Advances in DNA Methylation and Demethylation Regulation of Muscle Development: Postprint

Authors: Wang Bo, Luo Hailing

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Abstract

Muscle development is a complex biological process, and its regulatory mechanisms remain incompletely elucidated. In recent years, however, the regulatory role of epigenetic modifications in muscle development has gradually emerged as a prominent research area. Studies have demonstrated that DNA methylation and demethylation modifications play crucial regulatory roles in myogenesis and muscle development. DNA methylation modifications at specific loci in muscle stem cells influence the expression of key genes during muscle development, thereby regulating myogenic processes in early development. This article primarily discusses the dynamic changes of DNA methylation and demethylation modifications during muscle development, important methyltransferases and demethylases, and the role of nutrients in affecting myogenesis through DNA methylation modifications.

Full Text

Advances in the Regulation of Muscle Development by DNA Methylation and Demethylation

WANG Bo, LUO Hailing*
State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

Abstract

Muscle development is a complex biological process whose regulatory mechanisms remain incompletely understood. In recent years, the regulatory role of epigenetic modifications in muscle development has emerged as a hotspot, with studies demonstrating that DNA methylation and demethylation exert critical control over myogenesis and muscle development. Through DNA methylation modifications at specific sites in muscle stem cells, the expression of key genes during muscle development is affected, thereby regulating the myogenic process during early development. This review focuses on the dynamic changes in DNA methylation and demethylation during muscle development, important methyltransferases and demethylases, and the effects of nutrients on myogenesis via DNA methylation modifications.

Keywords: DNA methylation; demethylation; myogenesis; enzymes; nutrients

*Corresponding author, professor, E-mail: luohailing@cau.edu.cn

With the development of epigenetics, the corresponding epigenome has emerged. Epigenetic modification mechanisms primarily encompass three major components: DNA methylation [?], histone modifications [?, ?], and non-coding RNA [?]. DNA methylation, discovered in 1975 as an epigenetic modification [?, ?], represents the most extensively studied and deeply understood mechanism among epigenetic modifications. DNA methylation involves the covalent attachment of a methyl group from a methyl donor to the 5-carbon position of a cytosine nucleotide, forming 5-methylcytosine (5mC). This modification can exist relatively stably across plant, animal, and fungal models [?]. In animal models, this modification typically occurs in CpG-rich and symmetric regions of DNA strands, known as CpG islands, which are predominantly located in the promoter regions of housekeeping and developmental genes [?]. Methylation in these regions impedes transcription by preventing transcription factors and RNA polymerase from binding to the template strand through methyl-CpG-binding domain proteins, thereby inhibiting transcription [?], affecting gene expression levels in corresponding regions, and ultimately altering biological functions.

As a vital component of the body, muscle tissue development is also regulated by DNA methylation levels. Muscle tissue originates from mesodermal progenitor cells that proliferate, differentiate, fuse, and mature to ultimately form skeletal muscle fibers—a process known as myogenesis. Brunk et al. [?] demonstrated that the expression activity of the myogenic differentiation factor MyoD depends on a demethylated state, establishing the first direct link between DNA methylation and muscle cell differentiation. Subsequent studies on the transcriptional regulation of myogenesis by DNA methylation have attracted considerable interest, gradually clarifying the modification status of key genes and the critical regulatory enzymes involved. Moreover, with increasing attention to nutrition, nutrients have been found to influence muscle-related gene expression through DNA methylation, potentially altering physiological states. Therefore, investigating the relationship between DNA methylation and muscle development will enhance our understanding of muscle development processes and the regulatory effects of nutrition on muscle development.

1.1 DNA Methylation Mechanism

Numerous studies have confirmed that DNA methylation is a crucial epigenetic modification in mammals, participating in the regulation of X chromosome inactivation [?], genomic imprinting [?], silencing transposable elements to maintain genomic stability [?], and altering the transcription levels of numerous genes [?, ?]. The mechanism by which DNA methylation regulates gene expression has received the most attention. Early studies indicated that DNA methylation inhibits transcription factor binding to transcription start sites, thereby suppressing gene expression—a concept widely accepted by scholars. Recently, Schübeler [?] proposed five potential mechanisms for DNA methylation-mediated gene expression regulation (Figure [Figure 1: see original paper]): (a) methylation-insensitive transcription factors binding to transcription start sites can inhibit DNA methylation in that region; (b) transcription factors can bind to methylated start sites through a specific binding mode to enable transcription; (c) methylation-sensitive transcription factors cannot bind to regions where cytosines are methylated, thereby inhibiting transcription; (d) in CpG-rich start site regions (shaded in the figure), methylation facilitates binding to methyl-CpG-binding domain proteins (MBD), indirectly inhibiting transcription factor binding; and (e) methylation-insensitive transcription factors first bind to start sites, creating a hypomethylated binding site, after which methylation-sensitive transcription factors bind to ensure normal transcription initiation. Among these five models, models (c) and (d) are relatively well-established, while the other three require further validation.

