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REVIEW

Nuclear movement in multinucleated cells

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ABSTRACT

Nuclear movement is crucial for the development of many cell types and organisms. Nuclear movement is highly conserved, indicating its necessity for cellular function and development. In addition to mononucleated cells, there are several examples of cells in which multiple nuclei exist within a shared cytoplasm. These multinucleated cells and syncytia have important functions for development and homeostasis. Here, we review a subset of the developmental contexts in which the regulation of the movement and positioning of multiple nuclei are well understood, including pronuclear migration, the *Drosophila* syncytial blastoderm, the *Caenorhabditis elegans* hypodermis, skeletal muscle and filamentous fungi. We apply the principles learned from these models to other systems.

KEY WORDS: LINC complex, Cytoskeleton, Nuclear movement, Syncytia

Introduction

Nuclei dynamically traversing the dense cytoplasm is a visually engaging phenomenon and is conserved throughout the eukaryotic lineage. The conservation alone indicates the necessity of moving this large organelle, but the positioning of nuclei is crucial for the development and function of disparate cell types (Gundersen and Worman, 2013). Functionally, the position of the nucleus contributes to cellular mechanics (Stewart-Hutchinson et al., 2008), gene regulation (Kim et al., 2020; Petrany et al., 2020 preprint), the relative organization of cells within tissues (Miyata, 2015; Spear and Erickson, 2012; Kracklauer et al., 2007) and the segregation of the genome (Foe and Alberts, 1983; Varshney and Sanyal, 2019). As striking and functionally important as the movement of a single nucleus is, many cells are multinucleated, such as the early zygote before the first cell division (Siu et al., 2021), syncytial trophoblasts (Huppertz, 2008), the syncytial blastoderm that is characteristic of insect development (Johannsen, 1941), and both skeletal and cardiac muscle (Abmayr and Pavlath, 2012). Given that the position of a single nucleus can impact the behavior of a cell it is easy to envision that the movement of several nuclei relative to one another, and their ultimate spacing within a common cytoplasm, dramatically impact cell function and development. As in their mononucleated counterparts, there are many variations on similar themes that drive nuclear movement in multinucleated cells. Generally, the forces that move nuclei in multinucleated cells and mononucleated cells are provided by the actin and microtubule cytoskeletons. Similarly, in both mononucleated and multinucleated cells, the association of the nucleus with the cytoskeleton is facilitated by the linker of nucleoskeleton and cytoskeleton (LINC) complex (Bone and Starr, 2016; Cain et al., 2018). Forces are then applied to the nuclei by motor proteins that are either anchored at the cell membrane (Folker et al., 2012; Kotak et al., 2012), attached to other membrane-bound organelles (Cadot et al., 2012) or moving throughout the cytoplasm (Shinar et al., 2011). However, the presence of multiple nuclei adds a layer of complexity to nuclear movement. In addition to the interactions described above, each nucleus can also interact with the other nuclei (Collins and Mandigo et al., 2017), thus increasing the number of interactions that must be regulated in a properly organized multinucleated cell. The molecular mechanisms that facilitate these nuclear–nuclear interactions are not clear, but they likely involve cytoskeletal elements that are also used to move nuclei.

In the broadest terms, nuclear migration in multinucleated cells is driven by either cytoplasmic mixing or the direct application of force to individual nuclei (Box 1). In both cases, the forces that move nuclei are provided by the actin and/or the microtubule cytoskeleton. The defining characteristic of the cytoplasmic-mixing mechanisms is that the entire cytoplasm is moved and redistributed as a consequence of actin-myosin contractility (Deneke et al., 2019) or microtubule sliding (Lu et al., 2016). During cytoplasmic mixing, nuclei are not moved in isolation from other cellular components but are instead just one of the many components that are periodically redistributed. Thus, there is not a molecularly regulated link between the nucleus and the cytoskeleton. Whereas cytoplasmic-mixing mechanisms treat the nuclei similarly to other cellular components, the direct application of force typically proceeds through the LINC complex and is regulated. The LINC complex is formally composed of Sad1p, Unc84 (SUN)-domain proteins and Klarsicht, Anc-1, Syne Homology (KASH)-domain proteins. SUN-domain proteins span the inner nuclear membrane and interact with the nuclear lamina and chromatin within the nucleus, and with the KASH-domain proteins in the lumen of the nuclear envelope (Kracklauer et al., 2007; Lee et al., 2002). The KASH-domain proteins span the outer nuclear membrane and interact with the cytoskeleton in the cytoplasm (Starr and Han, 2002; Starr et al., 2001). Therefore, the LINC complex links the mechanical regulators of the nucleus and the cytoplasm, such that forces can be applied across the nuclear envelope. There are significant variations on this theme across cell types that are not the focus of this Review, but have been described well in previous reviews (Cain et al., 2018; Luxton and Starr, 2014). Although collectively grouped as the direct application of force, this force is applied to nuclei by at least four different mechanisms in various multinucleated cells (Box 1).

These broad mechanisms are used in various combinations to drive nuclear movement in multinucleated cells. We highlight multinucleated cells from various species in which there is a significant understanding of how and why nuclei move. Finally, we close with a brief discussion of several multinucleated cells for which nuclear position has been described but not explored from either a functional or mechanistic perspective. We apply the lessons from better-studied multinucleated cells to build hypotheses around these unexplored systems.

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Box 1. Mechanisms of nuclear movement by the direct application of force

Cargo transport
In this mechanism, molecular motors interact with the nuclei via the LINC complex and carry the nucleus along the microtubules or actin filaments in the same way that they carry more traditional membrane-bound cargos. In essence, in the cargo-transport mechanism the LINC complex serves as the adaptor for the nucleus as a cargo (Fridolfsson and Starr, 2010; Meyerzon et al., 2009; Wilson and Holzbaur, 2014).

Microtubule sliding
Here, the force is applied to nuclei by the sliding of antiparallel microtubules. This mechanism is reminiscent of the kinesin-dependent sliding of antiparallel microtubules during the elongation of the mitotic spindle (Vukušić and Tolić, 2021). The LINC complex typically functions to link the microtubule minus-ends to the nuclei in microtubule sliding mechanisms.

Cortical pulling
In cortical pulling, dynein is anchored at a site distant from the nucleus. Because dynein is anchored in place it pulls the microtubule minus-ends and the attached nucleus toward its location (Folker et al., 2012; Grava et al., 2011).

Cytoskeleton-based pushing
Cytoskeleton pushing can occur in one of two manners: microtubules or actin can polymerize from a distant site and bump into the nucleus, thus propelling it away from the polymerization site as they grow (Wang et al., 2015; Zhao et al., 2012); alternatively, the cytoskeleton can polymerize from the nucleus and into the cell cortex, pushing the nucleus away from the cortex as a consequence (Daga et al., 2006; Meaders et al., 2020).

Pronuclear migration

The first complex cellular behavior following fertilization is a form of nuclear migration. During animal development, the first nuclear movement, known as pronuclear migration, occurs before the first cell division and is crucial for the fusion of the male and female pronuclei, proper chromosome segregation and differentiation. Although all pronuclear migrations result in the meeting of the male and female pronuclei, the precise mechanisms driving pronuclear migration vary between organisms. We first discuss pronuclear migration in two historically important model systems, *Caenorhabditis elegans* and sea urchins, and conclude with a discussion of recent work in mouse zygotes that adds a new flavor to this age-old developmental biology question.

