Nuclear migration events throughout development
Courtney R. Bone and Daniel A. Starr*

ABSTRACT
Moving the nucleus to a specific position within the cell is an important event during many cell and developmental processes. Several different molecular mechanisms exist to position nuclei in various cell types. In this Commentary, we review the recent progress made in elucidating mechanisms of nuclear migration in a variety of important developmental models. Genetic approaches to identify mutations that disrupt nuclear migration in yeast, filamentous fungi, Caenorhabditis elegans, Drosophila melanogaster and plants led to the identification of microtubule motors, as well as Sad1p, UNC-84 (SUN) domain and Klarischt, ANC-1, Syne homology (KASH) domain proteins (LINC complex) that function to connect nuclei to the cytoskeleton. We focus on how these proteins and various mechanisms move nuclei during vertebrate development, including processes related to wound healing of fibroblasts, fertilization, developing myotubes and the developing central nervous system. We also describe how nuclear migration is involved in cells that migrate through constricted spaces. On the basis of these findings, it is becoming increasingly clear that defects in nuclear positioning are associated with human diseases, syndromes and disorders.

KEY WORDS: LINC complex, Development, Nuclear envelope, Nuclear migration

Introduction – nuclear positioning in model organisms
Nuclear migration and positioning are essential to a wide variety of developmental and cellular processes. Our current understanding of the molecular mechanisms of nuclear positioning originates from genetic screens in model organisms. The first screens for nuclear positioning defects were carried out to identify the mutant classes nuclear distribution (nud) and ropy (ro) in the filamentous fungi Aspergillus nidulans and Neurospora crassa, respectively. Mostly, these mutants were shown to possess a disruption of various subunits of cytoplasmic dynein or its regulators, including NudE (NDE1 and NDEL1 in humans) and NudF (LIS1 or PAFAH1B1 in humans) (reviewed in Morris, 2000). In the budding yeast Saccharomyces cerevisiae, nuclei must migrate to the bud neck prior to cell division. Forward genetics and imaging in budding yeast identified that dynein is recruited and anchored to the cortex of the bud tip. From the bud tip, dynein pulls on microtubules to drag the nucleus into the bud neck (reviewed in Moore et al., 2009).

The early studies in fungi set the stage for understanding nuclear positioning in multicellular eukaryotes. Genetic screens in Drosophila melanogaster eye discs and Caenorhabditis elegans embryonic hypodermal precursors led to the discovery of SUN- and KASH-domain proteins, which form LINC (linker of nucleoskeleton and cytoskeleton) complexes across the nuclear envelope (see Box 1) (Starr and Fridolfsson, 2010). The role of LINC complexes in moving nuclei is conserved across eukaryotes. For example, LINC complexes mediate nuclear migration in Arabidopsis thaliana and/or Physcomitrella patens root hairs, trichomes and pollen tubes (Folkes et al., 1997; Mathur et al., 1999; Miki et al., 2015; Tamura et al., 2013; Zhou et al., 2012, 2015; Zhou and Meier, 2014).

However, many nuclear migration events are mediated through mechanisms that are independent of LINC complexes. For example, in Drosophila oocytes, the oocyte nucleus is pushed from behind by the centrosome and growing microtubules (Zhao et al., 2012). Likewise, male pronuclei are pushed away from the cortex by a growing microtubule aster (Reinsch and Gonzalez, 1998).

A main take-home message of this Commentary is that a wide variety of molecular mechanisms exist to move nuclei. Moreover, more mechanisms are likely to await discovery. Some of the mechanisms described to date include nuclei that move along microtubules as cargo of kinesins and dynein, nuclei tethered to moving actin filaments, nuclei being pushed or pulled from a distance or passively displaced by other moving cells and, even, nuclei being moved by intracellular pressure. In this Commentary, we focus primarily on mechanisms that move nuclei in several vertebrate developmental contexts. We also discuss the difficulties of nuclear migration through constricted spaces. Given the important developmental processes that depend on nuclear migration, it should come as no surprise that defects in the nuclear migration machinery, especially in SUN- and KASH-domain proteins, have been linked to a variety of diseases (Calvi and Burke, 2015; Cartwright and Karakesisoglou, 2014) (see Table 1 for a brief list and further references). However, it is not yet understood how defects in nuclear migration contribute to disease pathologies. This important area of research will require close collaboration between cell and developmental biologists, engineers and biophysicists, and clinical researchers.

