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*Review*

# Histone Modification: A Mechanism for Regulating Skeletal Muscle Characteristics and Adaptive Changes

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**Abstract:** Epigenetics is getting increased attention in the analysis of skeletal muscle adaptation to physiological stimuli. In this review, histone modifications in skeletal muscles and their role in the regulation of muscle characteristics and adaptive changes are highlighted. The distribution of active histone modifications, such as H3K4me3 and H3 acetylation, largely differs between fast- and slow-twitch muscles. It is also indicated that the transcriptional activity in response to exercise differs in these muscle types. Histone turnover activated by exercise training leads to loosening of nucleosomes, which drastically enhances gene responsiveness to exercise, indicating that the exercise training transforms the chromatin structure to an active status. Furthermore, histone modifications play a critical role in preserving the stem cell lineage in skeletal muscle. Lack of lysine-specific demethylase 1 in satellite cells promotes the differentiation into brown adipocytes during muscle regeneration after injury. H4K20me2, which promotes the formation of heterochromatin, is necessary to repress MyoD expression in the satellite cells. These observations indicate that histone modification is a platform that characterizes skeletal muscles and may be one of the factors regulating the range of adaptive changes in these muscles.

**Keywords:** skeletal muscle; epigenetics; histone modification; exercise training; histone turnover



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## 1. Introduction

Skeletal muscle shows high adaptive capability in response to physiological stimuli and different environments. Mechanical stress applied to the skeletal muscles by resistance exercise training leads to muscle fiber hypertrophy. Previous studies [1–3] with younger adult human subjects reported that the resistance exercise with an intensity of 60–80% of one repetition maximum (1RM) or 8–12 RM, which was performed three times per week for 6–16 weeks, successfully induced 15–32% fiber hypertrophy (vs. pretraining level) in the thigh muscles. The differences in the effects of training among different individuals have also been reported. Bamman et al. [4] reported that subjects could be classified into extreme responders, modest responders, and nonresponders depending on the magnitude of muscle fiber hypertrophy induced by resistance exercise training; the mechano-growth factor and myogenin gene expression were found to be upregulated in both extreme and modest responders. It has also been reported that the muscle mass gain responses after resistance exercise training are positively correlated with the expression levels of a particular microRNA, miR-378 [5,6]. These observations indicate that the exercise-induced effects on skeletal muscles differ between individuals, and a muscular mechanism limits the adaptive changes.

Epigenetic regulation in skeletal muscle adaptation to exercise training is a current topic of interest; however, limited information is available regarding this topic. DNA methylation at CpG sites is known to act as a transcriptionally repressive modification. Several previous studies [7–9] have reported that the hypomethylation at CpG sites in the DNA of the skeletal muscles is induced by a bout of exercise training, which in turn is associated with the upregulation of the expression of particular genes. Histone modification

is also recognized as an epigenetic system that plays a crucial role in transcriptional activity in the cell nucleus. Methylation at lysine 4 of histone 3 (H3K4) and H3 acetylation typically marks transcriptionally active promoters and gene bodies in various cells [10–12]. For instance, the histones present at the MyoD-binding sites in skeletal muscle cells are acetylated during embryonic myogenesis, which allows MyoD to regulate the expression of skeletal muscle-specific genes [13]. However, limited information is available regarding the histone modifications and their role in the regulation of skeletal muscle characteristics in vivo. This review highlights the histone modifications induced in the skeletal muscles and their biological significance.

## 2. Histone Modifications in Fast- and Slow-Twitch Muscles

Slow-twitch skeletal muscles are characterized by higher-energy metabolism. It is also known that slow-twitch muscle-specific characteristics are acquired through exercise training. The enhanced capability of mitochondrial oxidative phosphorylation in skeletal muscles by exercise training has been demonstrated in numerous rodent models. However, the limit of adaptive changes to exercise training in the skeletal muscles has also been shown in several studies [14,15]. Running training attenuated the characteristics of fast-twitch muscles and produced muscle fibers that were more oxidative and insulin sensitive without showing any phenotypic transformations [14,15]. Other previous studies have tested the transgene models. For example, Tadaishi et al. [16], using a transgenic mouse model, demonstrated that skeletal muscle-specific overexpression of peroxisome proliferator-activated receptor $\gamma$ coactivator-1$\alpha$ isoform b (PGC-1$\alpha$-b), which is known to be upregulated in response to exercise-related stimuli, exhibited the enhanced oxidative capacity of the mitochondria in skeletal muscle and longer endurance capability, whereas the gene expression of type I myosin heavy chain was not stimulated in the gastrocnemius muscle of the transgenic mice. Myozenin 1, a muscle-specific modulator of calcineurin activity, is exclusively expressed in fast-twitch fibers of the skeletal muscle [17,18]. Frey et al. [19] have reported that the phenotypic shift of muscle fibers from type II to type I did not occur in the fast-twitch gastrocnemius muscle of myozenin 1-deficient mice, although the distribution of type IIa fibers increased in these mice. Furthermore, lineage tracing performed using mice with *Myh7*-dependent reporter gene expression by Wang et al. [20], revealed that all fibers in the soleus muscle, but only a subset of fast-twitch fibers in the extensor digitorum longus muscle, activated type I myosin heavy chain gene transcription. These observations indicated that the limit for the fiber-type transformation capability lies between fast- and slow-twitch skeletal muscle fibers.

