HIGHLIGHTED TOPIC | Signals Mediating Skeletal Muscle Remodeling by Activity

Histone modifications and exercise adaptations

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McGee SL, Hargreaves M. Histone modifications and exercise adaptations. J Appl Physiol 110: 258–263, 2011. First published October 28, 2010; doi:10.1152/japplphysiol.00979.2010.—The spatial association between genomic DNA and histone proteins within chromatin plays a key role in the regulation of gene expression and is largely governed by post-translational modifications to histone proteins, particularly H3 and H4. These modifications include phosphorylation, acetylation, and mono-, di-, and tri-methylation, and while some are associated with transcriptional repression, acetylation of lysine residues within H3 generally correlates with transcriptional activation. Histone acetylation is regulated by the balance between the activities of histone acetyl transferase (HAT) and histone deacetylase (HDAC). In skeletal muscle, the class II HDACs 4, 5, 7, and 9 play a key role in muscle development and adaptation and have been implicated in exercise adaptations. As just one example, exercise results in the nuclear export of HDACs 4 and 5, secondary to their phosphorylation by CaMKII and AMPK, two kinases that are activated during exercise in response to changes in sarcoplasmic Ca2+ levels and energy status, in association with increased GLUT4 expression in human skeletal muscle. Unraveling the complexities of the so-called “histone code” before and after exercise is likely to lead to a greater understanding of the regulation of exercise/activity-induced alterations in skeletal muscle gene expression and reinforce the importance of skeletal muscle plasticity in health and disease.

chromatin; gene transcription

SKELETAL MUSCLE is a highly plastic tissue that adapts to exercise/activity interventions by increasing its metabolic capacity and/or mass. Over the last 10–20 years, numerous studies have demonstrated that exercise increases expression of various myogenic and metabolic genes, usually by increasing rates of transcription. The importance of chromatin remodeling in regulated gene expression has also become evident, with a focus on post-translational modifications to histones that determine the spatial relationships between genomic DNA and the histone core and access of transcriptional regulators to the promoter regions of target genes. This review briefly summarizes recent insights into histone modifications in response to exercise and implications for exercise/activity-dependent alterations in skeletal muscle gene expression.

CHROMATIN AND REGULATED GENE EXPRESSION

In all eukaryote cells, DNA is wound around a core of histone proteins, with the resulting structure referred to as chromatin. The functional unit of chromatin is the nucleosome, which is comprised of a 147-bp section of DNA wrapped ~1.7 turns around a core octamer of histone proteins that includes two of each of the histones H2A, H2B, H3, and H4 (20). Early research viewed chromatin as a static structure that was primarily involved in DNA packaging and compaction; however, in recent years this paradigm has changed (33). It is now recognized that chromatin, and in particular the nucleosome, exhibits dynamic properties that play a central role in regulated gene expression. Indeed, it is well recognized that regulated genes in a transcriptionally repressed state contain nucleosomes at their transcription start site (4). This impairs the ability of the transcriptional initiation complex (TIC) and RNA polymerase II (Pol II) to occupy this region and hence impairs transcription. Nucleosomes within a promoter region also compete with transcription factors for occupancy of cis-regulatory elements that are critical to the initiation of transcription (4). Where a cis-regulatory element is located near the middle of a nucleosome, it is inaccessible to transcription factors (4). In this situation, modification of chromatin structure that alters the spatial relationship between histones and DNA must occur to allow transcription factor binding. Once bound to DNA, transcription factors can recruit and promote assembly of the TIC on the promoter region (4; Fig. 1). Transcriptional elongation also involves chromatin modifications as Pol II requires access to DNA to traverse and read the coding region of a gene (16). However, these mechanisms appear to be distinct from those that regulate chromatin structure at promoter regions (16). Chromatin structure can be modified through a number of mechanisms, which together are termed “epigenetics.” These
mechanisms include, but are not limited to, DNA methylation, histone variants, and histone modifications (12). While these processes are well studied in a number of cell systems, their role in skeletal muscle biology is only just beginning to be explored (1), with the role of histone modifications in the regulation of skeletal muscle gene expression receiving the most attention.