Nuclear migration in polarizing, adherent tissue culture cells
It is challenging to study nuclear migration events within a three-dimensional (3D) in vivo context. It was, therefore, an important breakthrough for the field when a tissue culture model was developed to study nuclear migration. On the edge of a scratched ‘wound’ in an otherwise confluent monolayer, NIH3T3 fibroblasts polarize to place their centrosomes in front of nuclei prior to migration into the wound. Live imaging of fibroblast polarization showed that the centrosome stays in place in the center of the cell while the nucleus actively migrates rearward. This rearward nuclear migration was shown to require actin flow, myosin and Cdc42 (Gomes et al., 2005). Today, this nuclear migration in polarizing fibroblasts is one of the mechanistically best understood nuclear migration events in any system.

Nuclear migration in cultured fibroblasts also helped to uncover a mechanism for nuclear migration that involves transmembrane actin-associated nuclear (TAN) lines (Luxton et al., 2010). TAN lines are found on the apical surface of an adherent, cultured cell and connect to actin cables that orient parallel to the wound edge. The initially identified components of TAN lines are the SUN-domain
protein Sun2 and the KASH-domain protein nesprin-2 (expressed from the SYNE2 locus) (Fig. 1A). Here, Sun2 and the giant isoform of nesprin-2, nesprin-2G physically tether the nucleus to actin cables that undergo retrograde flow, thereby moving the nucleus rearward and behind the centrosome (Luxton et al., 2010). At the nucleoplasmic side of TAN lines, lamin A (Lmna) and emerin (Emd) are required to anchor nuclei to TAN lines but are not involved in TAN line formation. Although mutations in lamin A or emerin result in non-moving nuclei, TAN lines still glide along the nucleoplasmic side of TAN lines, lamin A (Lmna) and emerin (Emd) are required to anchor nuclei to TAN lines but are not involved in TAN line formation. Meanwhile, the nucleoplasmic domains of SUN-domain-containing proteins (yellow) interact with structural components in the nucleoplasm, including lamins, to dissipate forces in the nucleoskeleton (Bone et al., 2014; Haque et al., 2016). Besides their role in nuclear positioning discussed here, LINC complexes also function during homolog pairing in meiosis (Chakravarti et al., 2006; Ding et al., 2007; Sato et al., 2009), DNA damage repair (Lei et al., 2012; Lottersberger et al., 2015; Swartz et al., 2014) and mechanotransduction (Chamblis et al., 2013; Lombardi et al., 2011). Thus, LINC complexes are required for a variety of cell and developmental functions, such as those described here. Given these functions, it is not surprising that mutations in SUN- and KASH-domain proteins have been linked to a number of human diseases, syndromes and disorders (Table 1).

Table 1. Diseases, syndromes and disorders associated with mutations in LINC complex components

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disease, syndrome, disorder</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nesprin-2 and Sun1</td>
<td>Emery-Dreifuss muscular dystrophy</td>
<td>Li et al., 2014; Puckelwitz et al., 2009; Zhang et al., 2007a</td>
</tr>
<tr>
<td>Nesprin-1/2</td>
<td>Cardiomyopathies</td>
<td>Banerjee et al., 2014</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Myogenic autosomal recessive arthrogryposis</td>
<td>Attali et al., 2009</td>
</tr>
<tr>
<td>Nesprin-2</td>
<td>Myopathies</td>
<td>Duong et al., 2014</td>
</tr>
<tr>
<td>Nesprin-2</td>
<td>Meckel-Gruber syndrome</td>
<td>Dawe et al., 2009</td>
</tr>
<tr>
<td>Nesprin-4</td>
<td>High-frequency hearing loss</td>
<td>Horn et al., 2013a</td>
</tr>
<tr>
<td>Nesprin-2 and Sun1/2</td>
<td>Visual impairment</td>
<td>Yu et al., 2011</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Autosomal recessive cerebellar ataxia</td>
<td>Gros-Louis et al., 2007; Noreau et al., 2013; Razafsky and Hodzic, 2015</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Bi-polar disorder</td>
<td>Green et al., 2013; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Recurrent major depression</td>
<td>Green et al., 2013; O’Roak et al., 2011; Yu et al., 2013</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Autism</td>
<td>Green et al., 2013; O’Roak et al., 2011; Yu et al., 2013</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Sterility</td>
<td>Ding et al., 2007</td>
</tr>
<tr>
<td>Nesprin-2</td>
<td>Ovarian cancer</td>
<td>Doherty et al., 2010</td>
</tr>
<tr>
<td>Nesprin-2</td>
<td>Breast cancer</td>
<td>Matsumoto et al., 2015; Sjöblom et al., 2006</td>
</tr>
<tr>
<td>Nesprin-1</td>
<td>Colorectal cancer</td>
<td>Sjöblom et al., 2006</td>
</tr>
<tr>
<td>Nesprin-2</td>
<td>Gastrointestinal stromal tumors</td>
<td>Schoppmann et al., 2013</td>
</tr>
<tr>
<td>Sun2</td>
<td>Lung cancer</td>
<td>Lv et al., 2015</td>
</tr>
</tbody>
</table>

