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SPOTLIGHT

Muscle tensions merge to cause a DNA replication crisis

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In this issue, Wang et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201708137) show that disruption to different mechanical domains of muscle cells converge at the linker of nucleoskeleton to cytoskeleton complex to affect DNA endoreplication potentially via barrier to autointegration factor–mediated epigenetic mechanisms.

In the postgenomic era of biological research, it has become evident that cell mechanics are not only important for cell and tissue functionality (for example, muscle cell contraction) but also are crucial in providing signals to the genome to generate rapid nuclear responses to external stimuli. This mechanosignaling occurs via a network of sequentially tethered structural proteins that span the ECM through the cytoplasm and directly into the nucleus. Mechanotransduction via this pathway can be viewed as alternative or complementary to classical soluble cell signaling.

In recent years, mechanobiology has come to the forefront of cell biology and molecular genetics with the discovery that chronic application of mechanical signals could influence physiological cell responses such as cell fate (Swift et al., 2013). Much of this work has been driven by the discovery of the nuclear envelope (NE) linkers of the nucleoskeleton to cytoskeleton (LINC) complex proteins comprising nesprins, Sad1p and UNC-84 (SUN) domain proteins, and the nuclear lamins, which span the NE and form a physical bridge between the cytoskeleton and DNA (Crisp et al., 2006). This linkage is crucial for efficient integration of mechanical signals that mediate a growing number of nuclear processes including transcriptional regulation, splicing, epigenetics, nuclear pore transport, and DNA damage repair.

For example, several studies have shown that disruption and mechanical manipulation of the LINC complex at both the single-cell and tissue level can exert profound changes on gene expression, which correlate with perturbations in chromatin organization including loss of heterochromatin. LINC complex disruption can also impact chromosome dynamics and positioning to modulate tissue-specific gene expression (Zuleger et al., 2011; Robson et al., 2017). In addition, nuclear shuttling of specific transcription factors was found to be dependent on actin dynamics, which in turn is dependent on lamin A/C function (Ho et al., 2013).

Tissues that are under constant mechanical stress, such as heart and skeletal muscle, have been the main focus of mechanobiology as these tissues are most often affected in human genetic conditions caused by mutations in components of the LINC complex. Emerging studies in these tissues have begun to unravel even more complex responses and functions that are regulated by mechanosignals, and they have also begun to tease out direct mechanopathways. One such study showed that manipulation of mechanical environments in vitro using quantum dot–labeled polydimethylsiloxane nanopillar arrays of varying stiffness can govern the gene expression response and subsequent phenotypic outcome of neonatal rat cardiomyocytes independent of nonmechanical stimuli (Pandey et al., 2018).

Wang et al. build on these studies by using a more reductionist approach, assessing the impact of separate mechanical subdomains of muscle cells on DNA endoreplication as well as nucleus size, nuclear position within the muscle syncytium, and gene expression.

They used a model of larval muscle development in the common fruit fly, *Drosophila melanogaster*, and performed genetic knockdowns of the LINC complex proteins klarsicht (*klar*) and klaroid (*koi*), which are the invertebrate versions of nesprin and SUN domain proteins, respectively. This led to dramatic myonuclear positioning defects as well as overall smaller nuclei. However, they additionally observed a huge variability in nuclear size when compared with WT nuclei. They speculated that this nuclear size variability could be caused by differences in DNA content, and using EdU incorporation, they showed that DNA endoreplication was affected when *klar* and *koi* were disrupted.

Next, they examined the effects on cell cycle progression by investigating the degradation of E2F1, a crucial cell cycle regulator. Consistent with a role for DNA endoreplication, LINC complex mutants exhibited variable and overall higher mean fluorescence intensity of a GFP-tagged E2F1 construct, implying impaired G1–S cell cycle progression between myonuclei of individual myofibers.

