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Review

Going nuclear: Molecular adaptations to exercise mediated by myonuclei

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ABSTRACT

Muscle fibers are multinucleated, and muscle fiber nuclei (myonuclei) are believed to be post-mitotic and are typically situated near the periphery of the myofiber. Due to the unique organization of muscle fibers and their nuclei, the cellular and molecular mechanisms regulating myofiber homeostasis in unstressed and stressed conditions (e.g., exercise) are unique. A key role myonuclei play in regulating muscle during exercise is gene transcription. Only recently have investigators had the capability to identify molecular changes at high resolution exclusively in myonuclei in response to perturbations *in vivo*. The purpose of this review is to describe how myonuclei modulate their transcriptome, epigenetic status, mobility and shape, and microRNA expression in response to exercise *in vivo*. Given the relative paucity of high-fidelity information on myonucleus-specific contributions to exercise adaptation, we identify specific gaps in knowledge and provide perspectives on future directions of research.

Background

Skeletal muscle comprises approximately 40% of body mass in adult humans and plays an integral role in whole body energy metabolism, glucose homeostasis, and locomotion.[1] Due to its abundance and central role in human health, biomedical research elucidating underlying mechanisms regulating muscle mass and function is of great importance. It is well established that exercise regulates the maintenance of healthy skeletal muscle throughout the lifespan.[2,3] Regular physical activity reduces the risk of chronic disease, thus lowering burden on the health care system.[4–8] Skeletal muscle is a plastic tissue that adapts in response to many stimuli and demonstrates distinct adaptations to varied exercise modalities (e.g., resistance, endurance, and concurrent or combined exercise). Muscle adaptations to exercise include loading-induced muscle growth (e.g., hypertrophy) as well as metabolic and contractile transformations (e.g., fiber-type transitions and mitochondrial accumulation/remodeling to accommodate the demands placed on the muscle cell).

At the center of adaptations in skeletal muscle are the nuclei- the "brains" of the cell- called myonuclei. Most cell types throughout the body have a single nucleus; however, the long, cylindrical, and voluminous skeletal muscle fibers (myofibers) contain hundreds to thousands of myonuclei. Myofiber multinucleation is the result of the fusion of numerous mononucleated precursor cells during development.[9,10] These precursor cells ultimately become muscle stem cells, or satellite cells (SCs), in adult skeletal muscle. The myofiber syncytium is believed to be post-mitotic. Myonuclei do not undergo division, nor is the myofiber thought to divide or "split" at an appreciable level.[11,12] Thus, fusion of SCs is required if additional myonuclei or myonuclear replacement is required. The precise cellular and molecular contributions of resident myonuclei versus SC-derived myonuclei in exercise adaptation are incompletely understood, making it an area of ongoing investigation.

Only recently have robust models emerged that allow for the dissection of specific molecular contributions from myonuclei to *in vivo* mammalian adult skeletal muscle exercise adaptation.[13] The purpose of this review is to outline contemporary knowledge on the role of myonuclei, both resident and SC-derived, during exercise adaptation *in vivo*. Many studies infer myonuclear contributions to exercise adaptation from tissue samples, which is a reasonable approach, but we aim to focus on studies specifically and intentionally examining myonuclei in adult muscle using direct measures. In presenting this overview, we identify

