Published in final edited form as:

Nat Cell Biol. 2017 September 29; 19(10): 1148–1150. doi:10.1038/ncb3618.

Myofibrils put the squeeze on nuclei

Jonathan Rosen and Mary Baylies

Program in Developmental Biology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, New York 10065

Abstract

During muscle development, nuclei travel from the center of the myofiber to the periphery, a process defective in certain diseases. A new study reveals that this movement is due to centripetal forces imposed on nuclei by the crosslinking and contraction of myofibrils.

Whether in high school classrooms or scholarly journals, schematics generally depict the nucleus as a circle that sits in the center of a cell. In real cells, however, nuclei often occupy specific subcellular localizations. Developing skeletal muscle cells, which can contain hundreds of nuclei each, provide an extraordinary system for investigating nuclear positioning. In healthy myofibers (fully differentiated muscle cells) nuclei are positioned around the cell periphery. In certain diseases, such as centronuclear myopathies (CNM), nuclei are instead found in the cell interior¹. These observations made by pathologists, combined with recent mutant analyses in model systems, have contributed to a growing appreciation that correct myonuclear positioning is essential for muscle function². But what mechanisms normally localize nuclei to the fiber periphery? And why do these mechanisms fail in CNM? On page ____ of this issue of *Nature Cell Biology*, Roman *et al.*³ report a novel mechanism for the peripheral movement of myonuclei: centripetal forces exerted by the crosslinking and contraction of myofibrils squeeze nuclei from the fiber center to the periphery.

Over the course of muscle development, nuclei undergo a series of discreet movements to arrive at their ultimate destination, the fiber periphery (Figure 1). Multi-nucleated muscle cells form by the iterative fusion of myoblasts (mononucleated muscle precursor cells) to the growing myotube. Following fusion, each newly added nucleus travels from the periphery to a growing cluster of nuclei in the myotube's center. Next, the nuclei spread out and become evenly spaced along the longitudinal axis of the cell, remaining in the cross-sectional center. Then, nuclei travel *back* from the fiber center to the periphery, where they remain⁴.

In recent years, the Gomes Lab has studied the molecular mechanisms underlying myonuclear movement using *in vitro* systems. As with other examples of nuclear positioning, the main players in myonuclear positioning are cytoskeletal components. Interestingly, however, the cytoskeletal-based mechanisms of nuclear positioning differ for each phase of nuclear movement. The initial movement of nuclei to the myotube's center following fusion requires microtubules (MTs), the MT motor Dynein, Cdc42, Par6, and Par3⁵. The subsequent spreading of nuclei along the myotube's long axis also requires MTs, but the motors and microtubule-associated proteins (MAPs) are different; this phase of nuclear movement requires Kinesin and MAP7/Enscosin². Interestingly, the next step of

nuclear movement—from the myotube center to the periphery—occurs by a completely different mechanism. Here, MTs are dispensable. Instead, peripheral nuclear movement requires actin, the actin nucleation promoter Neural Wiskott-Aldrich synapse protein (N-WASP), and the N-WASP regulator Amphiphysin-2 (Amph2)⁶. While elucidation of the N-WASP-Amph2-actin pathway for peripheral nuclear movement was exciting, the underlying molecular mechanism remained mysterious.

In this issue of *Nature Cell Biology*, Roman *et al.* investigate how actin and its regulators drive peripheral nuclear movement and propose a novel mechanism: extrusion of nuclei from the myofiber center by the physical forces exerted by myofibrils (Figure 2). This conclusion is partly based on dramatic spinning disk microscopy time-lapse images that track nuclei and myofibrils during peripheral movement. Before peripheral movement, although myofibrils are present in the fiber, they are absent from a longitudinal stretch surrounding the nucleus. Then, immediately prior to peripheral nuclear movement, myofibrils on either side of this gap crosslink in an orderly progression, starting from the positions furthest from the nucleus. Like zippers, the cross-linking spreads from both sides toward the nucleus, ultimately squeezing the nucleus out of that plane and into the cell periphery. During this process, the nucleus undergoes deformations that reflect the forces being applied to it.