C. elegans

Pronuclear migration has been most extensively studied in *C. elegans*. The male pronucleus brings with it a centrosome that nucleates microtubules, forming the sperm aster, immediately after fusion. Microtubules emanate in all directions, with many extending toward the female pronucleus. Cytoplasmic dynein, which is localized to the nuclear envelope of the female pronucleus by a LINC complex-dependent mechanism, binds to these growing microtubules and carries the nucleus toward the male pronucleus (Malone et al., 2003; Minn et al., 2009) – a clear example of the cargo-transport mechanism (Box 1) (Fig. 1A). Crucially, the male pronucleus also moves towards the cell center as a consequence of the viscous drag created by the moving female pronucleus (Kumar et al., 2015; Tawada and Sekimoto, 1991).

Sea urchin

The simple mechanism employed by *C. elegans* also applies to the movement of the pronuclei during sea urchin development. However, whereas *C. elegans* zygotes are ∼50 µm, oblong and polarized, the sea urchin zygote can be up to 200 µm and is spherical. The consequence of this increased size and symmetry is that pronuclear migration proceeds in two distinct phases in sea urchins. The first phase is slow and occurs before the significant expansion of the microtubule sperm aster when there are few, if any, interactions between the male and female pronuclei because the distance between them is greater than the length of the microtubules. Nevertheless, the male pronucleus moves slowly toward the cell center before the pronuclei interact. This first phase of slow movement is driven by a microtubule-pushing mechanism (Box 1). Because microtubules polymerize in all directions, a subset extends toward the cell cortex where the sperm fused and they push against this cell cortex as they continue to grow, moving the aster and the associated male pronucleus toward the cell center (Fig. 1B). In the second phase, as the male pronucleus moves, microtubules continue to polymerize and the size of the microtubule aster increases, enabling an interaction between the male and female pronuclei that results in a dynein-dependent cargo-transport mechanism (Box 1) of nuclear movement similar to that in *C. elegans* (Meaders et al., 2020).

Mouse

Mouse development provides a more complicated solution to pronuclear migration. Following fusion of the sperm and oocyte, an actin-based pushing mechanism (Box 1) propels the male pronucleus away from cortex and toward the cell interior. In this initial movement, actin polymerization stimulated by the activity of Ras-related protein (Rab11a) and Spire, an actin nucleator, assembles actin between the cortical actin network and the male pronucleus (Scheffler et al., 2021). Once the male pronucleus is driven into the cell interior, the male and female pronuclei move together by a microtubule- and dynein-dependent pulling mechanism (Box 1). However, this is a unique form of microtubule-dependent transport because the microtubules are not organized with their minus-ends focused towards the nucleus. Instead, microtubules are nucleated at many sites throughout the cytoplasm and dynein localized to the pronuclei moves randomly along these microtubules of various orientations that are themselves pushing against the cortex of the zygote (Scheffler et al., 2021). Thus, it is a combination of microtubule pushing forces, as seen in large zygotes such as the sea urchin (Meaders et al., 2020), and a cargo-transport mechanism in which the nucleus is carried along microtubules that are not part of a nucleus-associated array.

Together, these three examples show that this first movement of nuclei, which occurs before the first cell division in animal development, is driven by multiple mechanisms that are layered to efficiently move nuclei. Furthermore, there are variations on common themes between organisms such that it is crucial to investigate these movements in many contexts to build a comprehensive understanding of how pronuclei move.

Drosophila syncytial blastoderm

Early development in *Drosophila* and many other insects presents a unique example of a multinucleated cell. After fertilization, pronuclei move toward one another by mechanisms similar to those described in *C. elegans* (Endow and Komma, 1998; Williams et al., 1997). However, after pronuclear fusion, nuclei divide in the absence of cell division resulting in an embryonic blastoderm that contains ∼6000 nuclei before cellularization. Functionally, the spacing of nuclei is crucial for cellularization and the proper differentiation of cell fates (Petkova et al., 2019). A seminal paper in 1983 described these movements in exquisite detail using differential interference contrast microscopy (Foe and Alberts, 1983), determining that the nuclei in

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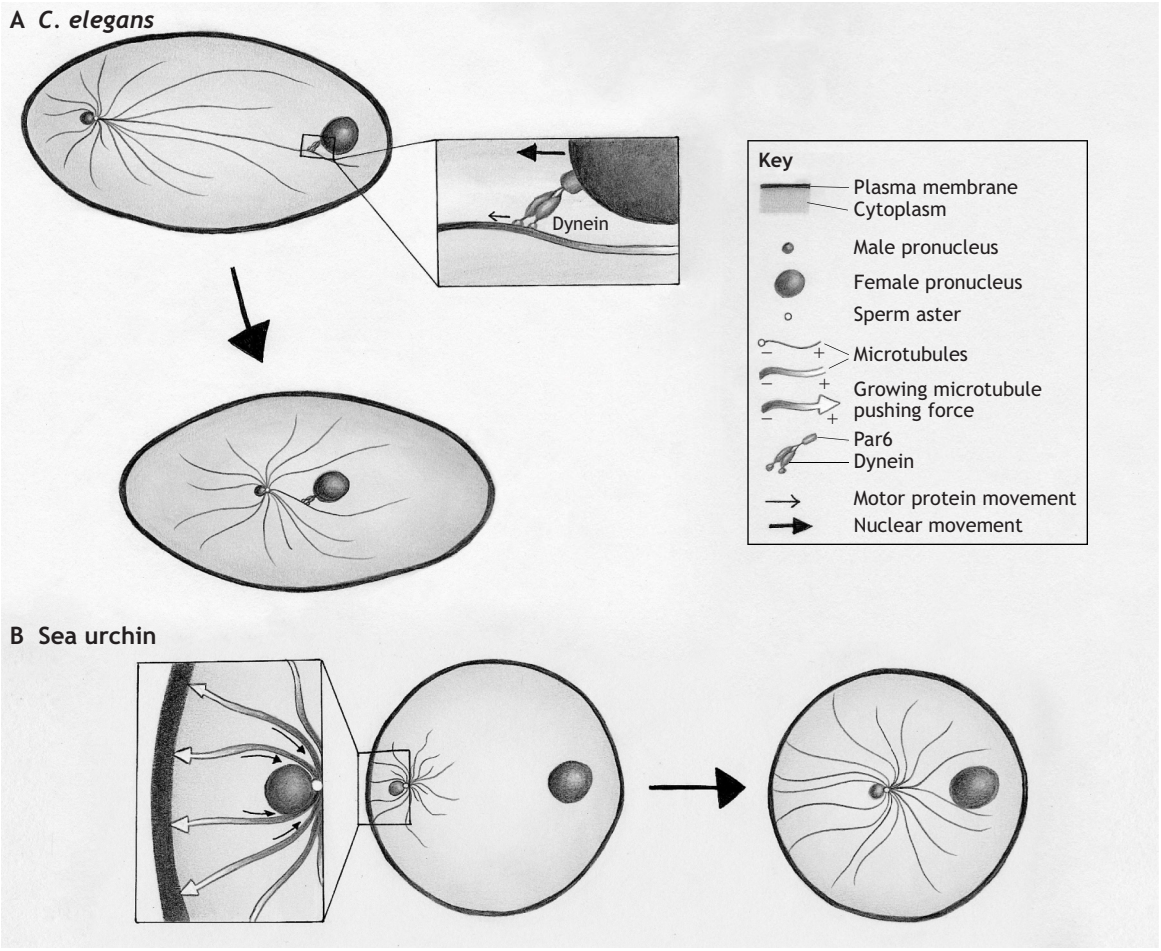

Fig. 1. Pronuclear movement. (A) Cargo-transport mechanism of nuclear movement in *C. elegans*. The female pronucleus is coated with dynein, which interacts with microtubules that extend from the sperm aster that is associated with the male pronucleus. Dynein then travels toward the minus-ends of the microtubules carrying the female pronucleus. (B) Microtubule pushing mechanism in sea urchin. The microtubule aster associated with the male pronucleus grows into and pushes against the cortex (indicated by arrows) of the cell to propel the male pronucleus toward the center, independent of its interactions with the female pronucleus.

this blastoderm undergo at least two mechanistically distinct movements.