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Abbreviations

Satellite cells (SCs)
Fibro/adipogenic progenitors (FAPs)
Ribonucleic acid (RNA)
Ribosomal RNA (rRNA)
Synergist ablation-induced mechanical overload (SA)
Progressive weighted wheel running (PoWeR)
Resistance exercise (RE)
Endurance exercise (EE)
Deoxyribonucleic acid (DNA)
MYC proto-oncogene (*Myc*)
RNA-sequencing (RNA-seq)
Extracellular matrix (ECM)
Rho guanosine triphosphate hydrolyze enzyme (Rho-GTPase)
Myosin heavy chain (MyHC)
Tumor necrosis factor (TNF)
TNF-like weak inducer of apoptosis (TWEAK)
TNF receptor superfamily member 12A (*Tnfrsf12a*)
Fibroblast growth factor-inducible 14 (*Fn14*)
Wingless-related integration site (Wnt)
Nuclear Factor Kappa B (NF-κB)
Assay for transposase-accessible chromatin using sequencing (ATAC-seq)
Myostatin (*Mstn)*
Insulin-like growth factor 1 (*IGF1)*
Mechano-growth factor (*MGF)*
Matrix metallopeptidase 9 (*MMP9*)
Peroxisome proliferator-activated gamma coactivator-1 alpha (*PGC-1α*)
Peroxisome proliferator-activated receptor gamma (*PPAR-δ)*
Pyruvate dehydrogenase kinase 4 (*PDK4)*
Kilodaltons (kDa)
Electrical pulse stimulation (EPS)
MicroRNAs (miRNAs or miRs)
Myofiber enriched microRNAs (myomiRs)
Phosphoinositide 3-kinase (PI3k)
Protein Kinase B (AKT)
Glucose-6-phosphate dehydrogenase (*G6pdx)*
Histone 2B- Green Fluorescent Protein (H2B-GFP)
Single myonucleus RNA sequencing (smnRNA-seq)

specific gaps in knowledge and provide guidance on future directions of research.

A brief primer on heterogeneity of nuclei in skeletal muscle

The skeletal muscle environment contains a diversity of nuclei found in mononuclear cells outside of the multinuclear myofiber. These cells include SCs, fibro/adipogenic progenitors (FAPs), immune cells, endothelial cells, and tenocytes, to name a few. Under resting conditions, myonuclei comprise ~50%–70% of all nuclei within the muscle tissue.[14,15] When murine muscle is subject to acute mechanical overload (a rapid hypertrophic stimulus), the myonuclear proportion can drop to ~30% of all nuclei.[14] This shift in proportion is primarily due to infiltration and proliferation of non-muscle cell types such as fibrogenic and immune cells (e.g., macrophages and neutrophils). The relative proportion of myonuclei at rest, as well as the changes that can occur under dynamic muscle loading conditions highlights the complexity of myonuclear contributions to adaptation. It can be reasonably inferred that certain genes are being expressed by myonuclei in muscle tissue since they are muscle fiber-specific (e.g. myosin heavy chains, skeletal muscle actin, myoglobin, muscle creatine kinase, etc.), but advances in single cell RNA-sequencing technology reveal the diverse influence of mononuclear cell types to the overall gene expression profile of muscle in stressed conditions.[16] Furthermore, myonuclei have specificity for maintaining specialized regions of the myofiber; examples include the cell body, neuromuscular junction, and myotendinous junction-associated myonuclei.[17,18] Myonuclear subpopulations can complicate the interpretation of myonuclear contributions to exercise. There are clear differences in myonuclear density according to myosin fiber type,[19–22] which points to fiber type-specific differences in myonuclear behavior. The specific influence of SC-acquired myonuclei during exercise adaptation are also poorly understood. Overall, the molecular contributions of myonuclei to exercise adaptation is an area of open inquiry.

The regulation of transcription according to myonuclear number in response to loading