We have previously determined the genome-wide differences in histone modifications between fast-twitch plantaris and slow-twitch soleus muscles in adult rats using chromatin immunoprecipitation (ChIP) followed by next-generation sequencing analysis [21]. Active histone modifications, such as H3K4me3 and H3 acetylation, were prevalent at the transcriptionally activated loci in the plantaris muscle (Figure 1A,C). However, no correlation was observed between the distribution of active histone modifications and gene expression in the soleus muscle (Figure 1B,D). It was also noted that the distribution of total H3, which might reflect the level of nucleosome deposition, was slightly less at both upstream and downstream locations from the transcription start site of muscle-related loci in the soleus muscle than that observed in the plantaris muscle (Figure 1E,F). Enhanced muscle activity in the plantaris muscle by synergists' tendon transection resulted in significant upregulation of slow-twitch muscle-specific genes, while acetylated H3 distribution at these loci was decreased [21]. However, the expression of fast-twitch muscle-specific genes was not lowered by enhanced muscle activity due to H3K4me3 level maintained at these loci. Interestingly, we also found that fast-twitch muscle-specific genes, in which active histone modifications were prevalent, were already upregulated at birth, independent of the muscle activity [21]. Conversely, activation of other slow genes started after the beginning of voluntary locomotion, although the magnitude of the upregulation was less pronounced when compared to that of the fast-twitch muscles. These results suggested

that slow-twitch skeletal muscles have a unique epigenetic system that regulates the gene expression, which might be closely associated with muscle activity. It is also suggested that the gene expression unique for fast-twitch muscle is robustly regulated by histone modifications and disturbs the complete shift of characteristics toward slow-twitch type.