Table 1. Diseases, syndromes and disorders associated with mutations in LINC complex components

Known as TMEM201 is an additional component of TAN lines and functions, in part, to connect Sun2 to lamin A (Borrego-Pinto et al., 2012). On the cytoplasmic side of TAN lines, the formin FHOD1 plays a structural role. FHOD1 is not necessary for the formation of the actin cables or their retrograde movement but, instead, acts as a second connection between nesprin-2G and actin thus reinforcing the coupling of the nucleus to actin cables (Kutscheidt et al., 2014). On the basis of these data, we have a solid understanding of the molecular mechanism and players, including actin cables and lamins, that connect nuclei to retrograde flow. However, although the importance of TAN lines is quite clear in 3T3 fibroblasts and cultured myoblasts (Chang et al., 2015a; Gundersen and Worman, 2013), their broader in vivo significance remains to be demonstrated. We expect that recent developments in live microscopy, especially lattice light-sheet microscopy (Chen et al., 2014), will enable researchers to test the extent to which TAN lines function or exist in vivo.

Additional insights into the function of nuclear positioning have recently been obtained from studying cultured cells in a 3D-matrix. Fibroblasts can migrate through 3D-matrices in multiple ways (recently reviewed in Petrie and Yamada, 2015), one of which is by using blunt, cylindrical protrusions called lobopodia that are dependent on Rho, but not Rac or Cdc42 (Petrie et al., 2012). Lobopodia migration relies on the existence of a cellular pressure gradient. Forward movement of the nucleus creates high pressure in front of the nucleus and a low-pressure region in the rear of the cell (Petrie et al., 2014). Actomyosin contractions and a RhoA–ROCK–myosin II pathway move nuclei forward. To maintain the pressure gradient, nuclei are attached to the cytoskeleton through the...
Pronuclear migration in the newly fertilized zygote

A striking example of nuclear migration is when male and female pronuclei must find one another in a newly fertilized zygote. In numerous organisms, from C. elegans to rhesus monkeys, dynein is recruited to the nuclear envelope of prounuclei to mediate their movement. The molecular mechanisms of pronuclear migration are particularly well-characterized in C. elegans. Here, the KASH-domain protein ZYG-12 and the divergent SUN-domain protein SUN-1 (Matefin) are required for centrosome attachment to male pronuclei, and pronuclear migration (Fig. 1B) (Malone et al., 2003; Minn et al., 2009; Zuela and Gruenbaum, 2016). ZYG-12 binds to the dynein light intermediate chain and so recruits dynein to the surface of both male and female pronuclei (Malone et al., 2003). SUN-12 and SUN-1 are required to keep the centrosome attached to the male pronucleus. Centrosome capture is also dependent on the surface area of pronuclei. Mutants with abnormally small pronuclei lack sufficient surface area to allow dynein to interact with both centrosomes and, therefore, often have a detached centrosome, resulting in pronuclear migration defects (Meyerzon et al., 2009b). Once the centrosome is attached to the male pronucleus, the female pronucleus captures a microtubule from the aster that is associated with the male pronucleus before dynein on the female pronucleus pulls the two pronuclei together (Fig. 1B) (Malone et al., 2003; Rose and Gonczy, 2014).