An intuitive task that the myonucleus performs to support exercise adaptations is transcription of muscle-specific genes coding for contractile elements, as well as excitation-contraction coupling, extracellular matrix, metabolism, and ribosomal genes. The latter is especially prevalent since ~85% of RNA in muscle is ribosomal RNA (rRNA).[23] One potential method to increase transcription of protein coding genes and rRNA as adult myofibers adapt to exercise is more myonuclei. It is well-established that myonuclear accretion occurs via fusion of SCs to the myofiber following endurance, resistance, and concurrent exercise, in the presence or absence of myofiber hypertrophy.[19,24–37] Irrespective of the cause of myonuclear addition in response to exercise, how new myonuclei contribute to myofiber adaptation at the molecular level can be difficult to discern.[38] The reason for a lack of specific evidence is largely technical; it is difficult to track, isolate, and interrogate myonuclei in a syncytial cell *in vivo*.[39] Using different genetically modified mouse models, recent evidence suggests that newly-fused myonuclei contribute specific transcription factors and ribosomal proteins to growing myofibers as a consequence of synergist ablation-induced mechanical overload (SA) or high-volume hypertrophic progressive weighted wheel running (PoWeR).[40,41] Apart from these recent studies, it is assumed that increased myonuclear number generally amplifies transcriptional potential, which could support adaptation in myofibers.[35,42] Interestingly, on a per-nucleus basis, global transcription rate in the early phases of loading-induced hypertrophy appears lower when myonuclei are added to an adult muscle fiber than when not added.[43] Apart from the regenerative roles of SCs in response to highly damaging exercise,[38,44] there is minimal evidence regarding the specific myonuclear contributions to adult muscle fiber hypertrophy in the context of exercise.

Myonuclear transcriptional responses to resistance-type exercise *in vivo*

Following SA mechanical overload of the mouse plantaris, which is a well-documented hypertrophic stimulus, transcription upregulates up to 7-fold in muscle tissue within a few days.[43] Up to 14 days after overload, the majority of transcription in muscle during hypertrophic loading is reportedly myonuclear.[43] Without myonuclear accretion from SCs, resident myonuclei can upregulate transcription during myofiber hypertrophy, providing evidence of myonuclear transcriptional reserve in growing adult muscle.[43] Given most RNA in muscle is rRNA, and rRNA increases dramatically in response to resistance exercise (RE) but not endurance exercise (EE),[45] it can be inferred that a large proportion of myonuclear transcription is ribosomal. Ribosome biogenesis is a process thought to be essential for sustained loading-induced muscle growth.[46–48]

Global epigenetic profiling of myonuclei after acute SA in mice further points to growth-related transcription by myonuclei.[14] Generally, myonuclear DNA hypomethylation occurs after acute short-term loading.[14] Specifically, hypomethylation of the promoter region of the oncogene and ribosome biogenesis-related transcription factor *Myc* occurs with SA, along with promoter sites in numerous muscle growth and autophagy genes.[14,45] There is also differential methylation in areas of ribosomal DNA.[14,45] It is unclear whether myonuclear methylome changes after an acute or short-term phase of muscle loading (hours to days) are persistent for a long period of time after loading has ceased. RNA-sequencing (RNA-seq) in myonuclei of short-term overloaded muscle corroborates upregulation of *Myc* at the gene expression level.[49] Recent evidence suggests the powerful transcription factor *Myc* is central to the muscle hypertrophy-associated gene expression program.[49] Myonuclear RNA-seq further reveals robustly elevated levels of extracellular matrix (ECM) and Rho-GTPase genes by the myofiber during the early phase of rapid muscle hypertrophy.[49] Although myonuclei may account for a large portion of transcription in muscle tissue during hypertrophy, other cell types such as SCs and fibro/adipogenic progenitors also contribute to muscle gene expression in various ways and to varying degrees.[49] Using single myonuclear RNA-seq, evidence indicates the presence of SCs may influence myonuclear transcription in response to acute PoWeR exercise independent from fusion to the myofiber.[17,50]

In addition to murine studies evaluating the acute myonuclear gene expression contribution to myofiber growth, human studies report gene expression in isolated myofibers following a bout of RE and with training. It is assumed most genes detected by this method are transcribed by myonuclei. In young adults, fast-twitch myosin heavy chain (MyHC) 2A fibers are more responsive than MyHC 1 fibers to acute RE at the gene expression level; this included a variety of genes implicated in myofiber hypertrophy.[51,52] One gene, the TWEAK receptor (*Tnfrsf12a* or *Fn14*), is highly upregulated by acute RE as well as training in pools of MyHC 2A fibers. Young adults are also more responsive than older adults to acute and chronic RE, regardless of myofiber type- in line with the observation that older adults are less responsive to RE training.[52–54] However, caution should be used when interpreting isolated myofiber gene expression. Mononuclear cells such as SCs can adhere to myofibers after mechanical isolation,[14] potentially influencing gene expression profiles. To this point, activated muscle stem cells are the cell type most enriched for *Fn14* in skeletal muscle.[16]