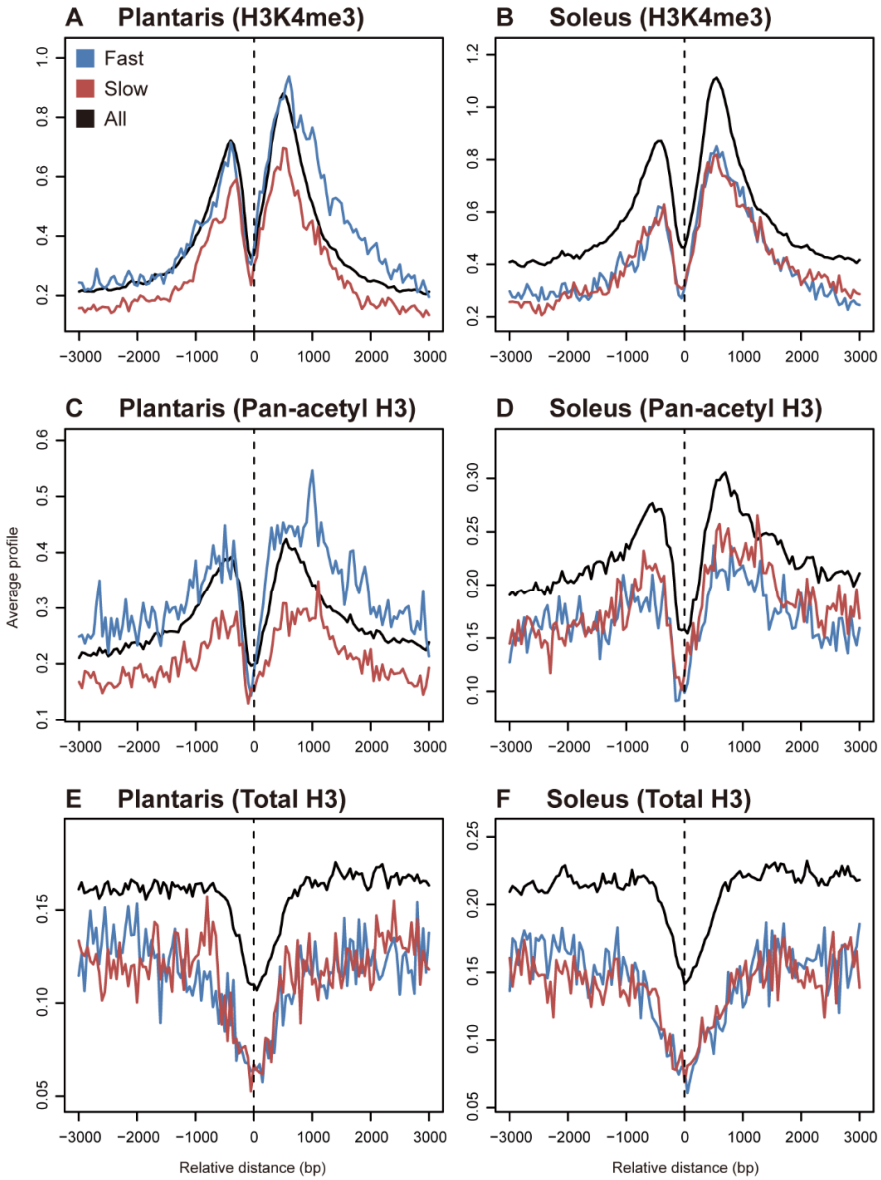


**Figure 1. Different histone modifications in fast- and slow-twitch-specific genes of rat plantaris and soleus muscles.** Distributions of transcriptionally active histone modifications, H3K4me3 (**A**,**B**), H3 acetylation (**C**,**D**), and total H3 (**E**,**F**) near the transcription start site (0 position) in the plantaris (**A**,**C**,**E**), and soleus (**B**,**D**,**F**) muscles were analyzed using ChIP followed by next generation sequencing. The distributions were averaged in fast-twitch-specific (blue), slow-twitch-specific (red), or all (black) genes. (**A**–**D**) were reproduced from Kawano et al. [21]. (**E**,**F**) were obtained from additional analysis of the results using the samples obtained from Kawano et al. [21].

Muscle-type-specific histone modifications affect the responsiveness of genes to exercise-related stimuli. Masuzawa et al. [22] reported that acute running exercise upregulated *PGC-1α* mRNA expression in both plantaris and soleus muscles in adult rats, whereas the transcripts from proximal exonic sequences were further enhanced in the plantaris muscle compared to those in the soleus muscle (Figure 2A,B). They also found that acute exercise-associated accumulation of RNA polymerase (Pol) II was significantly enhanced

at the proximal region of the *PGC-1α* locus in the plantaris muscle; H3 acetylation in this region was found to be more prevalent in the plantaris muscle than that in the soleus muscle (Figure 2C). In the soleus muscle, H3K27 acetylation (H3K27ac), which accelerates the transition of Pol II from initiation to elongation by altering the phosphorylation status at serine 5 to serine 2 [23], was enhanced throughout the *PGC-1α* locus in response to transcriptional activation by exercise, suggesting that elongating Pol II was capable of traveling through to the end of the locus. The sites at which the distribution of total and acetylated H3 and Pol II were synchronously elevated after acute exercise was observed in both plantaris and soleus muscles (Figure 2C), suggesting that the recruitment of Pol II was closely related to nucleosome deposition as well as histone acetylation.