**HISTONE MODIFICATIONS, ACETYLATION, AND HATs AND HDACs**

Histone modifications involve a number of different post-translational modifications to the lysine rich tail regions of histones, in particular H3 and H4 (16). These modifications include phosphorylation, ubiquitination, methylation, and acetylation (16). Each modification targets different amino acid residues and can have differential effects on transcription. For example, methylation at H3 lysine 4 is associated with transcriptional activation (40), while methylation at lysine 36 is associated with transcriptional repression (13). In addition, interdependency between different modifications also exists, such as phosphorylation of H3 serine 10, which facilitates acetylation of H3 lysine 9 (18). Recognizing and characterizing all of the potential interdependencies between these histone modifications in a context-dependent manner, which has been termed “the histone code,” will be one of the great challenges to modern biology. However, in general, histone lysine acetylation is associated with transcriptional activation (16). Lysine acetylation neutralizes the positively charged side chain on these residues and disrupts the electrostatic interactions with the negatively charged phosphate backbone of DNA (16). The resulting rearrangement in the spatial relationship between the nucleosome and DNA allows transcription factors access to cis-regulatory elements and initiation of the transcriptional cascade (16; Fig. 1). The opposing enzymes that regulate histone acetylation are histone acetyltransferases (HATs) and histone deacetylases (HDACs). As their names suggest, HATs catalyze the addition of acetyl groups to lysine residues, while HDACs remove them (22). As such, HATs are generally seen as transcriptional coactivators, while HDACs are considered transcriptional corepressors. It is thought that a balance in HAT/HDAC activity at a given promoter determines the level of histone acetylation and therefore transcription (22). Therefore, understanding the regulation of these enzymes is requisite to understanding regulated gene expression. While there is a scarcity of information on HATs, a growing body of evidence suggests that HDACs, in particular the class IIa subfamily of HDACs, play a fundamental role in skeletal muscle physiology.

**THE CLASS IIa HDACa AND SKELETAL MUSCLE**

The class IIa HDACs consist of HDAC4, 5, 7, and 9 and although ubiquitously expressed, they are enriched in striated muscle (22). These HDACs function by associating with the myocyte enhancer factor 2 (MEF2) family of transcription factors to repress MEF2 dependent transcription (19). The class IIa HDACs themselves do not possess intrinsic HDAC activity, but instead recruit a larger repressive complex that contains HDAC3 for this purpose (11). Whole body knockout of individual class IIa HDAC isoforms in mice produces phenotypes related to skeletogenesis (HDAC4; 39), endothelial...
dysfunction (HDAC7; 7), and exacerbated cardiac hypertrophy (HDAC5 and 9; 5). However, the class IIa HDACs appear to have redundant roles in regulating muscle gene expression, as an increase in the proportion of type I oxidative fibers is observed only when any two class IIa HDACs are ablated in skeletal muscle (31). This suggests that the class IIa HDACs play a fundamental role in muscle phenotype by repressing a program of oxidative genes. Indeed, some of the genes that are thought to be regulated by the class IIa HDACs include peroxisome proliferator activated receptor gamma coactivator 1α (PGC-1α), carnitine palmitoyltransferase 1 (CPT-1), medium chain acyl-CoA dehydrogenase (MCAD), hexokinase II (HKII), glycogen phosphorylase, and ATP synthase β (10). As an increase in the expression of many of these oxidative genes in skeletal muscle is a common response following exercise, this raises the possibility that the class IIa HDACs could play a role in skeletal muscle adaptations to exercise. Indeed, this question has been tested in Eric Olson’s (31) laboratory, where they examined muscle fiber type transitions in response to 4 wk of exercise training in wild-type mice or mice overexpressing HDAC5 in an inducible manner. These experiments showed that an increase in skeletal muscle HDAC5 was sufficient to impair the increase in type I oxidative fibers following exercise training (31). Although fiber type transitions are not often observed in human training studies, these data provide genetic evidence to suggest that regulation of the class IIa HDACs could be important in controlling gene expression responses to exercise.

