which is governed by the levels of the nuclear envelope proteins lamin A/C and chromatin organization. These findings are particularly relevant to immune cells and to invading cancer cells, which move through tissues with pore sizes smaller than the size of the nucleus and often have altered expression of lamins and other nuclear envelope proteins. Despite recent advances, many open questions remain. For example, can cells dynamically adjust their nuclear stiffness to facilitate cell migration through tight spaces, possibly by phosphorylation and/or degradation of nuclear lamins? Are some cells particularly well-suited for 3-D migration, either by having more deformable nuclei or by pulling/pushing harder on the nucleus? What are the precise molecular mechanisms by which cells translocate the nucleus through tight constrictions? And what are the biological consequences resulting from the large nuclear deformations, which could include changes in chromatin organization, DNA damage, and altered gene expression? Such mechanically induced events could not only affect migration itself, e.g., by altering nuclear stiffness and cytoskeletal dynamics, but also impact various other cellular functions and even viability. To drive new discoveries, it will be crucial to combine new imaging tools, such as fluorescence resonance energy transfer (FRET)-based intracellular force probes [76], with microfabricated environments that mimic physiological environments while providing defined geometries and enhanced live-cell imaging conditions, as well as single cell based assays to measure cell viability, gene expression, and epigenetic modification. Further insights into the role of the nucleus in 3-D migration will not only improve our understanding of the physical constraints during migration in physiological environments, but may ultimately lead to new strategies to better target invasive cancer cells and to reduce or eliminate metastatic spreading.

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Box 1

Development of tools to study migration in confined environments

Microfabrication techniques are finding increased application to study cell migration in confined environments, complementing existing approaches such as transwell plates and collagen invasion assays [10,46,77,78]. Besides micropillars, polymer scaffolds, and electrospun matrices [25,26,79,80], microfluidic devices made from polydimethylsiloxane (PDMS) by soft lithography [81,82] have proven particularly powerful in investigating cell migration through tight spaces by providing precisely defined microscale structures and constrictions with cross-sections from $100\ \mu m^2$ to less than $5\ \mu m^2$ [15_21,23,24,64,66,83]. These devices, which often include features to apply stable chemotactic gradients [14–16,18,19,24], allow for user defined geometries ranging from simple straight channels [15,16,24,66] to more intricate designs mimicking physiological environments [18_21,23,83]. The migration devices can be functionalized with a variety of ECM proteins to control cell adhesion. Since the devices are made of transparent PDMS and mounted on thin coverslips, they provide superb imaging conditions for live-cell imaging with high spatial and temporal resolution [81,82].

Box 2

Lamins, nuclear shape, and disease

Mutations in lamins cause a large spectrum of human diseases, ranging from muscular dystrophy and dilated cardiomyopathy to premature aging [84,85]. Increasing reports indicate that altered lamin expression (rather than mutations) is found in many cancers, and often correlates with negative clinical outcomes [47,86,87]. For example, reduced expression of lamin A/C has been reported in breast [88,89] and cervical cancer [90], and is correlated with an increased recurrence of stage II and III colon cancer [91] and reduced disease free survival in breast cancer [89]. However, in other cases, increased A-type lamin expression is associated with disease progression, specifically in prostate, colon and ovarian cancers [92,93]. Given the multiple function of lamins, changes in their expression are expected to have pleiotropic effects, affecting not only nuclear stiffness but also proliferation, survival, and gene expression [47,86,87,93,94]. Nonetheless, it is intriguing that more invasive breast cancer cell lines such as Hs578T and MDA-MB-231 are capable of more extensive nuclear deformation than normal and non-malignant controls [95], and move faster through migration devices designed to mimic tight spaces inside the body [15]. Future studies should be directed at characterizing the effects of altered lamin levels on cell migration, as well as other cellular functions, in more detail.
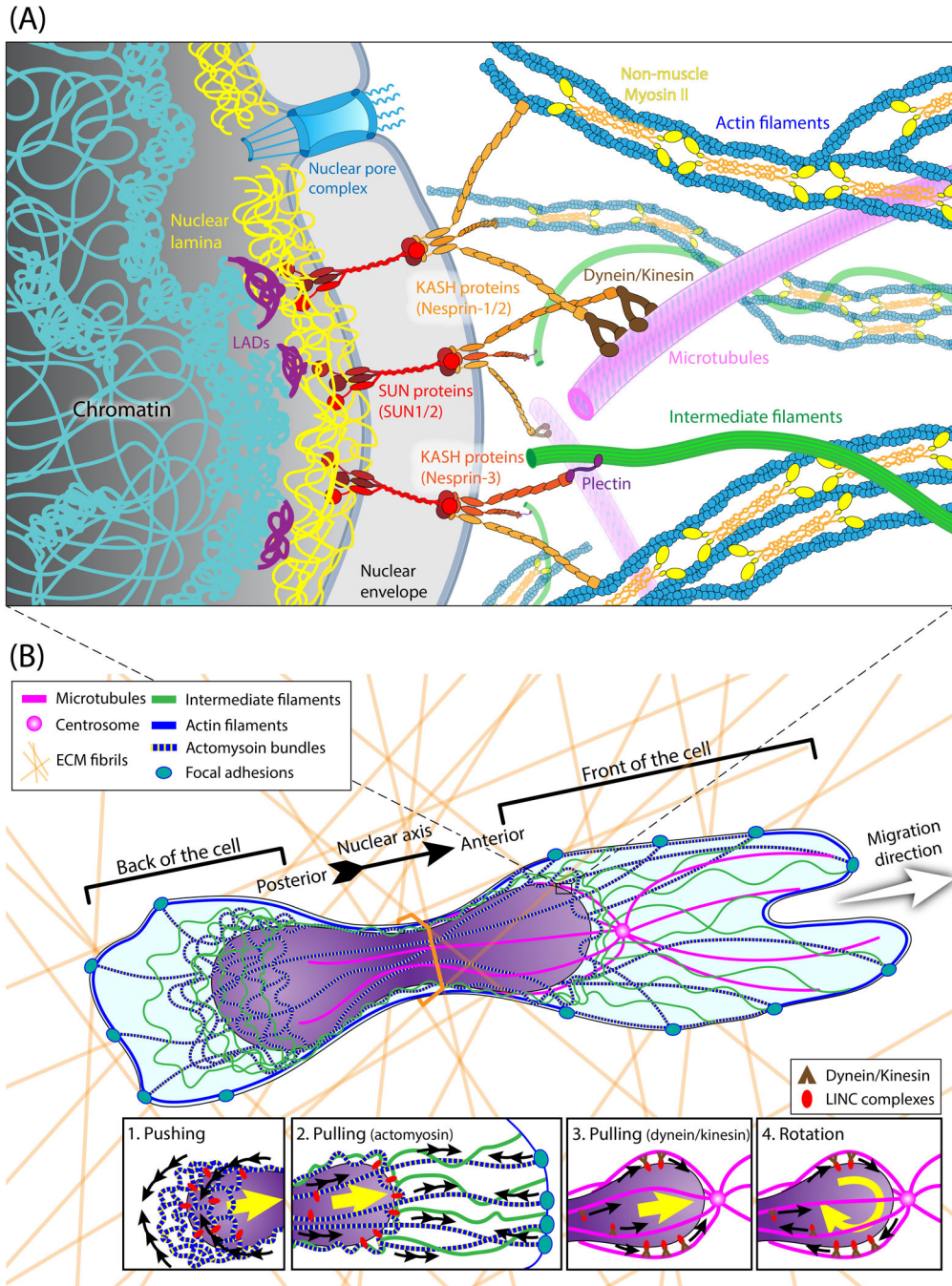
Figure 1. Schematic overview of the physical connections between the nucleus and cytoskeleton, and their roles in moving the nucleus through confined spaces