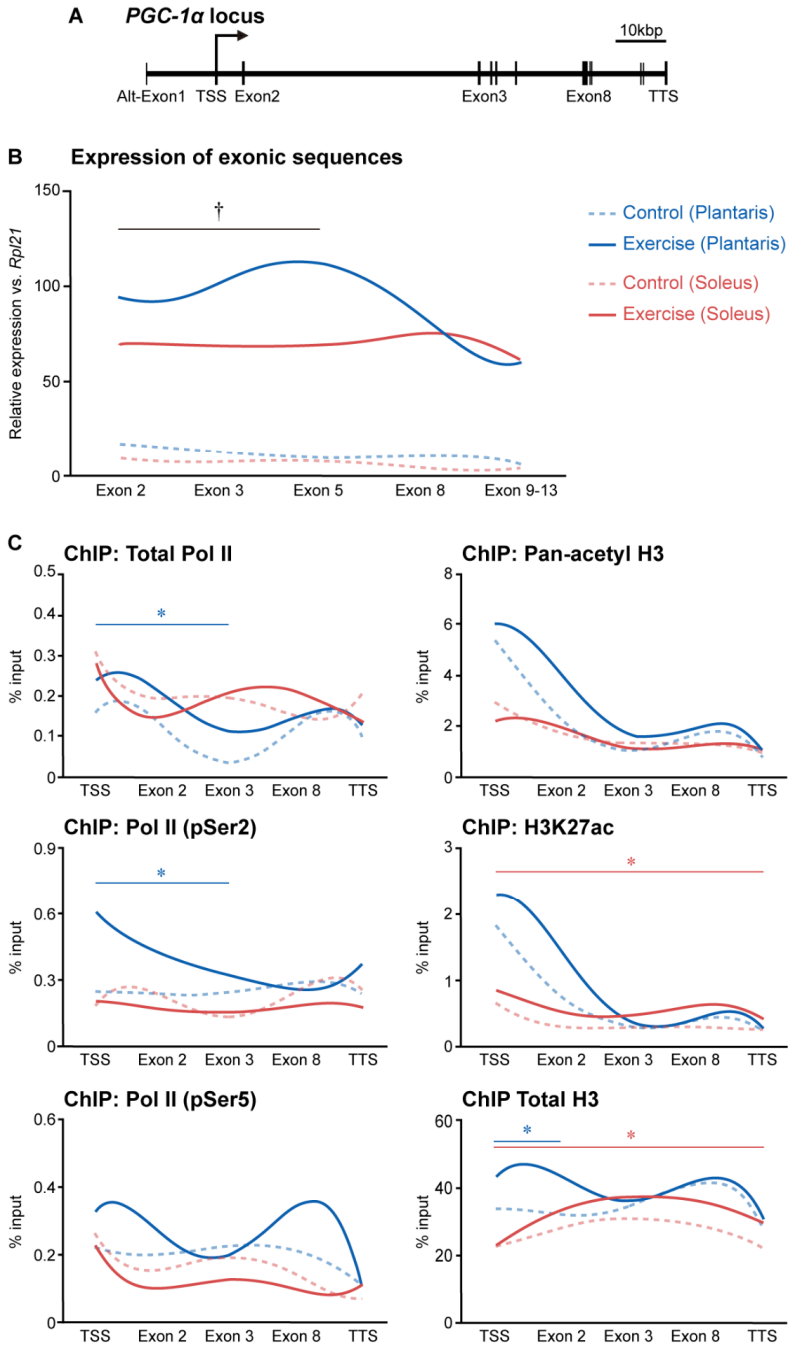


**Figure 2. Different response of *PGC-1α* gene expression to exercise in rat plantaris and soleus muscles.** (**A**) Rat *PGC-1α* locus showing the position of exons targeted for analysis. (**B**) Differential

upregulation of exonic sequences between the plantaris (blue) and soleus (red) muscles. Note that the expression of proximal sequences was more enhanced in the plantaris muscle, indicating higher transcriptional activity than soleus muscle. †: $p < 0.05$ in the plantaris vs. soleus muscles after exercise. (**C**) Distribution of RNA polymerase II (Pol II) and histone modifications analyzed by ChIP-qPCR, and their changes in response to exercise. Note that the accumulation of Pol II in response to exercise was significantly induced at the proximal sequences in the plantaris muscle, in which histone acetylation was prevalent. *: $p < 0.05$ in control vs. after exercise in the plantaris (blue) or soleus (red) muscles. The maximum length of the continuous regions from the TSS was selected by one-way ANOVA (**B**,**C**). The data were obtained from Masuzawa et al. [22] and combined between plantaris and soleus muscles.

## 3. Histone Modifications Regulated by Exercise

Acute and chronic resistance exercise alters histone modifications in human skeletal muscles. Lim et al. [24] reported that acute resistance exercise significantly upregulated the expression of 153 genes with gene ontology terms including muscle development, stress response, metabolism, cell death, and transcription factors. Upregulation of the distribution of acetylated H3, H3K4me1, and H3K27me3 and downregulation of that of histone variant H3.3 were observed at these loci after acute resistance exercise compared to the pre-exercise levels. After 10 weeks of resistance exercise training, the expression of these genes was maintained at a higher level than the pretraining level. The distribution of acetylated H3 at these loci was found to be upregulated after resistance exercise training. These results indicated that histone modifications in the skeletal muscles robustly responded to acute exercise, although chronic exercise training transformed the skeletal muscle chromatin toward the transcriptionally active status.

Nakamura et al. [25] reported that disuse atrophy was not induced in the hindlimb muscles of adult rats with prior running training experience in early life. They also found in RNA sequencing analysis of the plantaris muscle that a subset of genes that were upregulated in sedentary rats after unloading were less responsive in the rats with prior running training experience. The distribution of total H3 was significantly decreased at these loci during the training period (Figure 3A), suggesting that nucleosome formation was diminished by running training. The nucleosomes were restored after the detraining period, although the incorporation of histone variant H3.3 was noted in the rats with prior running training experience (Figure 3A). This result also shows that histone H3 is accumulated with age, independent of the experience of exercise training. Ohsawa et al. [26] further tested the effects of total amount or duration of running training on histone modifications in rat plantaris muscle. An exercise training-associated decrease in total H3 distribution was observed to be dependent on the amount of daily exercise when the ChIP-qPCR analysis was performed at the same loci targeted by Nakamura et al. [25] (Figure 3B). The results also showed that acetylated H3 accumulated during the running training if the total H3 distribution remained unchanged. These observations suggested that exercise training stimulated the dissociation of histones from nucleosomes as well as the exchange of histone components with newly synthesized histones such as H3.3, a phenomenon known as “histone turnover.”