REGULATION OF THE CLASS IIa HDACs

At present, two main regulatory mechanisms are thought to govern class IIa HDAC function. The best characterized is phosphorylation-dependent nuclear export. This mechanism is initiated when the class IIa HDACs are phosphorylated on multiple serine residues (21). These phosphate groups serve as docking domains for the 14–3–3 family of chaperone proteins, which export the HDAC out of the nucleus via a CRM1-mediated mechanism (21). When first identified, this was thought to be a calcium-dependent mechanism, but subsequent identification of multiple class IIa HDACs has established that this mechanism is also sensitive to perturbed energy balance and oxidative stress. Kinases that are known to mediate HDAC nuclear export include the calcium-calmodulin-dependent protein kinases 1, 2, and 4 (CaMKI, II, IV; 2, 21), protein kinase D (PKD; 6, 14), the AMP-activated protein kinase (AMPK; 26), and the AMPK related kinases, salt inducible kinase (SIK1; 3, 37) and Mark2 (6). Exercise is known to activate some of these kinases in skeletal muscle, suggesting that phosphorylation-dependent class IIa HDAC nuclear export could be a mechanism mediating muscle adaptation to exercise. The second regulatory mechanism controlling class IIa HDAC function is through ubiquitin-mediated proteasomal degradation. This process involves an E3 ubiquitin ligase attaching ubiquitin peptides to the HDAC, which then tags this protein for degradation by the proteasome (31). While this mechanism has not been extensively studied and the relevant E3 ubiquitin ligase has not yet been identified (31), it has been suggested to control skeletal muscle fiber type. Both of these regulatory mechanisms are thought to reduce the amount of functional HDAC at a promoter region, which would shift the balance of enzymatic activity toward HATs and transcriptional activation.

HISTONES, HDACs, AND MUSCLE PLASTICITY

As reviewed recently (1), epigenetic modifications have been implicated in the regulation of muscle development and in the adaptive responses of mature skeletal muscle to alterations in activity. In cultured, adult skeletal muscle fibers, electrical stimulation at 10 Hz, to mimic slow-twitch fiber activity, resulted in translocation of HDAC4, but not HDAC5, from the nuclear fraction to the cytoplasm (17, 35). This was associated with increased CaMKII activation and MEF2 transcriptional activity and the HDAC4 translocation in response to electrical stimulation was abolished by the CaMK inhibitor KN-62 (17). Caffeine, which increases sarcoplasmic reticulum Ca2+ release and activates CaMK, induces HDAC5 efflux from the nucleus and histone hyperacetylation at the MEF2 site of the GLUT4 promoter in C2C12 myocytes (29). Again, these effects were completely abolished in the presence of CaMK inhibitors. Reduced neuromuscular activity, as occurs with denervation, has been shown to increase the nuclear abundance of HDAC4 and alter the expression of genes encoding myogenic factors and the acetylcholine receptor (8, 9). These authors observed expression of both HDAC4 and CaMKII at the neuromuscular junction and hypothesized that HDAC4 could act as a “sensor” of local neuromuscular activity (8). HDAC9 also appears to be regulated by neuromuscular activity and influences chromatin acetylation and the expression of activity-responsive genes (28). Finally, recent work from Baldwin and colleagues has demonstrated that histone H3 acetylation and methylation are linked to the expression of myosin heavy chain (MHC) genes under normal conditions and the altered expression of type I, IIx, and IIb MHC genes in soleus muscle during hindlimb suspension, a model of reduced muscular activity (30).