Distribution of nuclei during early nuclear division cycles

The first several nuclear division cycles take place within the interior of the developing embryo, with the nuclei repositioning along the anterior-posterior axis after each division cycle (Callaini et al., 1992; von Dassow and Schubiger, 1994; Wheatley et al., 1995). The force to spread the nuclei axially is generated by a cortical actin-myosin network, the contraction of which is linked to the nuclear division cycle. These contractions generate cytoplasmic flow through the center and back toward the anterior and posterior poles of the embryo (Fig. 2). This cytoplasmic flow mixes the cytoplasm and evenly distributes the cellular components, including nuclei, throughout the developing blastoderm and represents a model cytoplasmic-mixing mechanism.

Actin-myosin driven cytoplasmic mixing specifically regulates the spacing when nuclei are few because the density of nuclei is sufficiently low that interactions between neighboring nuclei have a minimal impact on spacing (Foe and Alberts, 1983); however, these movements are still regulated by their linkage to the cell cycle. Specifically, during mitotic exit, the activity of protein phosphatase 1 (PP1) increases and locally inactivates cyclin-dependent kinase 1 (Cdk1) in the region of nuclei. The inactivation of Cdk1 results in actin-myosin contractility, again near the nuclei, to create cytoplasmic flows that spread nuclei along the anterior-posterior axis of the embryo (Deneke et al., 2019) (Fig. 2). This creates a feedback loop in which the position of nuclei, or more precisely the position at which nuclei exit mitosis, dictates the position of myosin contractility, which consequently redistributes the nuclei. Crucially, these movements appear to be LINC complex-independent and are driven purely by cytoplasmic flow and the physics of dispersion (Deneke et al., 2019).

Distribution of nuclei during later nuclear division cycles

During the later cycles of nuclear division, the concentration of nuclei is sufficiently high that nuclei are near, and interact with, their neighbors via the microtubule networks that are associated with each nucleus (Callaini et al., 1992). These microtubule-dependent interactions between adjacent nuclei are crucial to their spacing. A series of interconnected short-range repulsive interactions result in the even spacing of nuclei (and the associated mitotic spindles) throughout the blastoderm and the alignment of spindles relative to their neighbors (de-Carvalho et al., 2022) (Fig. 3). It is worth noting that the spacing of nuclei in this system is not directly dependent on nuclei themselves and is instead established during mitosis. During

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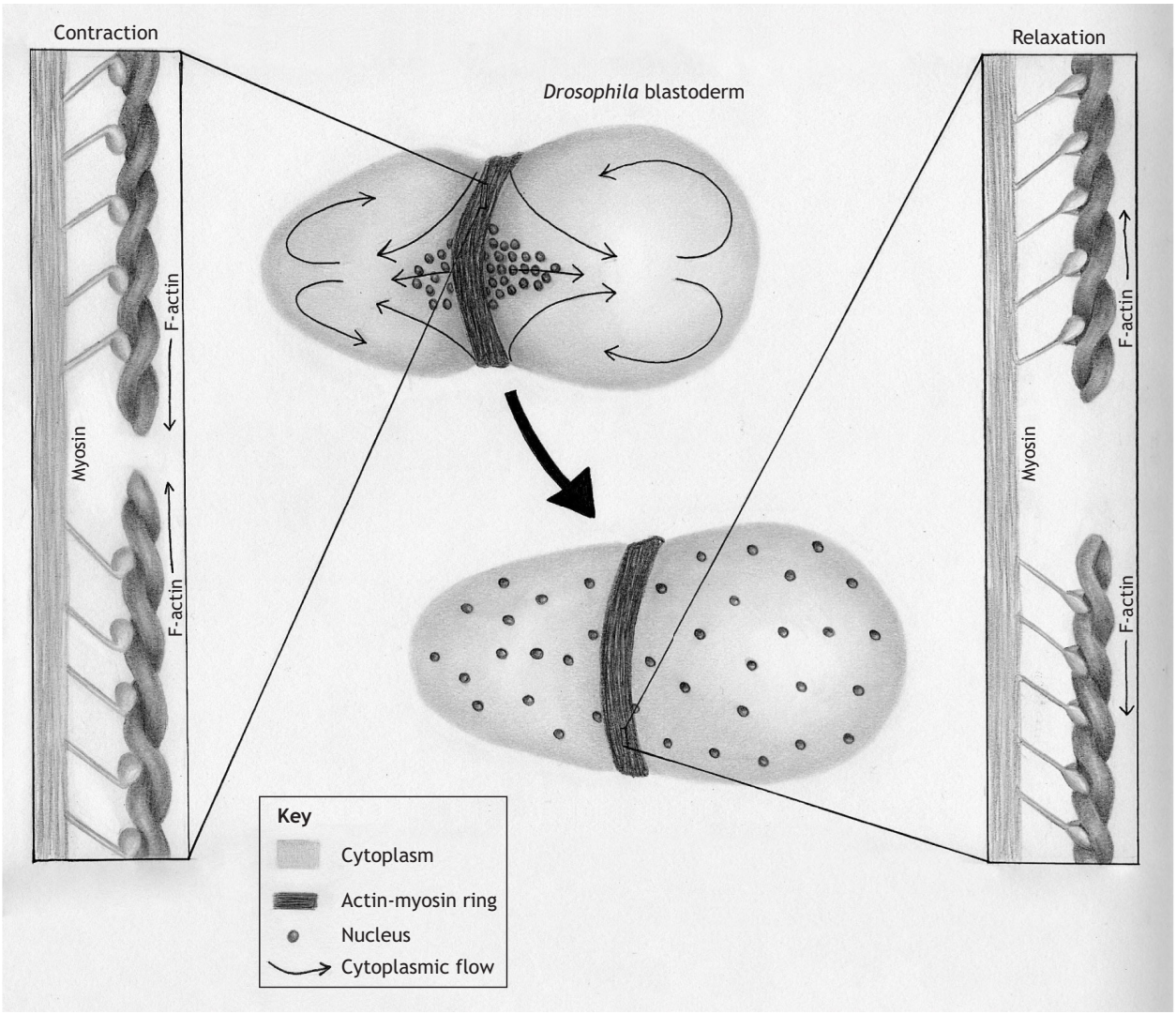

Fig. 2. Cytoplasmic mixing in the early stages of *Drosophila* blastoderm development. An early stage *Drosophila* syncytial blastoderm. A band of actin-myosin contractility near the center of the developing blastoderm creates cytoplasmic mixing that redistributes the nuclei based on dispersion physics. Left inset shows the contraction of the actin-myosin network that provides the force to mix the cytoplasm, whereas the right inset shows the relaxation of the actin-myosin network that correlates with stationary nuclei.

anaphase, the elongating mitotic spindles repel their neighbors to alter their position and maintain even spacing. This spacing requires a balance of forces between spindles across the blastoderm (Kaiser et al., 2018). Although the active movement occurs during mitosis, the removal of a single nucleus by laser ablation causes reorientation of the neighboring nuclei and their movement into the now voided region of the blastoderm, indicating that interactions between nuclei and their associated microtubule networks are not only crucial to the movement of spindles but also to maintain the spacing of nuclei during interphase (de-Carvalho et al., 2022).