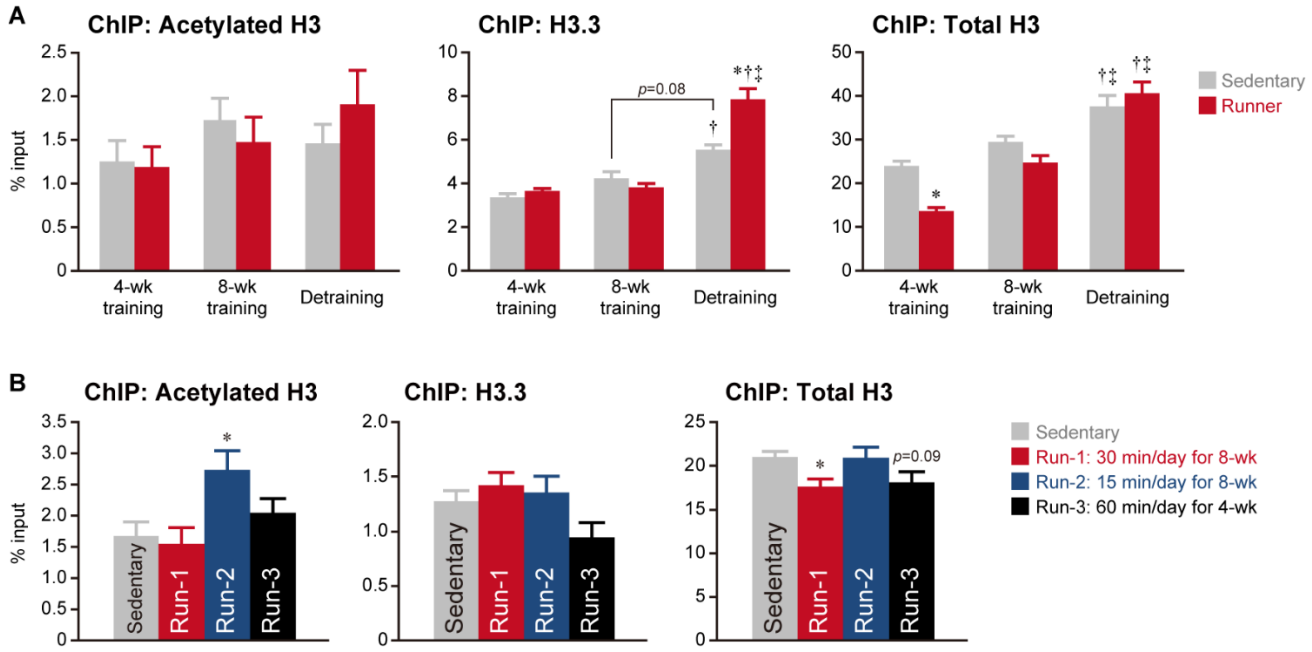


**Figure 3. Changes in histone modifications after exercise training in rat plantaris muscle.** (**A**) Distribution of histone modifications analyzed by ChIP-qPCR. Running training was performed using a treadmill (40 min/day) for 8 weeks followed by 8 weeks of detraining. The genes that were upregulated in response to muscular inactivation by tail suspension were targeted for the analysis. The data were obtained from Nakamura et al. [25] and re-analyzed for the statistics. Significant difference was tested by one-way ANOVA followed by Tukey's post hoc test. (**B**) Distribution of histone modifications analyzed by ChIP-qPCR after running training with different protocols. The data were obtained from Ohsawa et al. [26] and re-analyzed. The original result was represented as the relative distribution of acetylated H3 or H3.3 to the level of total H3. Since the alteration in total H3 distribution is also an important parameter, the data were separately shown in this review. *, †, and ‡: $p < 0.05$ vs. sedentary, and 4 wk- and 8 wk-trained group, respectively.

A nucleosome consists of DNA wrapped around an octameric protein complex composed of a histone H3-H4 tetramer flanked by two histone H2A-H2B dimers. It has been reported that H2A-H2B dimers and H3-H4 tetramers are independently exchanged in non-replicating yeast cells [27]. Histone turnover appears to correlate with gene transcription, as it regulates the accessibility of DNA to transcriptional machinery, such as transcription factors and Pol II [28,29]. Our recent study [30] has revealed that chronic exercise training activated histone turnover in the skeletal muscle fibers (Figure 4). Using a tetracycline-inducible H2B-GFP expression model, it was demonstrated that 4 weeks of running training significantly promoted the incorporation of H2B-GFP into nucleosomes as well as the dissociation of total histones at both transcriptionally upregulated and nonresponsive loci in mouse tibialis anterior muscle fibers; such effects were not observed upon 2 weeks of training. Gene expression responses to a single bout of running were significantly enhanced in 4 week-trained mice when compared to those in 2 week-trained mice. These results indicated that activation of histone turnover by exercise training led to the loosening of nucleosomes, which enhanced the gene responsiveness to exercise. It was further found that the levels of facilitates chromatin transcription (FACT), an H2A-H2B-specific chaperone, as well as several histone remodelers, including H3.3, H3K27me3, and H4K20me2, were upregulated in the myonuclei of the tibialis anterior muscle after 4 weeks of training. H3K27me3 [31] and H4K20me2 [32] are known to form a type of heterochromatin that represses transcription. Although it is still unclear how these heterochromatin-forming histone modifications are related to the activation of histone turnover in the skeletal muscle fibers, H3.3, which is also found to increase with postnatal age in rodent tissues [33], may play a key role in chromatin transformation during exercise training.