HISTONE MODIFICATIONS AND EXERCISE

Our interest in examining the role of histone modifications in the skeletal muscle adaptation to exercise arose from our observation that a single bout of exercise increased GLUT4 gene expression in human skeletal muscle (15). The logical extension to histone modifications came from studies demonstrating the importance of MEF2 for skeletal muscle GLUT4 expression (38) and the regulatory interactions between MEF2 and the class II HDAC5 (10). We observed that a single bout of exercise reduced nuclear HDAC5 abundance and MEF2-associated HDAC5 in human skeletal muscle (24; Fig. 2), changes that were associated with increased MEF2 DNA-binding activity (26) and GLUT4 mRNA (24). In a subsequent study, we observed reductions in the nuclear abundance of both HDAC4 and HDAC5 following exercise (23). This was associated with an increase in global H3 acetylation at lysine 36, a site linked with transcriptional elongation, but no change in global H3 acetylation at lysine residues 9 and 14, modifications that have been linked with transcriptional initiation. However, this does not rule out these acetylation sites as being important in the adaptive response to exercise, as global acetylation patterns might not reflect promoter-specific acetylation. Future studies need to examine such changes on histones associated with the promoter regions of specific genes to gain a more complete understanding of how exercise may differentially...
affect transcription within skeletal muscle. In addition, more frequent sampling may be required to further assess the temporal relationships between various histone modifications and activity-induced alterations in gene transcription.

As discussed in previous sections, the nuclear export of class II HDACs 4 and 5 occurs after their phosphorylation by upstream kinases. There are well described links between HDAC4 and CaMKII (2, 6, 9) and exercise increases both CaMKII activity and phosphorylation (23, 32). Furthermore, activation of CaMKII appears to be critical for histone hyperacetylation and increased MEF2 binding to the GLUT4 gene after swimming in rats (36). HDAC5 is not thought to be a direct target of CaMKII, but may gain responsiveness to this kinase via oligomerization with HDAC4 (2). Of note, we have observed nuclear export of both HDAC4 and 5 in human skeletal muscle after exercise (23). In relation to exercise adaptations, another potentially important HDAC kinase is AMPK, which increases in nuclear abundance (25) and activation (23) in human skeletal muscle following exercise. We undertook a number of cell-based experiments and demonstrated that AMPK is indeed an HDAC5 kinase and increases GLUT4 transcription via this mechanism (27). Thus both CaMKII and AMPK may be involved in the regulation of nuclear HDAC4 and 5 levels and histone acetylation during exercise (23). Any interplay between these kinases in targeting the class IIa HDACs during exercise could be dependent on a number of factors, including exercise mode, intensity, and duration. However, it appears that activation of any one kinase is sufficient for class IIa HDAC derepression, resulting in the potential for redundancy between these two kinases. It should also be noted that exercise fails to increase the activity of other

![Diagram of histone modifications and exercise/muscle contraction](Fig. 3. Histone modifications regulate transcription in response to exercise. Increases in the AMP:ATP ratio and intracellular calcium concentration activate AMPK and CaMKII, which in turn phosphorylate the class IIa HDACs, resulting in their nuclear export. In addition, through as yet unidentified mechanisms, class IIa HDAC ubiquitination by an E3 ubiquitin ligase results in HDAC degradation by the proteasome. Both of these class IIa HDAC regulatory mechanisms increase histone acetylation around MEF2-dependent genes and activation of MEF2 dependent transcription.)
Activity-induced alterations in skeletal muscle gene expression
increased skeletal muscle GLUT4 expression following exer-
phorylation by CaMKII and/or AMPK, is associated with
nuclear export of the class II HDACs 4 and 5 following
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SUMMARY
Post-translational modifications (acetylation, methylation,
(phosphorylation) of histone proteins within chromatin are
important in the regulation of gene expression. It is likely that
exercise results in numerous such modifications, which medi-
ate exercise-induced alterations in gene expression. For exam-
ple, hyperacetylation of some histone residues, secondary to
nuclear export of the class II HDACs 4 and 5 following phosphorylation by CaMKII and/or AMPK, is associated with
increased skeletal muscle GLUT4 expression following exercise. Unraveling the complexities of the so-called “histone code” before and after exercise is challenging, but likely to
lead to a greater understanding of the regulation of exercise-
activity-induced alterations in skeletal muscle gene expression and reinforce the importance of skeletal muscle plasticity in
health and disease.
DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.
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