With respect to resistance-type training, a few studies evaluated myonuclear epigenetic contributions to adaptation and show responses to chronic exercise may be partly distinct from acute exercise. Eight weeks of PoWeR alters the myonuclear DNA methylation landscape in resident myonuclei (e.g., myonuclei present at the start of training) of the plantaris muscle of young mice.[37] Genes in myonuclei with training-induced promoter region hypomethylation correspond with processes linked to protein turnover, mitochondrial biogenesis, and cellular remodeling, such as Wnt and NF-κB signaling. Following PoWeR in mice from 22 to 24 months of age, soleus myonuclear DNA methylation was characterized by global hypomethylation across genomic features including promoters.[36] A subset of genes with differential promoter methylation from training also have changes in gene expression corresponding with methylation status. Apart from these studies, there is little high-resolution myonucleus-specific *in vivo* information available regarding hypertrophic exercise adaptation.

The current evidence collectively indicates that myonuclear transcription contributes to muscle hypertrophy with resistance-type exercise *in vivo*, and that complimentary processes such as ECM gene expression and *Myc* induction are regulated in the myofiber. Some changes in myonuclear gene expression are linked to changes in the DNA methylation status of myonuclear DNA. Applying modern technologies such as single myonuclear RNA-seq and ATAC-seq to exercised muscle tissue *in vivo* will provide more granular insight on myonucleus-autonomous events regulating hypertrophy.

Myonuclear gene expression responses to resistance-type exercise *in vitro*

In vitro models of RE utilizing electrical pulse stimulation (EPS) have emerged as a method to study muscle adaptation to exercise.[55–57] Such models can provide insight into the specific contributions of myonuclei to hypertrophic exercise since only myogenic cells are used to generate myotubes in culture. In murine-derived C2C12 myotubes, these models reveal a decreased expression of *MSTN* and increased expression of *IGF-1, MGF, and MMP9*.[55,56] In one model, altered gene expression overlaps significantly between acute myotube stimulation in culture and *in vivo* RE in human muscle tissues; however, *in vitro* RE did not mimic the epigenetic response to *in vivo* RE.[56] The dissimilarity in epigenetic modifications between *in vitro* and *in vivo* RE may be due to contraction type and mechanical factors (e.g., deformation, maximum tension, and changes in muscle length that occur *in vivo*), or indicate that epigenetic modifications are differential *in vitro* versus *in vivo,* perhaps due to the influence of non-muscle cell types. Hypertrophic stimuli may also modulate translocation of nuclear proteins (e.g., transcription factors). Capsaicin-induced hypertrophy of myotubes increase the migration distance of high molecular weight (55–110 kDa) nuclear proteins derived from a given myonucleus in the syncytium. Uptake of proteins generated by neighboring myonuclei may function as a signal to coordinate transcription during growth, but more work is needed to understand the mechanisms of this process.[58]

Myonuclear gene expression responses to endurance-type exercise

To our knowledge, investigators have not performed *in vivo* models of EE in conjunction with myonuclear isolation. As a surrogate, exercise models utilizing EPS *in vitro* with C2C12 myotubes are used to mimic *in vivo* models of EE. These models involve varying stimulation frequency, amplitude, and duration used to simulate training in myofibers.[57,59–62] An *in vitro* approach can provide some insight on myonucleus-specific contributions to EE adaptation without the influence of other cell types. Using these models, upregulated transcription of the MyHC 1 gene, myogenic factors, and genes involved in mitochondrial biogenesis such as *PGC-1α* occurs in myotubes *in vitro*.[57,59–62] An *ex vivo* model of EE in isolated mouse soleus myofibers similarly demonstrates enhanced gene expression of *PGC-1α*, *PPAR-δ*, and *PDK4* concomitant with hypomethylation of their respective promoters.[63] The regulation of these genes in myofibers can drive fiber type transitioning and metabolic changes.[64–67] The gap in knowledge regarding direct measures of myonuclear gene expression *in vivo* with EE training warrants further attention.