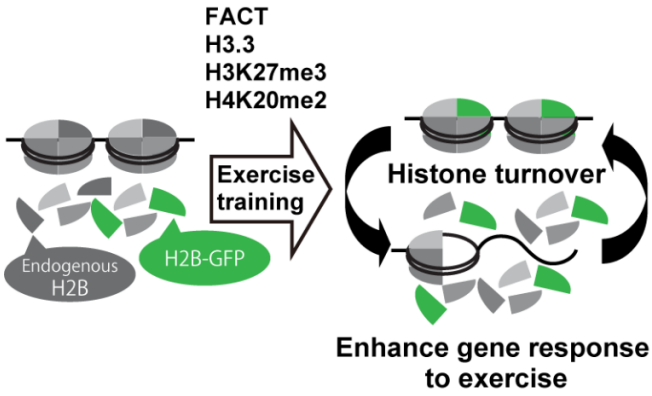


**Figure 4. Overview of exercise-induced histone turnover.** Tetracycline-inducible H2B-GFP expression model demonstrated the histone turnover in skeletal muscle fibers [30]. Since the histone turnover is referred to as the exchange of histone components with new histones, the incorporation of induced H2B-GFP into nucleosomes indicates the turnover of histone H2B. Upregulation of myonuclear facilitates chromatin transcription (FACT), and histone remodelers, such as H3.3, H3K27me3, and H4K20me2, were associated with the activation of histone turnover, which drastically enhanced the gene response to a single bout of exercise.

## 4. Skeletal Muscle Regeneration and Histone Modifications

Skeletal muscle has an excellent ability to regenerate after injury. Satellite cells, a postnatal source for the growth and regeneration of skeletal muscles, differentiate into muscle fibers following their proliferation and fusion, and/or incorporation into resident fibers [34,35]. Satellite cells in adult skeletal muscle are mitotically quiescent and express the paired-box transcription factor Pax7 [36]. Quiescent satellite cells become active in response to muscle injury. Upregulation of MyoD and myogenin expression in active satellite cells leads to the myogenic differentiation. A minority population downregulates MyoD, maintains Pax7, and returns to a quiescent state for self-renewal [37]. As the total number of satellite cells in adult muscles remains relatively constant following repeated muscle injury, the satellite cell population must be carefully maintained throughout the life. Histone modifications play a critical role in the self-renewal of satellite cells as well as their ability to regenerate skeletal muscle fibers.

Lysine-specific demethylase 1 (LSD1) is an epigenetic enzyme involved in transcriptional repression or activation of genes through the demethylation of mono- and di-methylated histone H3K4 [38,39]. Tosic et al. [40] have reported that satellite-cell-specific LSD1 knockout mice exhibited severe failure in muscle regeneration after injury. Surprisingly, loss of LSD1 in satellite cells promoted the differentiation into brown adipocytes. The researchers also confirmed this phenomenon in vitro using C2C12 myoblasts, in which H3K4me2 levels were significantly decreased at the locus of the pro-adipogenic factor Glis1, thereby enhancing the gene expression during muscle regeneration. Boonsanay et al. [32] demonstrated that SUV4-20H1, an H4K20 dimethyl transferase, controlled satellite cell quiescence by promoting the formation of facultative heterochromatin. Satellite-cell-specific SUV4-20H1 knockout mice exhibited decreased levels of H3K27me3 and H4K20me2 in the satellite cells, which triggered MyoD expression in these cells during muscle regeneration; loss of Pax7-positive quiescent satellite cells resulted in the severe failure of muscle regeneration. These observations indicated that histone modifications are necessary for fine tuning of gene expression that maintains the lineage and quiescence of muscle stem cells.

Canonical histones H3.1 and H3.2, and noncanonical histone H3.3 are incorporated into distinct loci and differentially function in association with unique histone modifications. H3.3 is exchanged with canonical histones at transcriptionally active loci, whereas it is also enriched at pericentric heterochromatin and telomeres in which the gene transcription is silenced [41]. It is known that transcription-activating H3K4me3 is prevalent in H3.1 and H3.2, whereas H3.3 is preferably modified by transcription-repressing H3K27me3 [42].

Harada et al. [43] reported that the incorporation of H3.3 into skeletal muscle gene loci was indispensable for the gene expression induced during myogenesis, suggesting that such incorporation potentiated the transcription of specific loci. Furthermore, H3.1 overexpression has been reported to replace H3.3 in the regulatory regions of skeletal muscle genes and induced a decrease in H3K4me3 and an increase in H3K27me3, resulting in the suppression of myogenic differentiation in C2C12 myoblasts [44]. They also showed that bivalent modification of H3K4me3 and H3K27me3 was frequently observed in H3.3-containing skeletal muscle genes in mouse embryos. Canonical and noncanonical histone-specific modifications and their selective incorporation into specific loci provide an appropriate balance of gene expression to maintain the lineage potential of muscle progenitors.