Wang et al. (2018) then attempted to delineate upstream cytoskeletal components that serve to sense and transmit environmental/mechanical cues to the nucleus via the LINC complex and potentially to regulate endoreplication. Most compellingly, they demonstrated that muscle-specific knockdown of β-position specific integrin at the plasma membrane led to a similar

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endoreplication defect, albeit with normal nuclear positioning, supporting a key role for mechanotransduction in DNA replication. In contrast, disruption of other components yielded surprising results. The microtubule (MT) network acting via NE attachments has been reported to be essential for myonuclei positioning (Wilson and Holzbaur, 2015). However, expression of spastin to sever MTs in fully differentiated myofibers unexpectedly had no impact on nuclear position or DNA endoreplication but did lead to enlarged nuclei, implying a role for the MT cytoskeleton in constraining nuclear size. Interestingly, prolonged expression of spastin eventually caused sarcomere disruption followed by nuclear positioning defects, suggesting that nuclear position is dependent on sarcomere structure. Knockdown of the sarcomeric protein sallimus (known as titin in mammals) resulted in nuclear positioning defects and smaller nuclei, in line with LINC complex disruption, although investigation of DNA replication proved inconclusive because of larval growth defects. Collectively, these data support the idea that specific mechanical domains are involved in diverse nuclear processes but that all potentially converge at the NE, which acts as a gateway for these mechanical stimuli.

To establish a mechanism that might account for the endoreplication defects, they compared RNA Pol II occupancy in *klar* mutant muscle with WT using Dam identification analysis. A large number of targets was identified, but of particular interest was the gene for barrier to autointegration factor (BAF), which was down-regulated in *klar*-mutant larvae. BAF is a chromatin-binding protein that is linked to the inner nuclear membrane via LEM domain–containing LINC proteins (Barton et al., 2014). Importantly, siRNA of BAF in muscle fibers led to an increase in DNA content, partially phenocopying the findings of the *klar* mutants and therefore suggesting they potentially act by a common pathway to facilitate proper DNA replication in muscle cells.

The data presented by Wang et al. (2018) point toward a link between mechanical input and epigenetic mechanisms in the regulation of nuclear functions in multinucleated systems. Wang et al. (2018) speculated that the endoreplication defects they observed were caused by alterations in chromatin compaction. Indeed, BAF has been shown to regulate histone modifications in part via interactions with SET/I2PP2A and G9a, where overexpression of BAF in HeLa cells led to significant changes in histone acetylation and methylation (Montes de Oca et al., 2011). However, epigenetic modifications in *klar* and *koi* mutants warrant future investigations to shed more light onto potential target genes regulating critical nuclear functions in response to altered mechanical stimuli. Indeed, only BAF was explored in detail, but it could be envisaged that other genes such as troponin C may also be critical for some of the phenotypes observed in this study. In regard to the specific role or roles of BAF, unresolved questions include whether overexpression of BAF can rescue endoreplication defects observed in BAF-deficient systems as well as *klar/koi* mutants. It would also be important to determine whether integrin disruption reduces BAF and therefore is acting via the same pathway as LINC complex disruption. This would provide direct evidence that the LINC complex is a mechanotransducer responsible for the relay of mechanical cues from the cell exterior to affect gene expression.

The *klar* mutant system also opens up relevant questions pertaining to nuclear mechanotransduction and how it might