Mechanistically, the force to space nuclei in the syncytial blastoderm is provided by sliding of antiparallel microtubules from adjacent spindles (Box 1). In this specific example, the chromokinesin, kinesin-like protein at 3A (Klp3A), in concert with the microtubule crosslinking protein, Falsetto (Feo; also known as protein regulator of cytokinesis 1, PRC1), is localized to the regions of overlapping antiparallel microtubules and provides the force to move spindles apart (Deshpande et al., 2022). Thus, the spreading of nuclei in the syncytial blastoderm is mechanistically similar to the separation of chromosomes during anaphase (Vukušić et al., 2019). Coincidently, the sliding of antiparallel microtubules occurs simultaneously with the elongation of the mitotic spindle (de-Carvalho et al., 2022). Thus, the spacing of the chromosomes and the spacing of the eventual microtubule asters and their attached nuclei are dependent on a network of repulsive interactions between spindles and within spindles mediated by kinesin and Feo (Fig. 3).

Although a syncytial blastoderm is unique to insect development, some of the lessons apply more broadly to multinucleated cells. For example, the generation of force from regions of overlapping antiparallel microtubules is conserved in other aspects of cell biology and could be relevant in any system where nuclei are evenly spaced. In fact, this specific mechanism for spreading nuclei has been hypothesized for skeletal muscle cells (Metzger et al., 2012), neurons (Guha et al., 2021) and in mitotic spindles (Risteski et al., 2021). More broadly, the syncytial blastoderm clearly illustrates that nuclei impact their neighbors and that the positioning of nuclei is coordinated across the entire cell by a series of short-range interactions. How these mechanisms of nuclear interactions are applied to and impact other multinucleated cells remains an important question.

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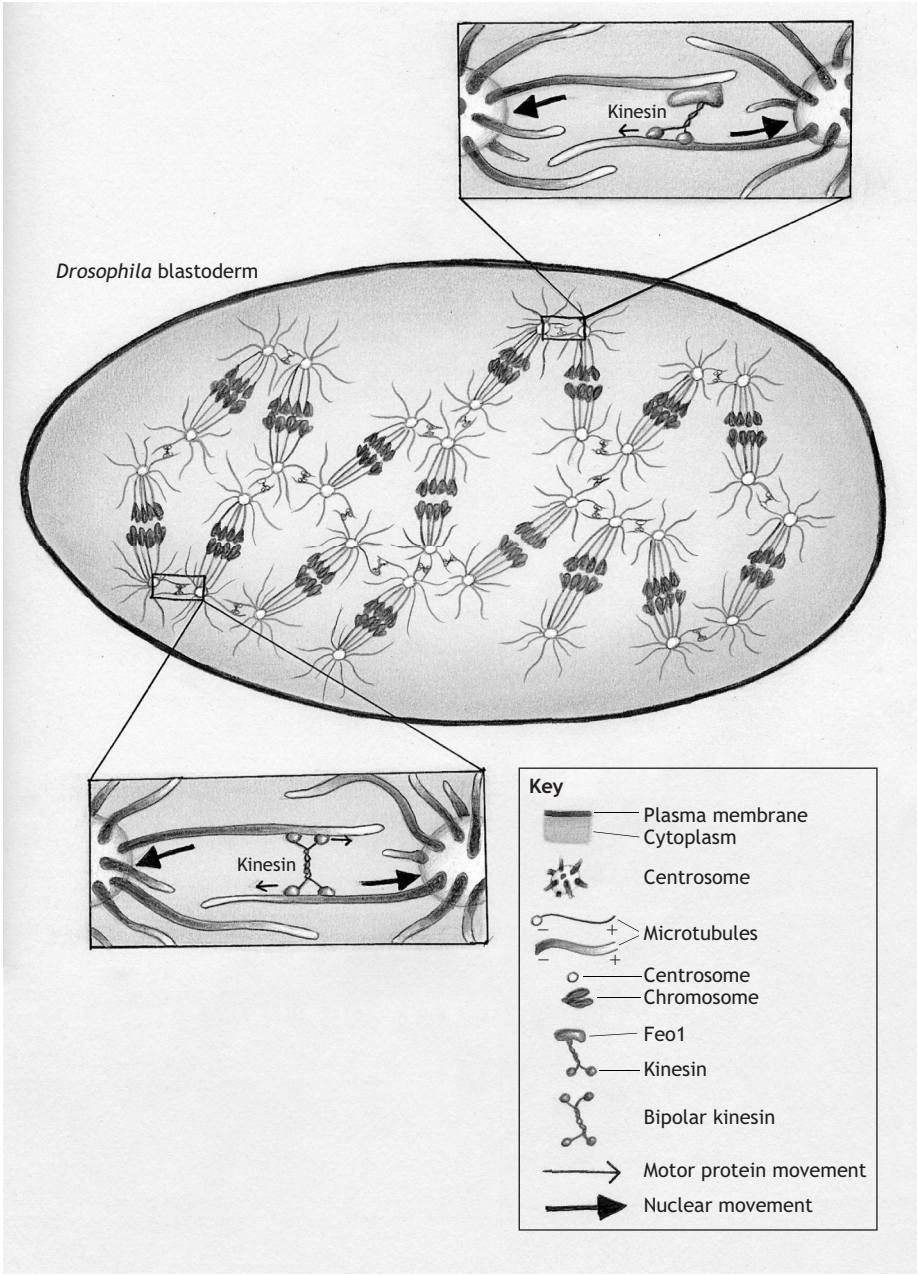

Fig. 3. Microtubule sliding in the *Drosophila* blastoderm. A late *Drosophila* syncytial blastoderm in which spindles are near enough to their neighbors that they interact. Mitotic spindles associate with their neighbors through their astral microtubules. Insets show kinesin localized to the regions of overlapping antiparallel microtubules. The inset at the top shows the organization in the *Drosophila* blastoderm in which Feo1 cooperates with kinesin to link and slide the antiparallel microtubules. The inset to the bottom shows an example where a bipolar kinesin can slide the antiparallel microtubules in the absence of another factor.

C. elegans hypodermal development

The hypodermal cells of *C. elegans* have historically been used to understand many of the mechanisms of nuclear movement and nuclear position (Starr, 2019). Hyp7 syncytial hypodermal cells have been a particularly useful cell type. Early screens identified that the KASH-domain proteins Anc-1 and Unc-83, along with the SUN-domain protein Unc-84, are necessary for nuclear spacing (Hedgecock and Nichol Thomson, 1982; Horvitz and Sulston, 1980). Subsequent molecular characterization and live imaging approaches have been used to demonstrate that an Unc-84/Unc-83 LINC complex is used to move nuclei and an Unc-84/Anc1 LINC complex is necessary for anchoring nuclei in place after they have moved (Malone et al., 1999; McGee et al., 2006; Starr and Han, 2002; Starr et al., 2001).

Mechanistically, nuclear movement is driven by a cargo-transport mechanism (Box 1) with LINC complex-dependent localization of kinesin and dynein to the nucleus, which then carry nuclei (Fridolfsson et al., 2010; Meyerzon et al., 2009). Because the microtubule network is polarized, kinesin-dependent transport toward the microtubule plus-ends dominates the movement of nuclei, with dynein-dependent transport of nuclei toward the minus-ends used to move around obstacles (Fridolfsson et al., 2010).