(**A**) At the nuclear periphery, chromatin interacts with lamins at lamina-associated domains (LADs). SUN domain proteins (SUN1 and SUN2) are anchored to the nuclear lamina and other components of the nuclear interior by their C-terminus. The N-terminal luminal long stalks and SUN domains of SUN1/2 form trimers that interact with KASH domain proteins located in the outer nuclear membrane (nesprin-1/-2/-3, along with the cell-type specific nesprin-4 and KASH5), forming the LINC complex [101]. The strong interaction between SUN domain trimers and the KASH domains provide the basis to mechanically couple the

nuclear interior with the cytoskeleton—nesprins interact directly with actomyosin bundles, or indirectly with microtubules and intermediate filaments via intermediary proteins (such as kinesin, dynein, plectin) [55]. Mechanical force transmission via nucleo-cytoskeletal coupling may also trigger mechanotransduction events, ranging from the recruitment of lamins to the LINC complex to changes in chromatin organization and gene expression, which may further impact cell migration processes. (**B**) Cytoskeletal organization and dynamics during migration in confined 3-D environments. As the cell passes through narrow pores, the nucleus separates the cell into front and back ends. The insets depict proposed mechanisms by which the cytoskeleton translocates the nucleus through confined spaces, including pushing via actomyosin contraction at the posterior of the nucleus (1), pulling via actomyosin contraction facilitated by intermediate filaments (2), pulling via microtubule-associated motors (3), and rotation via microtubule-associated motors (4).

Table 1

Overview of relevant molecular components involved in nuclear mechanics and migration in confining environments.

Components	Functions		References
Lamins	1	Major components of the nuclear lamina; determine nuclear shape and size; A-type lamins in particular are major contributors to nuclear stiffness.	[25] [37] [39] [40] [54]
	2	Contribute to anchoring LINC complex at the nuclear envelope	
	3	Bind to chromatin, particularly at lamin-associated domains (LADs) at the nuclear periphery; also interact with transcriptional regulators and DNA damage response proteins.	
Chromatin	1	Contributes to nuclear stiffness	[28] [52] [96] [97]
	2	Rearrangement can regulate gene expression in response to nuclear deformation	
LINC complexes	1	Physically connect the cytoskeleton and the nucleus, enabling force application and relaying of mechanical signal to the nucleus	[54] [55] [56]
Actomyosin bundles and non-muscle myosin II (NMII)	1	Actomyosin contraction is the major player of both actively pulling and pushing the nucleus	[10] [57] [60] [63] [64]
	2	Actomyosin contraction is crucial for relaying mechanical signal to the nucleus	
	3	Non-muscle myosin IIA participates in actomyosin contraction at cell anterior to pull the nucleus.	
	4	Non-muscle myosin IIB participates in actomyosin contraction at cell posterior to push the nucleus.	
Intermediate filaments	1	Vimentin filaments interact with actomyosin (NMIIA) bundles to facilitate pulling of the nucleus	[60] [98]
	2	Have the highest resistance to tensile force among the cytoskeleton, providing the strength needed for pulling the nucleus	
Integrins	1	Form focal adhesion at the leading edge of the cell, mediating traction forces needed for cell movement and nuclear translocation	[10] [99]
Microtubules and dynein/ kinesin motor proteins	1	Participate in nuclear rotation during 2D migration and in myotubes	[55] [62] [100]
	2	Possible role in nuclear rotation and pulling in 3D migration	