Initiation of embryonic and fetal myogenesis is preceded by the proliferation of Pax3-positive myogenic precursors [45,46]. During embryonic myogenesis, Pax3-positive cells migrate into the limb buds at E11–12, where most cells lack Pax7 expression [46]. Pax3 expression in the myogenic precursors is mostly downregulated before birth, and only a few cells in the lower leg muscles of adult animals appear to express Pax3 [47,48]. Previous studies [49,50] have also reported that Pax3- and Pax7-positive cells have a distinct function in regeneration. According to these observations, prenatal and postnatal cell sources might provide individual functions in the myonuclei after the differentiation into muscle fibers. We demonstrated that prenatally incorporated myonuclei in the transgenic mice with skeletal muscle-specific EYFP reporter expression driven by tetracycline administration persisted until at least 13 weeks of age, although these prenatal myonuclei were ablated by muscle regeneration after injury in adult mice [51]. We also tested the hypertrophic function in the regenerated fibers using the rat soleus muscle (summarized in Figure 5). Overloading by the tendon transection of synergists failed to induce compensatory hypertrophy in the regenerated fibers of adult rats, whereas unloading by tail suspension normally induced fiber atrophy. Loss of hypertrophying function correlated with the lowered histone acetylation at the *Igf1r* locus, which was one of the genes that did not respond to overloading in the regenerated muscle. These alterations in regenerated fibers were improved by the transplantation of cells harvested from juvenile soleus muscles of neonatal rats in association with enhanced histone acetylation at the *Igf1r* locus. These results suggested that prenatal myonuclei have a unique epigenetic feature that might enhance gene responsiveness to physiological stimuli later in the life.

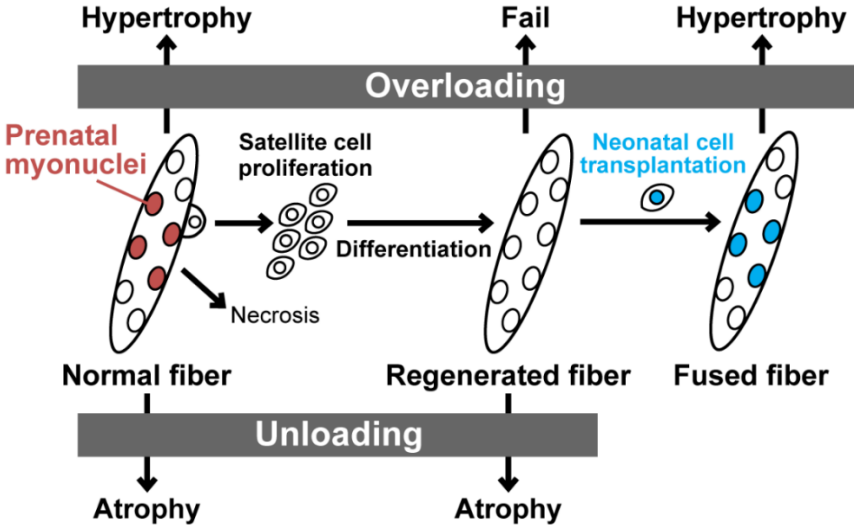


**Figure 5. Hypertrophic function programmed by prenatal myonuclei.** Myonuclei that are incorporated into muscle fibers during the development (prenatal myonuclei) persist in the adult muscle fibers. Muscle injury leads to a loss of the prenatal myonuclei. Although muscle fibers are regenerated via satellite cell proliferation and differentiation, the regenerated fibers fail to undergo hypertrophy in response to overloading. See the main text and Kawano et al. [51] for more details.

## 5. Muscular Disease and Histone Modifications

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant muscular dystrophy characterized by progressive wasting of facial, shoulder, and upper arm muscles [52]. FSHD1 (>95% of all cases) is caused by monoallelic partial deletion of D4Z4 repeat sequences at the subtelomeric region of chromosome 4q [53]. D4Z4 is a 3.3 kb macrosatellite repeat that contains an open reading frame for the double-homeobox transcription factor DUX4 retrogene, in which the transcription of DUX4 gene is strongly repressed in most adult tissues [54]. There are only 1–10 D4Z4 repeats in the contracted allele in FSHD1, in contrast to 11–150 copies in the intact allele. FSHD2 (<5% of all cases) is more rare form that exhibits no D4Z4 repeat contraction [55]. D4Z4 repeats contain transcriptionally repressive heterochromatin harboring DNA hypermethylation and H3K9me3 together with H3K27me3 [56,57]. The loss of DNA methylation and H3K9me3 at D4Z4 is the hallmark of both FSHD1 and FSHD2, strongly suggesting that FSHD is an epigenetic abnormality disease associated with the impairment of heterochromatin at D4Z4. Haynes et al. [58] also reported that H3K9 acetylation along with reciprocal loss of H3K27me3 at contracted D4Z4 was key epigenetic events that result in DUX4 expression. Lemmers at al. [59] found that the *SMCHD1* gene is mutated in >80% of FSHD2 cases. Zeng et al. [52] also found that the suppression of H3K9me3 results in displacement of SMCHD1 at D4Z4 and increases DUX4 expression in myoblasts. These observations indicate that epigenetic abnormalities possibly trigger a subtype of muscular dystrophy.