In zebrafish, pronuclear migration uses a similar mechanism. The KASH-domain protein lymphoid-restricted membrane protein (Lrmp) localizes to the nuclear envelope and centrosomes, and is required for correct pronuclear migration – probably through dynein (Lindeman and Pelegri, 2012). Thus, we speculate that C. elegans ZYG-12 and zebrafish Lrmp are functionally conserved in order to mediate pronuclear migration.

Although model organisms have provided great insight into pronuclear migration, there are fewer mechanistic studies of migration of mammalian pronuclei. This is probably due to the fact that rodents, which serve as the traditional mammalian model, are unique in that they use actin instead of microtubules to position nuclei in oocytes (Almonacid et al., 2015; Schatten, 1994). To better understand pronuclear migration in humans, studies have been conducted with fertilized bovine and rhesus monkey oocytes, in which dynamic microtubules are essential for pronuclear migration (reviewed in Schatten, 1982). Dynein and dyakin both function at the periphery of pronuclei to move the female pronucleus towards the male pronucleus (Payne et al., 2003). The mechanism for the recruitment of dynein to the surface of pronuclei is unknown but, given the role for Lrmp in zebrafish pronuclear migration and the fact that KASH5 (also known as CCDC155) is known to recruit dynein to the nuclear envelope of mouse meiotic cells (Horn et al., 2013b; Morimoto et al., 2012), we hypothesize that KASH5 mediates pronuclear migration in non-rodent mammals. If this is indeed, the mechanism for the recruitment of dynein to pronuclei through KASH5, Lrmp and/or ZYG-12 to mediate pronuclear migration is likely to be conserved from nematodes to fish to mammals – but not in rodents.

Nuclear movements and muscle development

The examples above focus on mechanisms of nuclear positioning in cells with a single nucleus (or two in the case of a fertilized embryo). It is a different challenge to position nuclei in a large syncytium with several or even hundreds of nuclei in a single cell. Here, we examine nuclear positioning in the development of mammalian skeletal muscle as an example. Throughout mammalian muscle development, nuclei undergo at least five mechanistically and temporally distinct nuclear movements, making them an excellent system to study various mechanisms of nuclear migration in syncytia (Cadot et al., 2015). In mammalian skeletal muscle,
hundreds of mononucleated myoblasts fuse together to create a giant syncytium that matures into a functional myofiber (Folker and Baylies, 2013). Normally, nuclei are evenly spaced along the length of the syncytium at the periphery of the myofiber to maximize the distance between nuclei (Bruusgaard et al., 2003). Moreover, a group of specialized nuclei localize to and anchor under the synapse at the single neuromuscular junction (Grady et al., 2005; Sanes and Lichtman, 2001). As in other examples of nuclear migration, some nuclear movements during muscle development are dependent on SUN- and KASH-domain proteins, whereas others are not. Further supporting the importance of nuclear positioning in muscle, nuclei are often mis-positioned to the center of the myofiber in wounded and diseased muscles, including in central myopathies (Romero, 2010).

The first nuclear migration event occurs prior to myoblast fusion when migrating cultured myoblasts polarize centrosomes in front of nuclei (Fig. 2A). This process requires actin, myosin, SUN-2 and nesprin-2G to form TAN lines in order to move the nucleus behind the centrosome (Chang et al., 2015a). Thus, myoblasts are polarized in a manner that is analogous to fibroblasts during wound healing.

Then, just after a myoblast fuses with the end of a developing myotube, the nucleus rapidly moves to the center of the myotube in a microtubule-dependent manner (Fig. 2B). This nuclear migration requires Cdc42 and its downstream effectors Par3 and Par6 (also known as Pard3 and Pard6, respectively), within a protein complex that has previously been implicated in spindle positioning and cell polarity (Chen and Zhang, 2013). Par6 functions at the surface of muscle nuclei to recruit dynactin and dynein to nuclei (inset in Fig. 2B). Dynein then pulls the newly added nucleus towards the middle of the myotube. In this model, microtubules are nucleated all around the surface of myotube nuclei with their plus ends extending towards the distal regions of the developing myotube (Fig. 2B) (Cadot et al., 2012). Defects in the function of the factors in this pathway lead to a decreased speed of nuclear migration (Cadot et al., 2012), which is likely to disrupt muscle development in vivo.