Myonuclear mobility and morphology with exercise

The myonucleus may modulate transcription to promote exercise adaptation by moving within the myofiber, changing shape, and functioning as a mechanosensor.[68–70] Muscle damage often occurs in response to unaccustomed exercise and eccentric contractions.[71,72] Emerging evidence suggests following acute contraction-induced muscle damage, myonuclei can move along the myofiber to the site of injury to aid in localized delivery of mRNA and enhance protein synthesis for muscle sarcomere repair.[73] Myonuclei are generally positioned at the periphery of the cell along the path of capillaries and will realign themselves as new capillaries form.[74] Myonuclear spatial organization may also "optimize" domains without physically disrupting the continuity of the myofibril network.[42,70,75,76] When myonuclei move from a more peripheral location to a site of damage after more severe injury, they may relocate to the center of the myofiber and disproportionally contribute to transcriptional activity.[77] Following a week of wheel running in mice, *bona fide* myonuclei that were genetically labeled prior to exercise are found in the center of myofibers.[78] Perhaps this

myonuclear translocation is associated with movement toward sites of sarcolemmal and/or sarcomere repair to facilitate adaptation to exercise. Recent evidence also suggests that myonuclei are less elongated with different types of exercise training in rodents.[78,79] Myonuclear shape change with exercise could be related to their post-training transcriptional status and role as a mechanosensor.[69] Some evidence suggests that *PGC-1α* expression, which is induced by exercise in muscle, can alter myonuclear shape.[80] More work is needed to elucidate the function of myonuclear mobility and shape change in the context of exercise adaptation.

Myofiber enriched microRNAs (myomiRs) and their role during exercise

MicroRNAs (miRNAs or miRs) are short, non-coding RNA that affect mRNA stability and translational efficiency, consequently changing protein levels without altering the genetic code. A subclass of miRNAs known as myomiRs are enriched in striated muscle and are sensitive to EE and RE training.[81–83] As myomiRs are expressed almost exclusively in skeletal muscle myofibers, it can be inferred they are produced mostly by myonuclei. MyomiRs appear integral to regulating muscle growth, atrophy, and fiber type switching, but much is yet to be discovered about their functions in muscle.[84–86] The most abundant myomiR in skeletal muscle, miR-1, is a presumptive negative regulator of muscle mass by inhibiting factors in the IGF-1/PI3k/AKT axis, consequently blunting protein synthesis.[79,87] MiR-1 also inhibits the gene target of glucose-6-phosphate dehydrogenase (*G6pdx)*, the rate-limiting enzyme in the pentose phosphate pathway, potentially contributing to metabolic reprogramming during muscle loading.[88,89] MiR-1 decreases acutely and chronically in response to RE training.[77,90–94] Conversely, acute bouts of EE can increase miR-1.[83,95] MiR-206 is another myomiR relevant to exercise adaptations and could facilitate fiber-type transitioning by inhibiting transcriptional repressors of the MyHC 1 gene.[82,84,90,96] Worth mentioning, though, miR-206 is also abundant in SCs and subsets of FAPs.[97–101] Therefore, its presence in muscle tissue may not always be attributable to myonuclear transcription.