The authors use siRNA-mediated knockdown and other molecular approaches to mechanistically define how myofibril “zipping” occurs. They find that the intermediate filament protein Desmin is essential for the myofibril crosslinking that contributes to nuclear extrusion. How are Desmin filaments properly localized at Z lines to perform this function? Knockdown experiments show that Desmin organization at the Z-line requires γ -actin and the Arp2/3 actin nucleation complex component Arpc5L, which co-immunoprecipitate and co-localize; knockdown of either prevents peripheral nuclear movement. Arp2/3 is regulated by N-WASP, which the Gomes Lab has previously shown is essential for peripheral nuclear movement, along with the N-WASP regulator Amph2⁶. Thus, this paper extends the actin pathway the Lab previously showed to be essential for nuclear movement to the periphery and defines its mechanism of action.

Along with their experimental work, the authors also mathematically model peripheral nuclear movement. Their model predicts that the myofibrils’ activity as molecular zippers is not sufficient for nuclear extrusion from the center: there must also be an increase in myofibril tension. Reasoning that this tension is likely caused by muscle contraction, the authors experimentally block muscle contraction with tetrodotoxin and observe an inhibition of peripheral nuclear movement. Moreover, through the use of optogenetics, they find that inducing contractions early in myofiber development causes precocious peripheral movement. Thus, contraction is necessary and sufficient for peripheral nuclear movement.

While it is impossible to know why this unusual mechanism evolved, one possibility is that it has value as a timing mechanism for muscle development. Because myofibril contractions can only occur after myofiber differentiation, this mechanism may ensure that nuclei will not travel to the periphery too early. It stands to reason that precocious peripheral movement could interrupt the previous phase of nuclear positioning—longitudinal spreading—and lead

to myofibers with peripheral nuclei that are clustered along the longitudinal axis of the fiber. *Drosophila* and mouse mutants have been described with nuclei clustered rather than evenly spread, and they all exhibit functional muscle deficits^{2,7,8,9,10}. The Gomes Lab previously showed that inhibition of MT/Kinesin/MAP7-dependent nuclear spreading ultimately prevents peripheral nuclear movement, even though that pathway is not required for peripheral movement *per se*, which led them to conclude that longitudinal nuclear spreading must occur before peripheral movement can take place⁶. This suggests that either myofibers lacking Kinesin or MAP7 cannot generate the force required to extrude nuclei to the periphery, or there are additional mechanisms ensuring the orderly progression of myonuclear movement.

The novel mechanisms of peripheral nuclear movement uncovered in this paper explain how nuclei can be left behind in the fiber's center. An open question is how such peripheral mispositioning of myonuclei affects muscle function. Roman *et al.*, by defining a molecular pathway for peripheral nuclear movement, are also expanding the toolkit available to researchers working on this question; the more precisely we can manipulate nuclear movements, the better we can characterize the downstream consequences. Ultimately, we will be able to explain not only how each stage of myonuclear movement occurs, but also how mispositioning affects both the mispositioned myonuclei and the whole myofiber.

The findings by Roman *et al.* also shed light on CNM. CNM is caused by mutations in a number of genes, including *Amph2*¹¹. The block in peripheral nuclear movement observed upon depletion of *Amph2* (or the other components of this actin pathway) recapitulates the centrally located nuclei observed in muscle samples from patients with CNM. While it has not been tested, it is highly plausible that myofibril “zipping” fails in CNM muscle, leading to the accumulation of centrally located myonuclei. The authors show that nuclear deformation and protrusion occur during peripheral nuclear movement in mouse *in vivo*; in the future it will be fascinating to observe to what extent their other observations and conclusions about normal nuclear movement are recapitulated *in vivo*, especially in human muscle. In the same way, it will be essential to know to what extent the defects they see in siRNA treated muscle *in vitro* recapitulate the cell biology of human CNM muscle.