After myoblast fusion and before nuclei move to the periphery of the myotube, a third nuclear migration event occurs, the spreading of syncytial nuclei (Bruusgaard et al., 2006). Nuclear spreading in the developing myotube is microtubule dependent, and both dynein and kinesin-1 motors are required (Fig. 2C) (Folker et al., 2012; Metzger et al., 2012; Wilson and Holzbaur, 2012, 2015). Here, the microtubule-binding protein known as Enscosin in Drosophila (microtubule-associated protein 7, MAP7, in mammals) binds to the tail of kinesin-1, which then walks towards the plus end of a second microtubule. Because microtubules are nucleated at the surface of nuclei in myotubes, the Enscosin/MAP7–kinesin-1 complex crosslinks microtubules and slides them apart (inset in Fig. 2C) (Metzger et al., 2012). Two independent dynein pathways function in Drosophila muscle to space nuclei (Folker et al., 2012). In the first, cytoplasmic linker protein 190 (CLIP-190), dynactin and dynein connect microtubules to the cortex and so pull nuclei away from each another. The second pathway involves Lis1 and is necessary for localization of dynein to the poles of muscle cells to establish proper muscle length. Impairment of either pathway results in locomotion defects, demonstrating the importance of nuclear positioning and appropriate muscle length in muscle function (Folker et al., 2012). In addition to its role in mediating microtubule sliding, kinesin-1 also functions on the surface of nuclei. Kinesin-1 is recruited to mammalian myotube nuclei through nesprin-2 or nesprin-1 (encoded by the SYNE1 locus). LEWD amino acid motifs in the nesprins directly interact with the kinesin light chain to target kinesin-1 to the nuclear surface (inset in Fig. 2C) (Wilson and Holzbaur, 2015). From its position at the nuclear envelope, kinesin-1 is then thought to walk towards the plus ends of microtubules, thereby helping to maximize the distance between nuclei (Wilson and Holzbaur, 2015).

The fourth nuclear migration event in developing myotubes is towards the periphery of the syncytium, similar to how nuclei move in myofibers that recover from injury (Falcone et al., 2014; Romero, 2010) (Fig. 2D). This nuclear movement is dependent on the correct spreading of the nuclei in the previous step, as well as a complex formed between the F-BAR protein amphiphysin-2 and the actin-nucleator neuronal Wiskott–Aldrich Syndrome protein (N-WASP) (Falcone et al., 2014). Furthermore, amphiphysin-2 is recruited to the surface of nuclei through an interaction with nesprin-2 (D’Alessandro et al., 2015). Nesprins are required for the timely nuclear movement to the periphery of myotubes (Falcone et al., 2014; Zhang et al., 2007a). Because N-WASP and amphiphysin-2 are also required to form normal triads, i.e. structures in which T-tubules are associated with the sarcoplasmic reticulum, it has been difficult to determine whether nuclear migration to the periphery directly depends on actin or only requires the correct formation of triads. However, because nesprins directly interact with amphiphysin-2 in other tissues, it is reasonable to propose that a complex between nesprin and amphiphysin-2 is responsible for nuclear movement to the periphery of myotubes (Fig. 2D). It remains to be determined how amphiphysin-2 and N-WASP regulate a local actin network that can generate the mechanical forces to move the nucleus.

The final nuclear migration event involves the localization of a subset of myonuclei to immediately underneath the neuromuscular junction (NMJ) (Fig. 2E) (Sanes and Lichtman, 2001), whereas the remaining nuclei move to the myofiber surface so they are closely associated with blood vessels (Ralston et al., 2006). LINC components are required for localization of myonuclei under the NMJ (Grady et al., 2005; Lei et al., 2009; Zhang et al., 2007a), as is lamina A (Méjat et al., 2009). However, it is unknown whether LINC complexes or lamins are required for initial movements or – more likely – whether they anchor nuclei after their arrival at the NMJ. It has been suggested that LINC complexes function redundantly with intermediate filaments to anchor nuclei in mature myofibers. For instance, in mice, knockouts of Sun1 and Sun2, nesprin-1 and nesprin-2, or the main muscle intermediate filament desmin lead to defects in nuclear anchorage (Fig. 2F) (Chapman et al., 2014; Lei et al., 2009; Ralston et al., 2006; Zhang et al., 2007a).