Nuclear position is maintained in the fully developed and moving animal via anchorage to an actin-dependent site on the cell cortex (Starr and Han, 2002). Furthermore, the anchoring in a moving animal requires a more mechanically stable LINC complex to connect the cytoskeleton and the nucleoskeleton. This difference necessitates the use of Anc-1, rather than Unc-83, which is used to move nuclei. Unc-84 and Anc-1 each have cysteine residues positioned at the SUN-KASH interface that form a disulfide bond, which allows this specific LINC complex to withstand the increased forces to anchor nuclei in place (Cain et al., 2018). These were some of the first data suggesting that distinct LINC complexes could

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regulate specific aspects of nuclear positioning within a single cell type and they further emphasized that distinct mechanisms can regulate nuclear position at different developmental stages (Bone and Starr, 2016). Although most of the active movements of nuclei have been investigated before fusion when the cells are mononucleated, the Unc-84/Anc-1-dependent anchoring of nuclei is crucial to maintain nuclear spacing after fusion.

One perplexing observation from early work was that nuclear positioning was more strongly disrupted in Anc-1 mutants compared with Unc-84 mutants (Cain et al., 2018; Jahed et al., 2019). In addition, earlier work demonstrated that mitochondria maintain their positions in Unc-84 mutants but not in Anc-1 mutants (Starr and Han, 2002). Together, these observations suggest that Anc-1 functions outside of its canonical LINC complex role. Recent work has demonstrated that Anc-1 functions independently of its localization to the nuclear envelope, changing the way in which both KASH-domain proteins and nuclear position must be considered. Specifically, Anc-1 can regulate nuclear position via its spectrin repeats, independent of the KASH domain, and therefore independently of its localization to the nucleus. In the absence of its KASH domain, Anc-1 is localized to the endoplasmic reticulum (ER) via its transmembrane domain, where it links the ER and mitochondria and regulates their relative positions and motion (Hao et al., 2021). Even with the KASH domain intact, some Anc-1 is localized to the ER, suggesting that this mechanism of action is relevant at many developmental stages. These data are intriguing beyond the specifics of the hyp7 cells and indicate that the positioning of all organelles is integrated and has ramifications for all cell types. While this hypothesis is not necessarily surprising, its demonstration is significant. When combined with the knowledge that mechanotransduction relies on feedback of mechanical and biochemical signals between the nucleus and cytoplasm, which is LINC-complex dependent, it is intriguing to propose that one major function of nuclear spacing is to distribute mechanocenters and thereby create a consistent mechanical environment.

Skeletal muscle

Nuclear movement and position have been extensively studied during skeletal muscle cell development because there is a clear correlation between nuclear spacing and muscle disease (Folker and Baylies, 2013; Roman and Gomes, 2018). Furthermore, the LINC complex and the proteins associated with the LINC complex are mutated in patients with the disease Emery-Dreifuss muscular dystrophy (EDMD) (Bione et al., 1994; Puckelwartz et al., 2009; Zhang et al., 2007). Importantly, from the perspective of this Review, several molecularly and temporally distinct nuclear movements occur during muscle development. These distinct movements combine many of the previously discussed general mechanisms such that understanding nuclear movement in muscle provides clues regarding the mechanisms in a variety of developmental contexts. Much of the work to understand nuclear movement during muscle development has relied on myogenic mammalian culture systems and *Drosophila*. Each system brings distinct strengths to the analysis, with mammalian cultures being more amenable to high-resolution imaging and biochemical analyses necessary to define molecular mechanisms. *Drosophila* provides an *in vivo* system in which nuclear movement occurs within the context of proper developmental signaling and the associated time constraints. Together these two systems have provided a significant understanding of how nuclei move during muscle development (Fig. 4).

Clustering of nuclei following myoblast fusion

Skeletal muscle cells become multinucleated through the iterative fusion of mononucleated myoblasts (Abmayr and Pavlath, 2012). Immediately following fusion, newly incorporated nuclei move in close approximation with the already incorporated nuclei in both *Drosophila* and mammalian culture systems (Cadot et al., 2012; Metzger et al., 2012) (Fig. 4A).

Experiments in mammalian cultures have established that the centering of newly incorporated nuclei occurs by a cargo-transport mechanism reminiscent of that seen during pronuclear migration in *C. elegans* and sea urchins (Meaders et al., 2020; Minn et al., 2009). Superficially, dynein associated with one nucleus binds the microtubules from a second nucleus and moves toward the microtubule minus-ends drawing the nuclei together. Experiments in mammalian cultures have identified a core regulatory mechanism in which cell division cycle 42 (Cdc42), Partition defective (Par) 6 (also known as Pard6a) and Par3 localize to the nuclear envelope and regulate the activity of cytoplasmic dynein (Cadot et al., 2012). As opposed to pronuclear migration in which only a single nucleus has an associated microtubule network, both the nuclei in the myotube center and the newly incorporated nucleus are active centers of microtubule nucleation. However, because there are many central nuclei, they are collectively larger than the single nucleus that was newly incorporated and therefore the net result is the movement of the new nucleus to the already established group of nuclei where it sits in alignment with the previously incorporated nuclei (Cadot et al., 2012) (Fig. 4A). Technical limitations have prevented understanding how nuclei come together during *Drosophila* myogenesis, but the nuclei do associate as a cluster before spacing. This single cluster is broken into two clusters that each migrate toward the opposite ends of the muscle (Metzger et al., 2012). The movement toward the ends is microtubule-, kinesin- and dynein-dependent and uses several regulators of kinesin and dynein including Ensconsin, Bsg25D (Nin), kinesin light chain, Dynactin complex, Partner of inscuteable (Pins), Sunday Driver (Syd)/JIP3, and Aplip1/JIP1 (Auld et al., 2018b; Elhanany-Tamir et al., 2012; Folker et al., 2012, 2013; Metzger et al., 2012; Rosen et al., 2019; Schulman et al., 2014). These various proteins move nuclei toward the muscle ends by at least two distinct mechanisms. The first is a microtubule-pulling mechanism (Box 1) similar to the mechanisms driving the oscillations of nuclei in *Ashbya gossypii*. In the case of *Drosophila* muscle development, nuclear migration toward the muscle end involves kinesin- and Syd-dependent transport of dynein to the cell end (Schulman et al., 2014), where dynein is anchored by Pins. Microtubules interact with the cell end, and presumably dynein, in a cytoplasmic-linker protein 190 (CLIP-190)-dependent manner. The anchored dynein then pulls the microtubule minus-ends, and the nucleus to which microtubule minus-ends are attached, toward the muscle end (Folker et al., 2012) (Fig. 4B).

During the same stages of nuclear migration, kinesin and dynein cooperate to drive nuclear movement by a cargo-transport mechanism. Live-embryo imaging has demonstrated that, *in vivo*, nuclei rarely change directions but dynamically change shape as they move (Folker et al., 2013), contrasting with the movements observed in culture that rely on the same proteins but are not directional (Gimpel et al., 2017; Wilson and Holzbaur, 2012, 2014). Both the directionality of nuclear movement and the shape changes observed *in vivo* are kinesin- and dynein-dependent, with the stretching of the nucleus at the leading edge being kinesin-dependent and the shortening of the nucleus at the trailing edge being dynein-dependent (Folker et al., 2013). These data suggest that kinesin acts at the front of the nucleus and carries the nucleus as