## 6. Age-Associated Changes in Histone Modifications

Epigenetics associated with age is one of the interesting fields of research and limited relevant information is available in the context of skeletal muscles. In a study on nonskeletal muscle tissue, Tvardovskiy et al. [33] established that the modifications on H3.3, which increased with age, varied with tissue types, in turn showing that H3K27me2K36me2 and H3K9me2K27me3 were negatively correlated. Yoshie et al. [60] showed that in the mouse tibialis anterior muscle, the incorporation of H3.3 into nucleosomes was significantly enhanced at lipid metabolism-related *Pdk4*, *Ucp3*, and *Zmynd17* loci in 13 month old mice compared to that observed in 3 month old mice. Zhou et al. [61] reported that H3K27 acetylation markedly increased in aged human skeletal muscles. This increase in H3K27 acetylation and enhancer activation was associated with the upregulation of genes related to the extracellular matrix in aged human skeletal muscles. Using a mouse model, they also showed that the treatment with an inhibitor of enhancer activation prevented the upregulation of extracellular matrix genes and restored the fibrogenic conversion of satellite cells. In skeletal muscle, the balance of histone modifications may shift toward a transcriptionally active status with age. Age-associated increase in H3.3 expression and its incorporation into nucleosomes are also unique alterations observed in the skeletal muscles as well as other tissues, which may contribute to changes in histone modifications. Further studies are needed to fully understand the age-associated changes in histone modifications and the mechanisms underlying skeletal muscle senescence.

## 7. Conclusions

This review focused on histone modifications in the skeletal muscles, which dynamically change in response to physiological stimuli. Overview of the roles of histone modifications in skeletal muscle is shown in Figure 6. Histone modifications, including the incorporation of histone variants into nucleosomes, alter the range of adaptive changes in the skeletal muscle fibers and satellite cells; the changes in histone modifications trigger enhanced gene responsiveness in the skeletal muscles and alter lineage-specific characteristics in satellite cells. Abnormal histone modification also leads to the pathogenesis and senescence in skeletal muscle. Interpreting the chromatin structure in the context of histone modifications can help us in understanding how an epigenetic regulation limits the adaptive changes in the skeletal muscles. However, more studies on the crosstalk be-

tween histone modification and DNA methylation are needed to understand the individual differences observed in epigenetic regulation in response to exercise and aging.

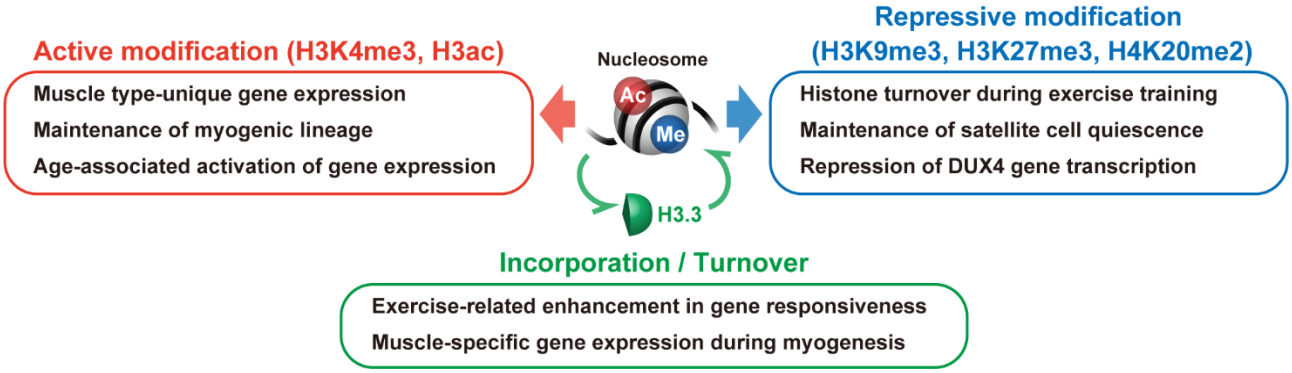


**Figure 6. Roles of histone modifications in skeletal muscle.** The roles of active and repressive histone modifications, and incorporation of newly synthesized histone, such as H3.3, into nucleosome and its exchange (turnover) are illustrated on the basis of this review.

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