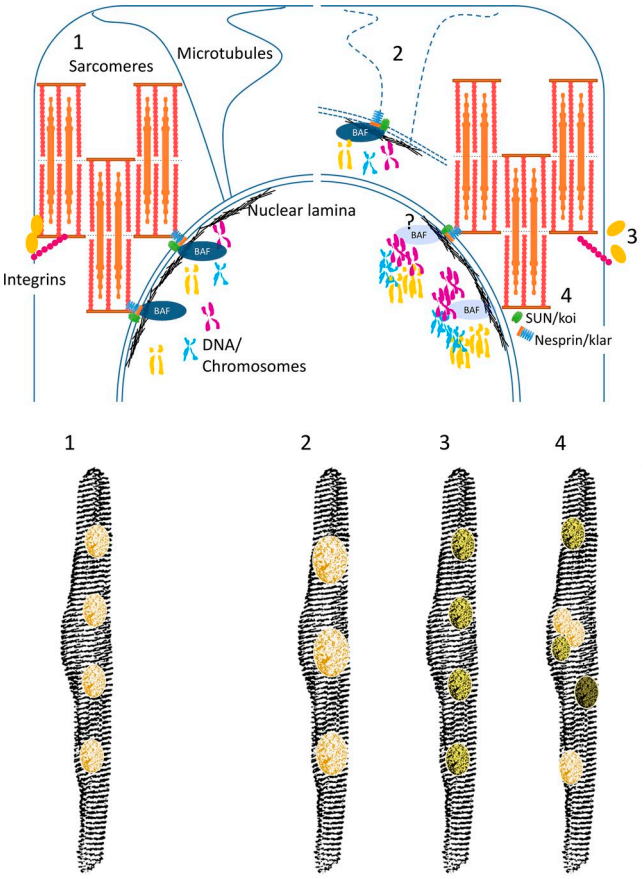

Figure 1. **Divergent mechanotransduction pathways regulate different nuclear processes.** 1: Intact cytoskeletal and LINC complex attachments are essential for normal regulation of DNA content and nuclear size and positioning in muscle cells. 2: MTs act to constrain only nuclear size in muscle cells. 3: Integrins regulate DNA content but not nuclear size or position. 4: The LINC complex regulates DNA endoreplication, myonuclear size, and positioning. The mechanical regulation of DNA endoreplication is mediated exclusively through the LINC complex, and expression of BAF appears to be critical.

impact on cellular functions such as endoreplication. By and large, the literature has amassed evidence that the cell perceives its microenvironment and transmits the physical signal via cascades of soluble factors in the cytoplasm across the NE (Wang et al., 2009). However, the study by Wang et al. (2018) calls attention to the intrinsic properties of the nucleus in mechanotransduction. In the future, it would be interesting to study the effects of *klar* silencing on the biophysical properties of the nucleus and how that impacts on transducing mechanical signals. The insights that would be obtained would be useful in answering fundamental questions in muscle biology, especially on how nuclear responses in a multinucleated cell/tissue are synchronized and also how selective manipulation of a single nucleus (for example, using optical tweezers coupled with micromanipulation) would affect neighboring nuclei in a multinucleated system such as the myofiber (Guilluy et al., 2014; Elosegui-Artola et al., 2017).

The use of E2F1 as an indicator for cell cycle defects also raises questions as E2F1 is known to be regulated by many post-translational modifications, which separately facilitate cell cycle progression and therefore help define the phase of cell cycle experimentally. How LINC regulates E2F1 function is also

an unanswered question by Wang et al. (2018). A limitation of the *Drosophila klar* mutant larvae model is the infeasibility of isolating single nuclei. It would be of interest and importance to explore and validate DNA replication defects in isolated nuclei to definitively and fully characterize cell cycle defects.

Collectively, the main message in this study is that DNA endoreplication and cell cycle is regulated by mechanical signaling mediated exclusively via the LINC complex. Outside the LINC complex, different mechanical domains of the cytoskeleton and plasma membrane are capable of eliciting different nuclear outcomes in muscle cells (Fig. 1). Work now needs to focus on these pathways in more detail, which may require different techniques and model systems such as fluorescence imaging of live single cells to determine the time course of outcomes initiated by disruption to each specific mechanical domain. Future work should also focus on the downstream molecular mechanisms that are impacted by different mechanotransduction pathways to regulate specific nuclear processes such as DNA replication.

The wider implications for health are that defects in transmission of mechanical signals may impair DNA replication and cell division in muscle cells and negatively impact upon development as well as muscle repair and growth after injury. Importantly, mutations in genes encoding both cytoskeletal and LINC complex components in humans are known to cause muscular dystrophy and cardiomyopathy (Puckelwartz et al., 2010; Bollen et al., 2017). This implies that pathological responses to mechanical strain such as those seen in progressive heart muscle diseases such as cardiomyopathy can be propagated by different mechanodomains via different mechanistic pathways to converge on the same phenotypic outcome of cardiomyopathy. Ultimately, a more in-depth dissection of the underlying disease mechanisms may facilitate design of treatment strategies. An encouraging example came from a recent study describing the role of mechanical tension in facilitating regeneration of zebrafish heart tissue, where it impacts on DNA endoreplication to elicit functional repair of tissue (Cao et al., 2017).

In summary, this intriguing study has left us with as many questions as answers, but it has opened up the possibility that by using simple model systems, differential mechanopathways can be dissected to reveal yet more surprising biological pathways that are regulated by physics.

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