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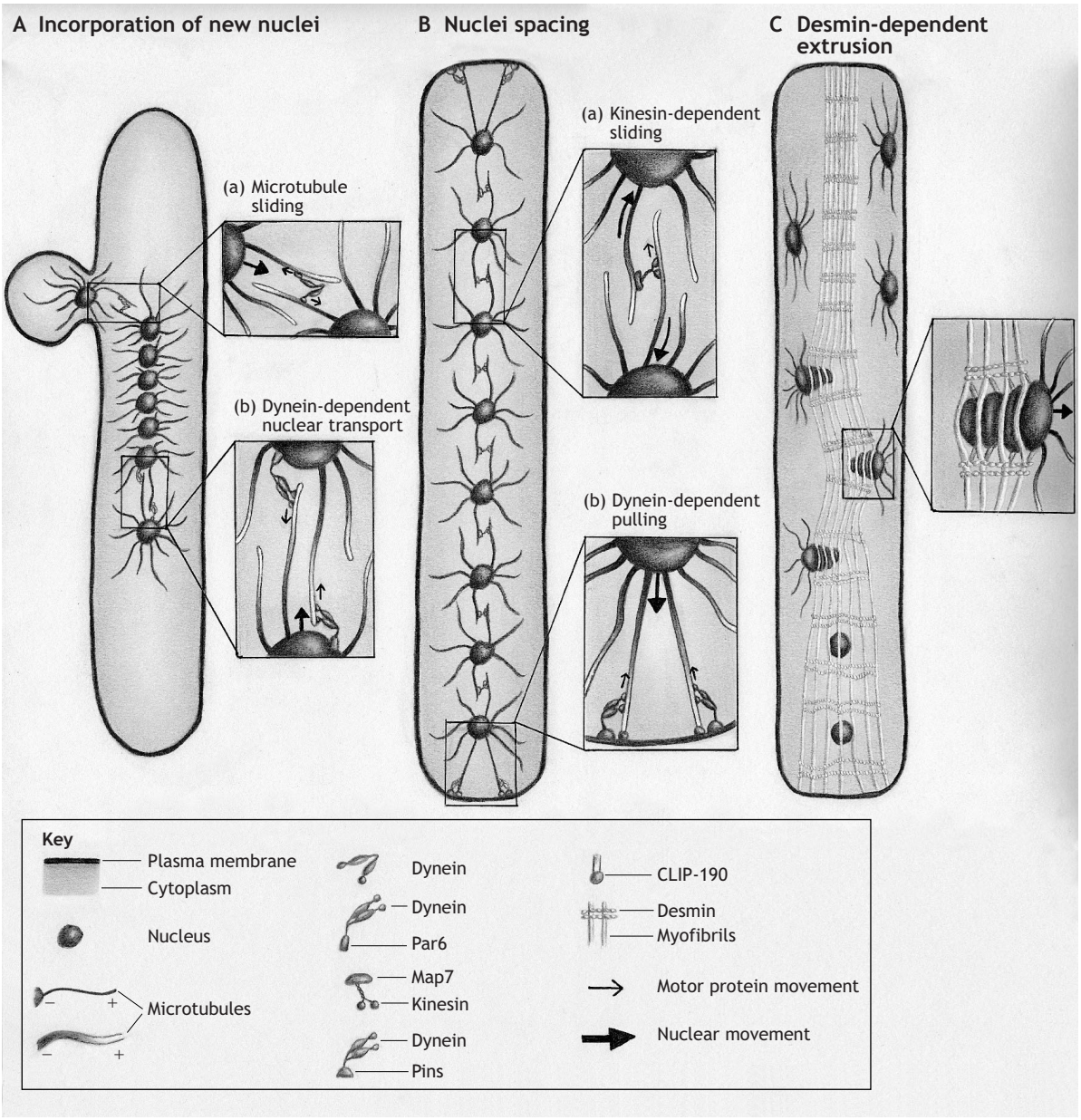

Fig. 4. Nuclear movement in muscle. This is a general model based on data from cultured myotubes and *in vivo Drosophila* muscles. (A) The microtubules associated with a newly incorporated nucleus associate with the microtubules from the already incorporated nuclei in the center of the muscle. (Aa) Hypothetically, a minus-end directed motor could interact with the overlapping antiparallel microtubules and slide these microtubules to move nuclei together. (Ab) Dynein localized to the nuclear envelope carries the newly incorporated nucleus toward the already incorporated nuclei. Crucially, when the active motor is kinesin, the cargo-transport model moves nuclei away from their neighbors. (B) Nuclei are spaced equidistantly from their neighbors. Two mechanisms have been proposed. (Ba) Kinesin-dependent sliding of antiparallel microtubules moves nuclei away. (Bb) Dynein-dependent cortical pulling moves nuclei away from the cluster. (C) Nuclei move to the periphery of the muscle when myofibrils are zippered together in a desmin-dependent mechanism. The density of the myofibril network excludes the large nucleus from the cell interior.

a cargo toward the microtubule plus-ends that extend towards end of the muscle. The role of dynein at the trailing edge of the nucleus is less clear. One hypothesis is that dynein carries the back of the nucleus along microtubules that have their minus-ends in the direction of nuclear movement. Alternatively, dynein may provide a means for the rear of the nucleus to avoid obstacles, similar to its role in *C. elegans* hypodermis (Fridolfsson and Starr, 2010), or a means to avoid neighboring nuclei, such as the dynein-dependent oscillations of nuclei in *A. gossypii* (Alberti-Segui et al., 2001). Although understanding the role of dynein in nuclear movement requires additional work, the important finding of these experiments is that the context of *in vivo* muscle development crucially impact the details of how nuclei move and, therefore, mechanistic studies in culture must be combined with *in vivo* analysis to build a comprehensive understanding of nuclear movement.

Beyond the application of force to nuclei, these experiments have shown that nuclei move as groups rather than individuals during much of embryonic *Drosophila* muscle development. Although the collective movement of nuclei has not been seen in mammalian muscle systems, interactions between nuclei are a crucial feature of mammalian muscle development. As previously discussed, the first nuclear movement in mammalian muscle leaves nuclei in the center

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of the developing muscle in alignment with the other nuclei. This movement and alignment occurs during both development and muscle repair, suggesting that the movement to the center and the association with other nuclei is a crucial feature of muscle development (Roman et al., 2021). Centrally positioned nuclei are a hallmark of muscle repair and are therefore used as the first indication of a muscle disorder (Sewry, 2010). The nature of the interactions between nuclei in mammalian muscles is not known. However, in *Drosophila* the clusters of nuclei are maintained by Amphiphysin. In the absence of Amphiphysin, nuclei dynamically separate from their neighbors and transiently populate the center of the developing myofiber (Collins and Mandigo et al., 2017). The consequence of this behavior is a poorly functioning muscle with mispositioned nuclei, indicating that the associations of the nuclei in clusters are crucial to muscle development. Although moving groups of nuclei have not been seen in mammals, Amphiphysin is crucial for nuclear spacing of nuclei in myogenic cultures and contributes by recruiting microtubules to the nuclear envelope (D'Alessandro et al., 2015), suggesting that maintaining the interactions between nuclei is a microtubule-dependent process. Furthermore, in culture Amphiphysin interacts with Wiscott-Aldrich syndrome protein (WASP), and the two proteins, together with dynamin 2, are essential for the extrusion of nuclei to the cell periphery (Falcone et al., 2014).

Nuclear spacing

Whether and how nuclei maintain their spacing during homeostasis is an important question. Work in mouse myofiber explants has demonstrated that nuclei in animals lacking dystrophin are more mobile than those in controls (Iyer et al., 2017), indicating that nuclei maintain their relative positions in functioning muscle, and that this ability may be crucial to muscle stability because dystrophin is linked directly to Duchenne muscular dystrophy (Gao and McNally, 2011). The mechanism by which dystrophin regulates nuclear position is not clear. However, dystrophin is known to bind both actin and microtubules (Prins et al., 2009; Rybakova et al., 2000), either of which could be used to anchor nuclei in place, similar to the mechanism by which Anc-1 anchors nuclei in the *C. elegans* hypodermis (Cain et al., 2018). Alternatively, a model in which microtubules emanating from a nucleus push against the neighboring nuclei (D'Alessandro et al., 2015; Wang et al., 2015) has been proposed, as has a microtubule-sliding mechanism (Metzger et al., 2012) (Fig. 4B).