DNA methylation can occur in multiple regions along the DNA strand, including both intragenic and intergenic regions, with different regional methylation exerting distinct effects. Studies show that intragenic methylation has diverse effects on transcription and can also regulate alternative splicing [?, ?], whereas intergenic DNA methylation inhibits gene activity by suppressing enhancers [?]. However, the function of non-CpG site methylation remains unclear. Thus, DNA methylation modifications play an important role in regulating gene expression and consequently affect tissue development processes.

1.2 Methyltransferases and Demethylases

DNA methylation and demethylation constitute a dynamic and plastic process during organismal development. In this process, DNA methyltransferases (DNMTs) and demethylases—the ten-eleven translocation (TET) enzyme family—play crucial roles in the formation, maintenance, and removal of methyl groups.

1.2.1 Methyltransferase Family

The primary function of DNMTs is to catalyze the transfer of methyl groups from S-adenosylmethionine (SAM) to the 5-carbon position of cytosine, forming 5mC [?]. The DNMT family includes DNMT1, DNMT3a, DNMT3b, and DNMT3L. DNMT1 mainly functions during mitosis, maintaining the DNA methylation pattern of the newly synthesized strand identical to that of the template strand during replication. DNMT3a and DNMT3b share high homology and primarily function in establishing new DNA methylation, though they act at specific developmental stages. DNMT3b mainly functions during early embryonic development, particularly during implantation, while DNMT3a acts primarily during later embryonic development and cell differentiation [?]. Additionally, some studies suggest that DNMT3a and DNMT3b also function to hinder DNMT1 activity during the maintenance of genomic methylation patterns in offspring [?]. DNMT3L assists DNMT3a and DNMT3b in establishing new methylation and promotes their action on chromatin [?]. Thus, the DNMT family plays a critical regulatory role in establishing and maintaining DNA methylation states.

1.2.2 Demethylation Regulatory Pathway

DNA methylation modifications can be eliminated through demethylation pathways in organisms. The TET family (including three isoforms: TET1, TET2, and TET3) plays an important regulatory role in this process. Under the action of TET enzymes, 5mC is first hydroxylated to 5-hydroxymethylcytosine (5hmC), then further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [?, ?], thereby eliminating 5mC, though whether 5caC can be converted back to cytosine through decarboxylation remains unclear. Additionally, the intermediate products 5fC and 5caC can be removed by thymine DNA glycosylase (TDG) through the base excision repair mechanism [?]. Seisenberger et al. [?] further proposed a possible mechanism for methylation clearance in organisms (Figure [Figure 2: see original paper]), including both passive and active demethylation pathways. Some processes in this pathway require further validation to complete the demethylation pathway. In organisms, both DNA methylation and demethylation processes involve key enzymes for regulation, and their specific regulatory processes may vary across different developmental stages to enable precise epigenetic modifications and maintain normal development. During myogenesis, DNA methylation and demethylation regulation also occur, and key regulatory genes are affected by epigenetic modifications.

2.1 Gene Networks Regulating Muscle Development

Skeletal muscle originates from mesodermal progenitor cells during embryonic development, which proliferate, differentiate, fuse, and mature to form skeletal muscle fibers. During muscle formation, cells progress through embryonic progenitor cells, satellite stem cells, committed satellite cells, myoblasts, myocytes, and myotubes/myofibers stages. Two major classes of regulatory factors participate in this process: myogenic regulatory factors (including Myf5, MyoD, Myf6, and MyoG) and upstream factors Pax3 and Pax7.

Pax3 and Pax7 belong to the evolutionarily conserved Pax family and play critical roles in regulating tissue differentiation and organ development, with expression that is not tissue-specific. During embryonic development, Pax3 participates in the formation and delamination of hypaxial trunk muscles and is required for the migration of myogenic progenitor cells to other myogenic regions such as limbs [?]. Pax3-expressing migratory cells give rise to Myf5- and MyoD-expressing cells, further promoting muscle generation and forming the satellite cell pool. During fetal muscle formation, Pax3 expression is downregulated, and Pax7 becomes the primary factor regulating all myogenic stem cells. In limbs, both Pax7 and Pax3 are initially expressed, and lineage tracing experiments indicate that all later Pax7-expressing cells originate from cells that previously expressed Pax3 [?]. Pax7 is also essential for maintaining the adult stem cell population. Before birth, Pax7 is not necessary for fetal muscle development, possibly because Pax3 can compensate for Pax7 function to some extent; however, after birth, Pax7 becomes essential for muscle development, likely due to functional divergence where Pax7 and Pax3 assume different regulatory roles in myogenesis. Thus, Pax3 and Pax7 are interdependent yet independent, coordinately regulating muscle development.