Many questions remain about the molecular mechanisms for how nuclei migrate in various steps of mammalian skeletal muscle development. First, do TAN lines function in vivo during myoblast migration? Second, how is the function of MAP7 in sliding microtubules apart coordinated with the nucleus becoming a kinesin and dynein cargo to position nuclei throughout the length of the developing myotube (Fig. 2C) (Folker et al., 2012, 2014; Metzger et al., 2012; Wilson and Holzbaur, 2012, 2015)? Third, how do LINC complexes and amphiphysin-2 connect nuclei to actin networks to mediate nuclear migration to the periphery? Finally, because some NMJs that completely lack associated myonuclei appear normal (Grady et al., 2005), what is the relevance of myonuclei aggregating under the NMJ?

The most crucial, remaining questions in the field focus on how defects in nuclear positioning in muscles contribute to diseases. Mutations in several SUN-, KASH-domain and lamin proteins have been correlated with muscular dystrophies (see Table 1) (Meinke et al., 2014; Puckelwartz et al., 2009; Tapley and Starr, 2013; Zhang et al., 2007a). Furthermore, presence of SUN-domain proteins...
enhance dystrophies that are associated with mutations of lamin A, because Sun1-knockout and lamin A double-mutant mice have less severe pathologies than those carrying lamin A single mutants (Chen et al., 2012; Meinke et al., 2014; Starr, 2012). In another example, knockdown of nesprin-2 in migrating myoblasts leads to a partial defect in the fusion of developing myotubes (Chang et al.,...
Actomyosin contracts in a zone (orange) behind the nucleus to push the nucleus to the apical surface of the epithelium just prior to mitosis. Apical movement during G2 phase; dynein (purple) is recruited to the nuclear surface at nuclear pores (light blue) to move nuclei on microtubules (green). Actomyosin actively move towards the apical side, they passively push G1-phase nuclei out of the way, resulting in their migration towards the basal surface. (B) Active apical movement during G2 phase; dynein (purple) is recruited to the nuclear surface at nuclear pores (light blue) to move nuclei on microtubules (green). Actomyosin contracts in a zone (orange) behind the nucleus to push the nucleus to the apical surface of the epithelium just prior to mitosis.
been described in the literature. For example, many basal nuclear migrations during G1/S phase are passive, cell non-autonomous movements, during which G1/S nuclei are forced basally when G2 nuclei of neighboring cells actively migrate apically and, therefore, push G1/S nuclei out of the apical regions (Fig. 3A) (Kosodo et al., 2011). LINC-independent mechanisms that underlie apical movements during G2 are better understood. Dynein, which is required for the rapid apical movement of nuclei within the mouse neocortex (Hu et al., 2013), is recruited to the surface of nuclei through interactions with nuclear pore components (Fig. 3B) (Bolley et al., 2011; Splinter et al., 2010). Dynein is specifically recruited during G2, just in time for the apical aspect of interkinetic nuclear migration (Baffet et al., 2015). Occasionally, a nucleus does not complete nuclear migration to the apical surface before the onset of mitosis. In this case, centrosomes are pulled away from the apical surface towards the nucleus to initiate mitosis (Spear and Erickson, 2012a; Strzyz et al., 2015). Shortly thereafter, before mitosis is completed, actomyosin contraction is required to complete the apical migration of the nucleus (Norden et al., 2009; Spear and Erickson, 2012a) (Fig. 3B). In summary, a variety of microtubule- and actin-based mechanisms function together to ensure interkinetic nuclear migration and development of pseudostratified epithelia. Future studies will focus on understanding the differences of the mechanisms in different models. What works for one interkinetic nuclear migration might not be sufficient for a different event in larger epithelia.

Nuclear positioning also plays a role in columnar epithelia. Polarized intestinal epithelial cells contain highly organized and polarized microtubules, essential for the correct positioning of nuclei and other organelles within the cell (Toya et al., 2016). A better-characterized nuclear migration event in columnar epithelia is that of hair cells in the inner ear. In these hair cells, nuclei migrate to a basal position within the cell in a kinesin-1-dependent mechanism. Nesprin-4 (encoded by the SYNE4 locus) and its partners (Sun1 or Sun2) recruit kinesin-1 to the surface of nuclei (Roux et al., 2009). Knockouts of either nesprin-4 or Sun1 in mice result in misposition of nuclei in inner hair cells, degeneration of outer hair cell, and hearing loss of high frequencies (Horn et al., 2013a). However, further studies are required to elucidate how exactly nuclear mispositioning contributes to the hearing loss. Interestingly, in certain Iraqi-Jewish families, mutations in the KASH-domain protein nesprin-4 are also associated with hearing loss at high frequencies (Horn et al., 2013a). Thus, mispositioning of nuclei in epithelia is likely to contribute to a variety of conditions in humans.