Functionally, it has long been thought that the spacing of nuclei was necessary so that transcriptional products could be efficiently delivered to the entire muscle. This hypothesis is supported by seminal work in cell culture showing that fluorescent proteins are restricted to the region of the myotube near the nucleus that encoded for them (Pavlath et al., 1989). However, the fact that the diffusion of products is restricted is not equivalent to an inability to transport proteins longer distances. One alternative hypothesis is that nuclear spacing is crucial to establish and maintain the mechanics of the myofiber. Indeed, early sarcomere assembly spatially segregates with the nuclei in *Drosophila* (Auld and Folker, 2016), nuclei migrate to the site of muscle injury and are essential for muscle repair (Roman et al., 2021), and the nucleus is a crucial integrator of mechanotransduction in a variety of cell types, such fibroblasts and epithelial cells (Isermann and Lammerding, 2013). Finally, an emergent hypothesis is that nuclear position provides a mechanism to specify nuclear functions. Different transcriptional patterns exist for nuclei within the same muscle fiber (Kim et al., 2020; Petrany et al., 2020 preprint). In addition, it has long been known that the nuclei at the neuromuscular junction have unique functions (Sanes et al., 1991) and it has recently been demonstrated that the positions of nuclei near the myotendinous junction and the neuromuscular junction have different positioning requirements (Perillo and Folker, 2018). Recent work has united these hypotheses, suggesting a role for the nucleus in mediating sarcomere assembly, the spacing of the nuclei for the delivery of transcriptional products and a role for the nuclear movement in muscle repair. Upon either contraction-based insult or laser cutting, nuclei in cultured myotubes moved to the site of damage and provided the necessary proteins to repair the damaged myotube (Roman et al., 2021).

The LINC complex serves a canonical and novel function during nuclear movement in muscle. As in many other systems, the LINC complex functions as an adapter for microtubule motors to associate with the nucleus to drive nuclear movement by the cargo-transport mechanism. In addition, the LINC complex is crucial for the association of microtubule minus-ends with the nucleus (Espigat-Georger et al., 2016; Gimpel et al., 2017; Minn et al., 2009) and is, therefore, crucial for the direct application of forces through microtubules so that nuclei can be moved by a microtubule-sliding mechanism or a microtubule-pulling mechanism (Box 1) (Fig. 4B). Recent work has proposed a third function for the LINC complex in regulating the separation of nuclei from their neighbors. This hypothesis was based on the elongated shape of nuclei in LINC complex mutants combined with their exaggerated response to ablation of adjacent nuclei (Collins et al., 2021). Both behaviors suggest that the nuclei are under increased mechanical tension when LINC complex function is compromised. These data suggest that the LINC complex is not contributing by regulating the application of force to nuclei but rather the separation of nuclei from their neighbors. Furthermore, previous work has shown that nuclei are longer and less dynamic in dynein mutants (Folker et al., 2013), raising the possibility that the LINC complex is specifically necessary to recruit dynein to the nucleus and that dynein-dependent movement of the back of the nucleus is necessary to separate nuclei from their neighbors.

Nuclear extrusion

The final type of nuclear movement in muscle, the extrusion of nuclei to the cell periphery, has been studied exclusively in cultured mammalian systems. This movement is mechanistically distinct from any of the nuclear migrations previously discussed, but is most reminiscent of a bulk-movement mechanism (Deneke et al., 2019). At the final stage in myofibril development, the aligned myofibrils are wrapped together by the muscle-specific intermediate filament, desmin. The consequence of the myofibrils being tightly wrapped is that they exclude other cellular components from their space and therefore squeeze nuclei out of the center of the myofiber and to the periphery (Roman et al., 2017) (Fig. 4C). The squeezing of nuclei to the periphery is absent from *Drosophila*, most likely because myofibers in *Drosophila* are flat rather than cylindrical (Auld et al., 2018a). However, the nuclei in *Drosophila* serve as a center for myofibril assembly (Auld and Folker, 2016), suggesting that the positioning of nuclei above the myofibrils could be linked to myofibril assembly in both systems. Alternatively, it could be proposed that the assembly of an actin-myosin based contractile network on or near nuclei would be able to directly push the nucleus away from the myofibrils and represent an actin-based pushing mechanism.

Filamentous fungi

Fungi are historically important organisms in understanding many of the genetic and cell biological mechanisms that

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regulate development, including the mechanisms and functions of nuclear movement (Varshney and Sanyal, 2019; Xiang, 2018). For example, mononucleated *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* move their nuclei to the bud neck and the cell equator to ensure the equal division of genetic material (Hagan and Yanagida, 1997; Jacobs et al., 1988). Studies in these species have been crucial in developing models of microtubule-based pulling of nuclei and in understanding how microtubule polymerization against a cell cortex can move a nucleus. In addition to these classic fungal systems, filamentous fungi have emerged as models that provide an opportunity to understand nuclear spacing in a multinucleated system.

Although there are long-range movements of nuclei in filamentous fungi, the spacing of nuclei as they divide is best understood in *A. gossypii*, in which nuclear spacing is microtubule- and dynein/dynactin-dependent (Grava et al., 2011), but distinct from the mechanism described for pronuclear migration. Rather than a cargo-transport mechanism, *A. gossypii* employs a cortical pulling mechanism (Box 1). In *A. gossypii*, dynein is anchored to the cell cortex by a num1p-dependent mechanism; because dynein is immobilized, it moves the microtubules against the cortex, pulling the microtubule minus-ends, and thus the associated nuclei, toward the dynein (Grava et al., 2011) (Fig. 5). Interestingly, despite the cell being multinucleated, the nuclei being in proximity and the nuclei moving past one another, the nuclei move and function independently of their neighbors (Grava et al., 2011), unlike in the example of the *Drosophila* blastoderm (Foe and Alberts, 1983). Dynein activity drives oscillatory random nuclear migration, which is crucial to maintain the spacing of nuclei, but the nuclei still move in the absence of dynein (Alberti-Segui et al., 2001; Dundon et al., 2016). Thus, dynein activity is not crucial for nuclear translocation but is instead necessary for nuclei to move around the other nuclei that they encounter. Indeed, dynein activity provides nuclei in *C. elegans* hypodermal development with the ability to move around obstacles during a directed nuclear migration that is otherwise kinesin-dependent, as discussed below (Fridolfsson and Starr, 2010).

Functionally, the importance of nuclear movement and spacing in filamentous fungi is not known. The position of the nuclei has little impact on at least several basic nuclear functions: specifically, the size of nuclei, the motion of nuclei and the transcriptional activity of nuclei are independent of whether a nucleus is in a cluster or isolated away from the cluster (Dundon et al., 2016). Thus, in filamentous fungi, nuclei are independent in both their migration and their function. Whether there is a link between nuclei moving independently of their neighbors and the independence of their functions is unclear.