Myonuclear histone modifications and exercise

Histone modifications, including acetylation, methylation, phosphorylation, and ubiquitination, may epigenetically regulate gene expression.[102–104] Literature regarding exercise-induced histone modifications exclusively in myonuclei is scant, limiting the depth of our commentary. In mice, four weeks of voluntary running increases histone turnover (proxied by incorporation of H2B-GFP into nucleosomes) in conjunction with fewer histones.[105] These histone adaptations cause loosening of nucleosomes and may lead to increased gene expression. Following acute SA, histone H3 acetylation, which typically coincides with increased transcription, was greater in myonuclei.[43] Further, acute bouts of forced eccentric muscle contractions and downhill treadmill running in mice induced a transient elevation in phosphorylation and acetylation of histone H3 in myonuclei.[106] These studies indicate that exercise may modify histones in a manner that allows for modulating accessibility to the DNA sequence and thus gene expression.

Myonuclear "memory" of past training adaptations

In the context of sport performance, "muscle memory" usually refers to rapid re-acquisition of muscular strength or sport skills. The mechanism for this re-acquisition is at the intersection of motor learning, neuromuscular adaptations, and longer-lasting changes to skeletal muscle fibers after prolonged periods of detraining.[107] Several lines of evidence direct the molecular explanation for "muscle memory" towards myonuclei. At the cellular level, one proposed mechanism for muscle memory is permanence of myonuclei acquired by SCs during exercise training.[108] Recent review papers and cross-talk debates have discussed the plausibility of myonuclear permanence with detraining, atrophy, and aging in great detail.[109–115] In summary, the current evidence suggests that myonuclei gained during exercise training may constitute a muscle memory in the short term (weeks to months), but are likely not permanent over the long term. The maintenance of myonuclei gained during exercise training may therefore not be a definitive explanation for muscle memory. That said, myonuclear loss may also have the prerequisite of significant muscle atrophy (> 30%) and be muscle-type or myofiber-type specific.[78,109] Technical issues may also contribute this contested area of inquiry. For example, results from a recent meta-analysis indicate that denervation- the most commonly used model of myofiber atrophy in rodents- causes a significant increase in the number of SCs. Satellite cell behavior and/or abundance may influence the process of maintaining myonuclear number during atrophy.[109] It is also imperative to clearly identify the myofiber cell border to ensure non-myonuclear cells are not mis-identified. The complexity of myonuclear identification may exacerbate challenges when quantifying myonuclei via cross section or isolated single fibers. To navigate these challenges, future investigations should use models of genetic myonuclear labeling to ensure exclusively resident myonuclei are quantified.[13,78]

While data are limited in the current literature, other prospective mechanisms for muscle memory are long-lasting changes to methylation in certain regions of myonuclear DNA, as well as altered miRNA expression. DNA methylation is a dynamic process and, as previously discussed, both EE and RE alter the DNA methylome. One rodent study reported that after chronic PoWeR training and a period of detraining (return to baseline myofiber size), myonuclear DNA partially maintains its differential methylation status months later, particularly in genes involved in muscle hypertrophy.[37] Upon a month of retraining, previously trained mice have accelerated muscle hypertrophy. Studies utilizing human muscle tissue corroborate long-term changes to muscle DNA methylation after training.[116–118] There is some reversion of modifications to the DNA methylome to pre-training state after detraining. However, when previously trained individuals retrain, they more rapidly restore modifications to the DNA methylome, along with muscle mass/strength and myofiber size compared to untrained individuals.[116,118] A similar pattern is shown with respect to miRNAs. After two months of PoWeR training in mice, miR-1 levels are significantly reduced, which persists after six months of detraining.[78] Perhaps myomiR expression is subject to epigenetic regulation as well.

Collectively, the concept of cellular muscle memory has an evidence-based foundation, but the mechanisms of muscle memory are not fully elucidated. Myonuclear permanence over extended periods of time is one possibility. An alternative explanation is that epigenetic modifications to myonuclear DNA allow for genes involved in hypertrophic adaptation to be more readily expressed once training resumes, thereby facilitating more rapid adaptation.