Myf5, Myf6, and MyoD are myogenic determination factors essential for muscle generation, while MyoG functions as a differentiation factor that, together with Myf6 and MyoD, regulates the differentiation of myotubes into mature muscle fibers [?]. Myf5 is the first myogenic regulatory factor expressed during muscle development and is crucial for skeletal muscle cell determination and differentiation during embryogenesis. Myf6 functions not only in skeletal muscle cell determination but is also activated during myotube differentiation, participating in this process. MyoD expression follows Myf5 expression in epaxial and hypaxial dermomyotomes, with its initial activity depending on Myf5 and Pax3, after which it can activate its own expression through feedback mechanisms [?]. MyoG expression is regulated by MyoD and Myf5, and during differentiation, MyoG and MyoD jointly activate terminal differentiation genes. As myogenic differentiation progresses, genes encoding structural and enzymatic muscle proteins such as -actin, troponin, tropomyosin, and creatine kinase are activated by MyoG. Therefore, myogenic determination and differentiation factors collectively promote myogenesis, with their distinct expression patterns and interactions finely regulating the muscle development process.

2.2 DNA Methylation and Demethylation During Myogenesis

During myogenesis, the methylation status of corresponding genes undergoes dynamic and complex changes. As cellular differentiation increases, overall methylation levels rise; however, gene expression becomes more specific, and methylation levels at certain special sites decrease in different cell types. This may occur because increased global gene methylation reduces cellular pluripotency, while cell type-specific gene expression during development requires decreased methylation at specific sites to ensure proper expression of specialized genes.

2.2.1 DNA Methylation

Different tissues and cells exhibit specific DNA methylation patterns. Comparing methylation patterns between muscle tissue and other tissues helps researchers understand how DNA methylation regulates muscle development. By comparing skeletal muscle with blood cells, sperm, brain tissue, and spleen cells, 178 specifically hypermethylated sites were identified in skeletal muscle [?]. Subsequently, Calvanese et al. [?] identified 47 genes significantly hypomethylated in skeletal muscle, some encoding contractile proteins such as obscurin, myocardin, and slow-contracting arrestin. Thus, different tissues display distinct methylation patterns to adapt to their unique structures and functions. Tsumagari et al. [?] compared methylation patterns between muscle cells and 30 non-muscle tissues, finding that 94% of differentially methylated sites showed hypomethylation in muscle, with 47% of these hypomethylated sites also present in myoblasts or myotubes, while only 3% of differentially methylated sites were hypermethylated in myogenic progenitor cells. This demonstrates that muscle methylation patterns are specific and dynamically change across myogenic cell stages to regulate stage-specific gene expression and ensure proper muscle development. The study also indicated that new methylation in muscle tissue occurs primarily before the myoblast stage, while demethylation occurs around the myotube formation stage, regulating transcription through this mechanism. TET1 and TET2 play important roles in activating demethylation and forming stable 5hmC products during this process. Tsumagari et al. [?] also reported that the Pax3 gene maintains high methylation levels in both myogenic cells and mature skeletal muscle, which may affect cell migration during myogenesis. However, Miyata et al. [?] found that during myogenesis, genomic methylation levels showed slight but significant increases from myoblasts to myotubes, with these hypermethylated sites located in promoter regions of genes (transcription factors ID4 and ZNF238) related to muscle contraction and other myogenic processes. This result differs from previous reports, possibly because their study used Illumina' s 450K DNA methylation bead chip, which did not distinguish between 5mC and 5hmC. These studies reveal that DNA methylation is specific and dynamic across different differentiation stages of myogenic cells, with methylation patterns varying even within the same stage, requiring further validation. Additionally, increasing attention has focused on DNA demethylation at specific sites to ensure proper expression of corresponding genes during myogenesis. Most scholars agree that methylation changes occur primarily before cell fate determination, with only minor modifications after developmental maturation, though the effects of post-maturation environmental stimuli remain poorly reported.