Finally, it is interesting to consider whether the mechanism of peripheral movement by physical extrusion could be relevant to other muscle diseases. In muscle disorders like Nemaline myopathy, muscle contraction is impaired¹². Is peripheral nuclear movement similarly affected? The modeling done by Roman *et al.* also predicts that nuclei must have a certain stiffness to be extruded properly, and they validate this experimentally by overexpressing Lamin A/C to increase stiffness or reducing Lamin A/C to decrease stiffness; both manipulations inhibit peripheral nuclear movement. Certain mutations in Lamin cause Emery-Dreifuss Muscular Dystrophy (EDMD), and in some cases myonuclei have diminished stiffness¹³. Does peripheral nuclear movement fail here as well? In the future it will be exciting to gain a better understanding of how intracellular mechanical properties are affected in various muscle diseases, and what the downstream consequences are for muscle structure and function.

Acknowledgments

JR and MB are supported by the NIH [GM078318, AR108981] to MKB and National Cancer Institute [P30 CA 008748] core grant to MSKCC.

References

1. Romero NB. Centronuclear myopathies: A widening concept. *Neuromuscular Disorders*. 2010; 20:223–228. [PubMed: 20181480]
2. Metzger T, et al. MAP and kinesin-dependent nuclear positioning is required for skeletal muscle function. *Nature*. 2012; 484:120–124. [PubMed: 22425998]
3. Roman, William, Martins, Joao P., Carvalho, Filomena A., Voituriez, Raphael, Abella, Jasmine VG., Santos, Nuno C., Cadot, Bruno, Way, Michael, Gomes, ER. Myofibril contraction and cross-linking drive nuclear movement to the periphery of skeletal muscle. *Nat Cell Biol*. 2017
4. Cadot B, Gache V, Gomes ER. Moving and positioning the nucleus in skeletal muscle - one step at a time. *Nucleus*. 2015; 6:373–81. [PubMed: 26338260]
5. Cadot B, et al. Nuclear movement during myotube formation is microtubule and dynein dependent and is regulated by Cdc42, Par6 and Par3. *Nat Publ Gr*. 2012; 13:741–74989.
6. Falcone S, et al. N-WASP is required for Amphiphysin-2/BIN1- dependent nuclear positioning and triad organization in skeletal muscle and is involved in the pathophysiology of centronuclear myopathy. *EMBO Mol Med*. 2014; 6:1455–1475. [PubMed: 25262827]
7. Folker ES, Schulman VK, Baylies MK. Muscle length and myonuclear position are independently regulated by distinct Dynein pathways. *Development*. 2012; 139:3827–3837. [PubMed: 22951643]
8. Schulman VK, Folker ES, Rosen JN, Baylies MK. Syd/JIP3 and JNK Signaling Are Required for Myonuclear Positioning and Muscle Function. *PLoS Genet*. 2014; 10
9. Zhang J, et al. Nesprin 1 is critical for nuclear positioning and anchorage. *Hum Mol Genet*. 2009; 19:329–341. [PubMed: 19864491]
10. Elhanany-Tamir H, et al. Organelle positioning in muscles requires cooperation between two KASH proteins and microtubules. *J Cell Biol*. 2012; 198:833–846. [PubMed: 22927463]
11. Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies. *Frontiers in Aging Neuroscience*. 2014; 6
12. Ottenheijm CAC, et al. Altered myofilament function depresses force generation in patients with nebulin-based nemaline myopathy (NEM2). *J Struct Biol*. 2010; 170:334–343. [PubMed: 19944167]
13. Davidson PM, Lammerding J. Broken nuclei - lamins, nuclear mechanics, and disease. *Trends in Cell Biology*. 2014; 24:247–256. [PubMed: 24309562]

Figure 1. Myonuclear movement during muscle development

A) Following myoblast fusion to the myotube, newly added nuclei move to the myotube center (dashed line). B) During nuclear spreading, myonuclei spread out along the longitudinal axis of the myotube. C) Finally, myonuclei move to the myofiber periphery. Black arrow shows longitudinal axis; green arrows show center-periphery axis.

Figure 2. Myofibril zipping causes myonuclear extrusion to the periphery

A) Myofibrils crosslink in a progressive fashion toward the nucleus. B) The force generated by myofibril crosslinking deforms the nucleus and causes it to extrude from the myofiber center. C) Following myofibril zipping, the nucleus is above the myofibrils, at the myofiber's periphery. All three schematics show a single plane at the center of a developing myofiber. Nucleus is red; Z-lines (running through numerous myofibrils) are in black.