Summary

Through recently developed technologies such as inducible myonuclear labeling and single myonuclear RNA-seq (smnRNA-seq), researchers can overcome the barrier of analyzing heterogenous nuclear populations in skeletal muscle. These advances can allow for the dissection of specific molecular contributions of resident and SC-derived myonuclei to exercise adaptation *in vivo*. Prior to the utilization of these modern technologies, it could be reasonably inferred that transcription is augmented in response to exercise partly due to fusion of SCs to the myofiber during both EE and RE training. Enhanced transcription may even occur in the absence of hypertrophy; some evidence suggests individual myonuclei in adult muscle can upregulate transcription several-fold. Alterations in myonuclear gene expression driving muscle hypertrophy with resistance-type exercise *in vivo* are linked to changes in DNA methylation status and myomiR expression, particularly in genes important for growth, autophagy, extracellular matrix remodeling, and ribosome biogenesis. Less is known about myonucleus-specific gene

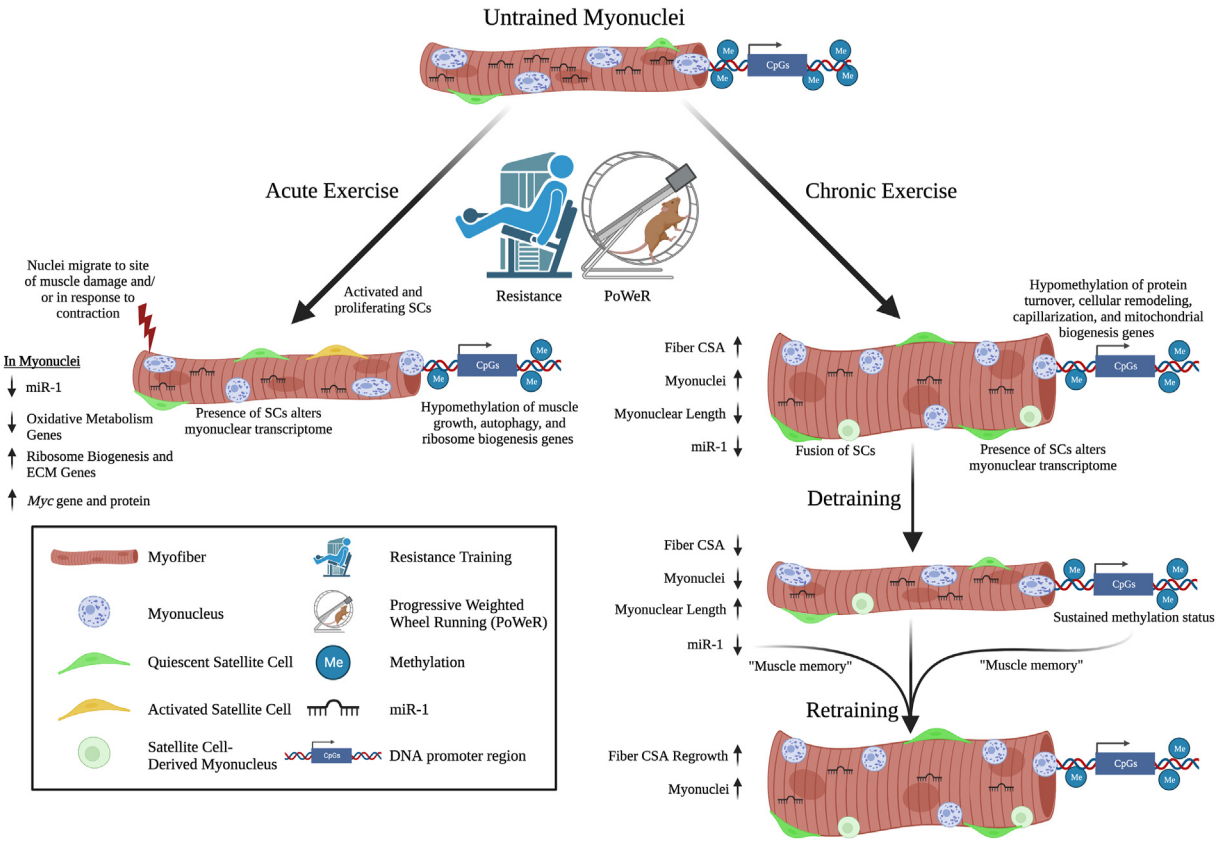

Fig. 1. With acute exercise, myonuclei can migrate along the myofiber to a site of damage and aid in localized mRNA delivery to facilitate muscle sarcomere repair. Myonuclear movement is likely of particular relevance with unaccustomed exercise featuring an eccentric (damaging) component, but some evidence also suggests that myonuclei can move as a consequence of contraction. Satellite cells activate and may proliferate in response to several acute exercise-induced signals. The presence of satellite cells can also influence the myonuclear transcriptome independent from fusion, as revealed by satellite cell loss-of-function studies. Epigenetic modifications that occur in myonuclear DNA after exercise include hypomethylation in promoter regions of genes involved in muscle growth, autophagy, and ribosomal biogenesis, along with lower expression of miR-1. Epigenetic modifications in muscle fibers may result in transiently reduced expression of oxidative metabolism genes, as well as increased expression of extracellular matrix and ribosomal biogenesis genes. With chronic exercise training, myofiber size and myonuclear density increase due to fusion of satellite cells, and a decrease in myonuclear length. The myonuclear transcriptome may be altered by the presence of satellite cells and epigenetic modifications to myonuclear DNA such as hypomethylation of genes involved in protein turnover, cellular remodeling, capillarization, and mitochondrial biogenesis, as well as chronically reduced expression of miR-1. Epigenetic modifications are sustained with detraining and may function as a "muscle memory" to potentiate a rapid re-acquisition of training adaptations upon retraining. Figure was generated using BioRender.