**Nuclear movements through constricted spaces**

In the examples above, nuclei remain relatively rounded throughout migration, suggesting that only minimal mechanical strains exist to deform nuclei during movements. In contrast, many cells migrate through narrow openings between other cells or in the extracellular matrix (ECM), thereby experiencing extensive external mechanical forces. For example, hematopoietic cells must migrate through a basement membrane to exit the bone marrow and enter capillaries (Junt et al., 2007; Shin et al., 2013). The nucleus is, typically, the largest organelle of a cell and is five to ten times stiffer than the surrounding cytoplasm (Dahl et al., 2004; Friedl et al., 2011). Therefore, nuclear deformability limits the ability of cells to migrate through constricted spaces (Fu et al., 2012; Wolf et al., 2013). In fact, pathologists have indirectly appreciated this for decades by using lobulated nuclei, which are indicative of a flexible and softer nucleus, as an indication for cancer cells (Chow et al., 2012).

The relative expression levels between different nuclear lamins have been shown to regulate nuclear stiffness and its deformability in a variety of cells, including neutrophils (Rowat et al., 2013), metastatic cells (Fu et al., 2012; Harada et al., 2014; Wolf et al., 2013), mouse embryonic fibroblasts (MEFs) (Davidson et al., 2014) and hematopoietic cells (Shin et al., 2013). Indeed, overexpression of lamin A increases the transit time through constrictions, whereas lamin A knockout cells migrate faster (Davidson et al., 2014; Rowat et al., 2013) (Fig. 4). Thus, lamin A plays a crucial role in increasing the stiffness and in reducing the deformability of migrating nuclei. Similarly, in bone marrow exit assays, cells with high ratios of lamin A to lamin B are retained in the bone marrow, suggesting a reduced ability of the nucleus to deform (Shin et al., 2013). Furthermore, an excess of lamin A has also been shown to impede matrix-metalloproteinase-independent migration, for which nuclear deformability is the main determinant in migration through the ECM (Wolf et al., 2013). These results are consistent with previous findings that levels of lamin A positively correlate with nuclear stiffness, as demonstrated by using micropipette aspiration (Dahl et al., 2006) or resistance to mechanical strain (Broers et al., 2004; Lammerding et al., 2006, 2004). In general, increasing the amount of lamin A makes the nucleus stiffer and less deformable, thereby resulting in slower migration of a cell through a constricted space.

In addition to what has been learned about the role of lamin A, future experiments following single cells migrating through constrictions are likely to yield additional mechanistic insights into nuclear migration. Recent data point to at least three areas that warrant additional studies. The first is the role of chromatin compaction during nuclear constriction. Condensed heterochromatin has been implicated in migration through constricted spaces, because blocking the formation of heterochromatin in breast cancer cells results in reduced migration (Fu et al., 2012). The second is the poorly understood aspect by how actin filaments in the nucleoplasm regulate nuclear stiffness. The small GTPase Rac1 has recently been implicated in nuclear stiffness because its enrichment in COS cell nuclei induced the formation of actin filament formation, resulting in nuclear deformations (Navarro-Lérida et al., 2015). The third one relates to events that occur in the cytoplasm at the rear of the nucleus such as actomyosin-based contraction, similar to those that take place during interkinetic nuclear migration. Non-muscle myosin IIB is...
crucial for generating forces that allow nuclei to squeeze through a narrow constricted space (Thomas et al., 2015).