2.2.2 DNA Demethylation

During myogenesis, different regulatory factors participate at various differentiation stages, and their expression is related to DNA methylation status. Studies show that the 110 kb enhancer region of myogenic regulatory factors Myf5/Myf6 contains numerous enhancer elements. Carrió et al. [?] compared this region across embryonic stem cells, skeletal muscle stem cells, myoblasts, and myotubes, finding hypermethylation in embryonic stem cells but hypomethylation in skeletal muscle stem cells, myoblasts, and myotubes. This hypomethylation increased Myf5 expression and confirmed that DNA methylation specifically regulates the Myf5 enhancer during cell differentiation stages, primarily controlling myogenesis. MyoD is a key gene in myogenesis, and its function is also related to methylation status. Brunk et al. [?] found that the MyoD distal enhancer region was unmethylated in all myogenic cells but showed an average methylation level of 50% in non-muscle cells and tissues (liver, heart, brain, etc.). The study noted that while MyoD distal enhancer methylation does not directly block embryonic MyoD activity, maintaining a demethylated state may be necessary to generate specific developmental signals, enabling the enhancer to respond to further signaling and activate MyoD gene expression. MyoG activity is also closely related to methylation status. Lucarelli et al. [?] showed that MyoG expression in differentiated muscle cells depends on a hypomethylated state, while it remains methylated in non-muscle tissues (spleen, brain) and proliferating myotubes. Treatment of proliferating myotubes with methylation inhibitors increased MyoG expression due to reduced methylation levels. The MyoG promoter region requires a demethylated state to bind transcription factors, while in the methylated state, MBD proteins bind to the promoter region and prevent normal transcription [?]. Recent studies have also found methylation and demethylation in non-CpG regions of myogenic cells, though the regulatory role of methylation in these regions during myogenesis remains unclear and requires further investigation.

2.2.3 Roles of DNMT and TET in Myogenesis

During myogenesis, the activities of DNMT and TET are important for understanding methylation and demethylation processes and are essential for normal development. Due to their functional specificity, DNMT and TET exhibit distinct expression patterns during development. DNMT1 expression is downregulated during myogenic cell differentiation, consistent with its function. Gene knockout experiments have confirmed that the DNMT family is essential for normal development: embryos with DNMT1 knockout show approximately 70% reduced methylation levels and cannot develop normally [?]; mice with DNMT3a knockout survive only 4 weeks during embryogenesis, while DNMT3b knockout mice are not viable [?], demonstrating that DNA methylation is essential for development. Dawlaty et al. [?] knocked out TET family genes and found increased overall 5mC levels, indicating increased methylation. Further analysis of hypermethylated promoters and correspondingly downregulated genes revealed that downregulated genes were primarily involved in embryonic development and differentiation, with significant enrichment in skeletal muscle development pathways. Thus, DNMT and TET can regulate gene expression by affecting methylation and demethylation status, thereby influencing normal developmental processes, with myogenesis requiring the participation of both.

3 Effects of Nutrients on Muscle DNA Methylation

Tissue and cell development depends on nutrient supply, making nutrients important regulators of developmental processes. In recent years, research on nutrients regulating organismal development through epigenetic modifications has gradually increased, particularly during embryonic development and preweaning stages. Maternal protein levels, high-fat diets, imbalanced gestational nutrition, and overfeeding can all cause abnormal offspring development and increase susceptibility to diseases such as diabetes, obesity, and hypertension through effects on DNA methylation levels [?]. However, research on nutrients affecting DNA methylation modifications during myogenesis remains relatively limited. In the DNA methylation process, methyl donors can be provided by nutrients such as folate, methionine, choline, and betaine as precursor substances to participate in DNA methylation level regulation. This process also requires ATP participation, and nutrients can generate ATP during metabolism, potentially affecting the methylation process. In demethylation, TET enzymes require the tricarboxylic acid cycle intermediate -ketoglutarate for participation. Thus, nutrients may affect DNA methylation processes by altering cellular metabolic pathways.

Studies show that gestational protein restriction may affect offspring muscle oxidative phosphorylation through DNA methylation modifications, causing muscle dysfunction [?]. Human health research indicates that short-term high-fat intake significantly increases DNMT3a and DNMT1 expression in muscle, substantially altering DNA methylation patterns with poor plasticity [?]. This suggests that the methylation status of mature muscle cells is significantly influenced by nutritional regulation, with long-lasting effects. Oster et al. [?] showed that supplementing gestational pigs with methyl donors such as methionine, folate, choline, and vitamin B6 can regulate the offspring muscle insulin-like growth factor pathway, increasing offspring birth weight, while also confirming that these changes in offspring muscle have poor plasticity to dietary stimuli and can persistently affect offspring development. As an important component of organismal development, muscle development is significantly influenced by nutrients, though many unknowns remain regarding how nutrients affect muscle development through DNA methylation pathways. Research in this area will help deepen our understanding of nutritional effects on muscle development, enabling better regulation of muscle development.

DNA methylation plays an important regulatory role in muscle development. Under the catalysis of methyltransferases and demethylases, appropriate levels of DNA methylation modifications at specific sites in cells at different myogenic stages ensure the temporal and spatial specificity of important gene expression, maintaining normal myogenesis. However, since DNA methylation levels during muscle development constitute a complex dynamic process, current research remains incomplete. Factors or signals that stimulate methylation and demethylation during myogenic cell differentiation, the role of non-CpG site methylation in regulating muscle development, and nutrient regulation of muscle development through DNA methylation all require further investigation. Exploring epigenetic modifications during muscle development will help us precisely understand the regulatory mechanisms of muscle development and promote healthy animal growth and increased meat production.

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