expression changes following endurance-type exercise *in vivo*. *In vitro* models show elevated mRNA expression of genes involved in fast-to-slow fiber type switching, myogenic factors, and mitochondrial biogenesis. Transcription profiles in resident and SC-derived myonuclei may be distinct and appear partly regulated by DNA methylation. Myonuclei may also have a muscle memory of past training adaptations. We posit this memory is more likely due to epigenetic modifications to the DNA methylome and/or changes in myomiR expression, and less likely myonuclear permanence. Precisely what drives epigenetic modifications in myonuclei is poorly understood. Taken together, the current evidence provides a preliminary understanding of the myonuclear functions that support exercise adaptations (Fig. 1), but there are still numerous gaps in knowledge.

Future directions in studying myonuclei

New models of murine exercise training are rapidly developing.[119,120] Combining murine exercise with pre-clinical models of genetic myonuclear labeling will provide a robust platform for studying myonuclear adaptations to different forms of exercise.[13,39] Developing new methods to track SC-derived myonuclei *in vivo* will further enhance these efforts. Directing effort toward understanding how SC-derived myonuclei contribute to specific fiber types could also be worthwhile since myonuclear addition with training can differ according to MyHC isoform.[25] In humans, the utilization of an antibody to specifically isolate myonuclei will enable high-resolution analysis of myonuclear adaptations to exercise,[121] although recent evidence suggests a more specific antibody may be required, specifically during times of muscle stress.[122] High-resolution sequencing of RNA, miRNA, chromatin accessibility, DNA methylation, and other molecular layers at single nucleus resolution in mice and humans will provide molecular maps of exercise adaptation across age ranges and sexes, and further reveal subpopulations and heterogeneity among myonuclei in dynamic conditions.

Submission statement

All authors have read and approve of the contents of the manuscript. While the manuscript is under review for this journal the manuscript will not be submitted elsewhere for review and publication.

Authors' contributions

Pieter J. Koopmans drafted the manuscript and created the figure. Kevin A. Murach and Kevin A. Zwetsloot provided critical feedback, edited, and revised the manuscript. All authors approve of the final version of the manuscript.

Conflict of interest

The authors have no conflicts to declare.

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