Other multinucleated systems to explore

The systems discussed thus far are multinucleated systems in which the spacing of nuclei has been investigated, but there are many other noted multinucleated systems for which the positioning of nuclei is

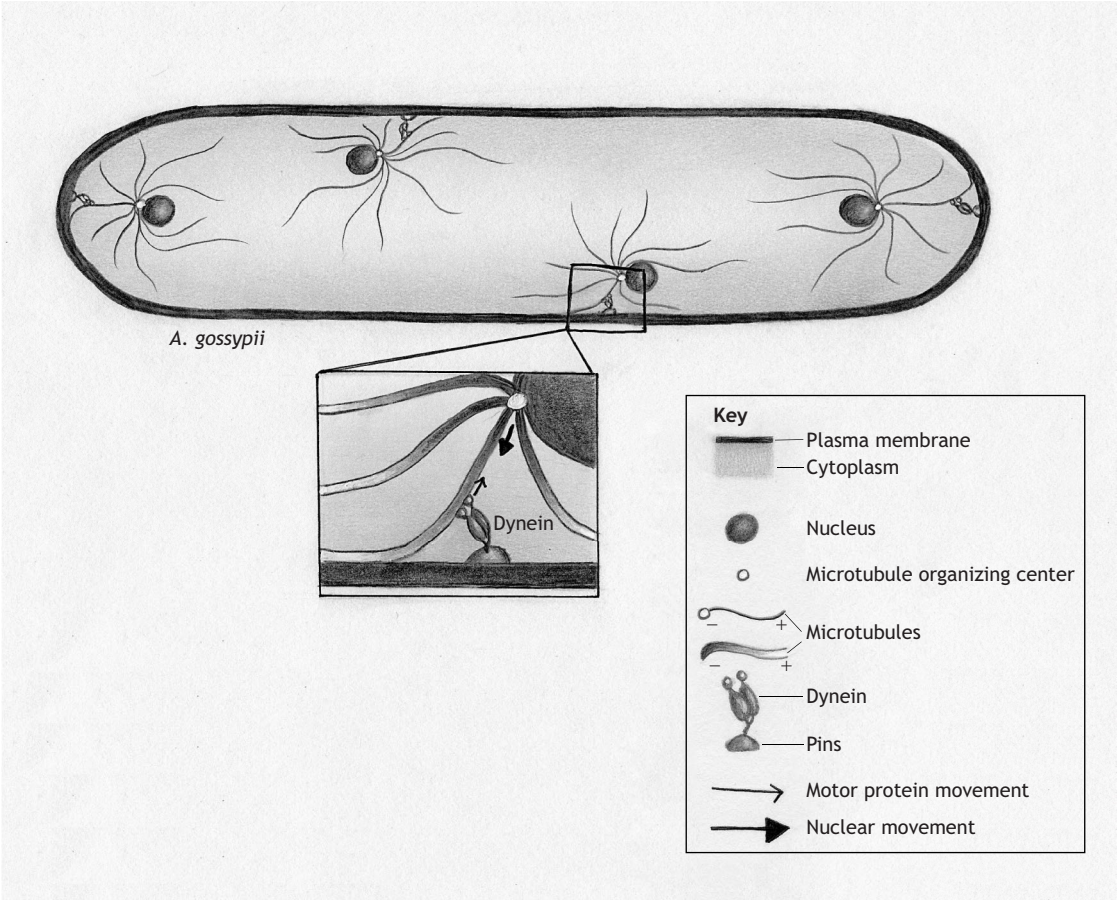

Fig. 5. Cortical pulling as seen in *Ashbya gossypii*. Microtubules extend from the microtubule-organizing center that is associated with a nucleus and interact with dynein that is anchored at the cortex. Because dynein is anchored and cannot move, it pulls the minus-end of the microtubule toward its position.

relatively unexplored. Nevertheless, these cells could provide crucial insights to understand the mechanisms and functions of distinct patterns of nuclear spacing.

Yolk syncytial layer in zebrafish

One system that was extensively studied many decades ago is the yolk syncytial layer (YSL) of the zebrafish embryo, which contains evenly spaced nuclei aligned at the interface between the yolk and the developing zebrafish embryo and is likely crucial for the flow of nutrients between the yolk and the animal (Carvalho et al., 2009). Furthermore, and consistent with the hypothesis above regarding a mechanical function for nuclear spacing, the nuclei of the YSL provide crucial mechanical cues for epiboly migration (Bruce and Heisenberg, 2020). The spacing of nuclei is microtubule-dependent, but the mechanism is unclear. Specifically, there are long microtubules that extend to the vegetal pole consistent with a pushing mechanism (Solnica-Krezel and Driever, 1994). Recent advances in imaging technology have revealed that the movement of YSL nuclei is kinesin-dependent and that nuclei move away from microtubule minus-ends, consistent with a kinesin-dependent cargo-transport mechanism (Fei et al., 2018). These data are further complicated by recent findings that YSL nuclei respond to the movements of the adjacent mesodermal and endodermal progenitors (Carvalho et al., 2009). How these mechanisms are coordinated and whether they are temporally restricted is a question for further investigation.

Placenta in mammals

In mammals, the syncytial trophoblast layer of the human placenta regulates the flow of nutrients and is also multinucleated. One interesting feature of this syncytia is that the nuclei are not evenly spread throughout the cytoplasm. Although the positioning of nuclei is poorly understood and not well-documented, nuclei have been found in a single cluster near the center of the cell or many smaller clusters throughout the cell (Calvert et al., 2016). This observation is reminiscent of the nuclei coming together in the center of a developing myofiber or moving as a cluster during *Drosophila* muscle development (Collins et al., 2021; Metzger et al., 2012), but whether nuclear interactions are regulated by similar mechanisms has not been explored. However, reactive oxygen species are thought to alter nuclear clustering in the syncytial trophoblasts, implying that the clusters are molecularly regulated and functionally important (Heazell et al., 2007).

Osteoclasts in vertebrates

Similarly, osteoclasts are multinucleated cells in which the nuclei form a single cluster. There are no data to indicate a mechanism of nuclear movement or clustering in osteoclasts. However, functionally, the nuclei are positioned away from the resorption site and there is robust kinesin-dependent transport of materials to the resorption site (Ferron et al., 2013; Lacombe et al., 2013). Thus, it appears that the benefit of nuclear clustering to the cell is that the obstacles are concentrated and, therefore, do not impede transport. Understanding how the interactions between nuclei are regulated in distinct tissues could identify conserved mechanisms and functions for nuclear clustering.

Tissue repair in various species

Finally, the formation of multinucleated cells in response to injury, infection and disease is evolutionarily conserved. Plants form a syncytium in response to parasites (Caillaud et al., 2008; Palomares-Rius et al., 2017), giant multinucleated cells form in many cancers (Brooks et al., 2019), a hallmark of infections from enveloped viruses is the formation of multinucleated cells (Aguilar et al., 2013) and, as discussed above, nuclei move to sites of injury in skeletal muscle (Roman et al., 2021). In addition, an early phase of the wound healing process in *Drosophila* epithelial tissue is fusion of the cells near the wound and the formation of a large multinucleated cell (Losick et al., 2013). To date, no work has been carried out to understand the spacing of nuclei and whether nuclear position is dynamic in any of these situations. Defining the mechanisms and functions of nuclear movement and the impact that distinct spacing patterns have on function could provide crucial insight into the selection of multinucleated cells in these contexts.

Conclusions

The phenomenon of nuclear movement has gained significant interest in recent years. Multinucleated cells provide particularly intriguing examples of nuclear movement, often with clearly repetitive final spacing patterns. In total, a small number of different mechanisms are used in different combinations to drive the movement and spacing of nuclei. Moving forward, identifying the similarities and the unique features of nuclear movement in multinucleated cells will likely provide the context necessary to understand the importance of the unique patterns that have emerged in different cell types. Perhaps most fundamentally, understanding the mechanisms by which nuclei interact with their neighbors, affect the position of one another and, as a consequence, affect the function of the other nuclei that share the common cytoplasm, is a crucial next step in understanding multinucleated cells.

Competing interests

The authors declare no competing or financial interests.

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