As cell biologists and engineers more broadly adapt and develop assays to image cell and nuclear migrations through narrow spaces, we expect additional factors involved in nuclear deformability, stiffness and shape changes to be identified and characterized. Although substantial insights have been made in the past five years by imaging individual cells migrating through constrictions, much less is understood about how groups of cells migrate collectively. Researchers are likely to need to utilize in vivo assays to study collective migrations, such as those developed for studying Drosophila border cells or zebrafish lateral line cells (Friedl and Gilmour, 2009). Of particular interest are future studies on how nuclear stiffness is related to metastasis of individual or groups of cancer cells, which then have the potential to lead to the development of cancer treatments.

Conclusions and future directions
In the past 15 years, substantial progress in describing and understanding the molecular mechanisms for the movement of nuclei has been made. The diversity of molecular mechanisms that mediate different nuclear migration events has been particularly surprising. Studies that used model organisms have identified the LINC complex, at the core of which is the interaction between KASH- and SUN-domain proteins that span the nuclear envelope. In order to move nuclei, LINC complexes connect them to microtubule motors or a dynamic actin network. However, there are a number of nuclear migration events that do not depend on LINC complexes. Alternative mechanisms include utilization of microtubules to push a nucleus, dynein recruitment to the nuclear envelope by the nuclear pore, and actomyosin contractions at the rear of the nucleus, among others (Bolly et al., 2011; Norden et al., 2009; Splinter et al., 2010; Zhao et al., 2012). The crucial nature of several of these nuclear migration events in development, and the small number of phenotypes observed upon mutation of LINC and other known nuclear migration components suggests roles for partially redundant pathways that are yet to be identified.

Despite the recent progress, a number of questions remain with regard to nuclear migration in a variety of developing mammalian tissues. For instance, the differences in interkinetic nuclear migration observed for different pseudostratified epithelia warrant further investigation. Likewise, the consequences of mispositioning nuclei during muscle function remain poorly understood. Moving back to the initial stages of development, even the mechanisms of pronuclear migration in humans remain poorly explored, especially, because in this case rodents are not useful models because they lack microtubule involvement for pronuclear migration (reviewed in Schatten, 1994). Furthermore, many details of the better-understood mechanisms of nuclear migration remain unknown. For example, some KASH-domain proteins recruit both dynein and kinesin to the surface of the nucleus (Fridolfsson and Starr, 2010), but it remains an open question how a single cargo regulates the switch between different molecular motors (Hancock, 2014).

It is likely that multiple mechanisms remain to be identified for moving nuclei. Recent studies in traditional models, such as Drosophila oocytes or cultured fibroblasts, have led to the discovery of entirely unexpected mechanisms for nuclear movements, including one, in which the nucleus is used as a piston to propel cells through 3D matrices, and another in which microtubules and centrosomes are pushing a nucleus from behind (Petrie et al., 2014; Zhao et al., 2012). Moreover, dramatic nuclear migrations take place in many ciliates and their mechanisms are almost completely unexplored (Mikami, 2000). Thus, additional, new models for nuclear migration need to be developed and studied.

Finally, more research is required to better understand how nuclear migration is related to human disease. Nuclear migration is often the rate-limiting step of metastasizing cells (Wolf et al., 2007), but how metastatic cell nuclei remodel to squeeze into narrow openings is poorly understood. Many exciting recent findings in this field come from studies of nuclei squeezing through constricted spaces in a variety of in vitro contexts. Since the in vitro nature of these studies is limiting it is essential to study nuclear squeezing events in vivo. One possible model is nuclear migration in C. elegans larval P-cells, in which the nucleus migrates from lateral to ventral through a constricted space of about 200 nm between the muscle and the cuticle (Chang et al., 2013b; Sulston, 1976). Such future in vivo studies are likely to expand our understanding of disease. Mutations in SUN- or KASH-domain proteins have been linked to a wide variety of human diseases, syndromes and disorders (Table 1). However, the underlying relationships between nuclear positioning and disease pathologies are almost completely unknown. In the near future, we anticipate that many of the molecular details on how nuclear migration contributes to human diseases will be uncovered. Such findings are expected to lead to translational studies for the treatment of associated diseases.

Acknowledgements
We thank members of D.A.S.’s lab for insightful discussions and the anonymous referees for their helpful comments.

Competing interests
The authors declare no competing or financial interests.

Funding
Research in the lab of D.A.S. is supported by the National Institutes of Health [grant numbers: R01 GM073874 to D.A.S. and T32 GM007377 to C.R.B.]. Deposited in PMC for release after 12 months.

References


