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Temporal regulation of chromatin during myoblast differentiation

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Temporal regulation of chromatin during myoblast differentiation

Akihito Harada a, Yasuyuki Ohkawa a, Anthony N. Imbalzano b,*

1. Introduction .................................................................................................................................................. 78
2. The chromatin state of repressed myogenic loci ...................................................................................... 78
   2.1. Histone modifiers and modifications prior to myoblast differentiation ........................................... 78
   2.2. Chromatin remodeling and histone variants prior to myoblast differentiation ................................. 78
3. The chromatin state at active myogenic loci ............................................................................................... 80
   3.1. Changes in histone modifiers and modifications upon myoblast differentiation ................................ 80
   3.2. Chromatin remodeling and histone variants upon myoblast differentiation ...................................... 81
   3.3. Changes in higher order chromatin organization during myoblast differentiation ............................ 82
4. Conclusions ............................................................................................................................................... 83
Acknowledgement ....................................................................................................................................... 83
References ...................................................................................................................................................... 83
1. Introduction

The discovery of MyoD as a factor that could reprogram cells of other lineages into skeletal muscle-like cells [1] established the concept of lineage-determining transcription factors and made skeletal muscle differentiation a model system for understanding basic tenets about cellular differentiation (see also the article by Lassar in this issue). Subsequent work determined that lineage determination for skeletal muscle is mediated by the family of related basic helix-loop-helix transcription factors that includes MyoD, Myf5, Mef4, and myogenin [2]. Each recognizes a DNA sequence called an E box (consensus: CANNCTG) common to regulatory sequences of genes expressed during skeletal muscle differentiation [3], but, interestingly, these factors are dependent on cooperating transcription factors. Specifically, the members of the MyoD family interact with members of the ubiquitous E–protein family to form functional heterodimers [4] and with members of the Mef2 family of transcription factors to synergistically activate myogenic genes [5]; see also the article by Hughes and Taylor in this issue). Additional transcription factors promote expression of myogenic genes [6], leading to numerous combinations of factors that cooperate with MyoD family member proteins to drive skeletal muscle formation.

The most remarkable feature of MyoD and its paralogues is the ability to drive new gene expression programmes that are required to develop the skeletal muscle cell phenotype. Such activity necessarily involves activating silent genes, which of course are incorporated into cellular chromatin. Thus differentiation-specific gene expression requires mechanisms to alleviate the effects of repressive chromatin and additional mechanisms to promote the activity of transcription factors and RNA polymerase II in a chromatin environment conducive to active gene expression.

The basic unit of chromatin is the nucleosome, which is composed of 147 bp of DNA wound −1.8 turns around a globular octamer of histone proteins containing two copies of each of the four core histones: H2A, H2B, H3 and H4 [7]. Nucleosomes are arranged linearly on the DNA as arrays, and fold into higher order structures in conjunction with the linker histone H1, which is present stoichiometrically with nucleosomes. Detailed structural and biophysical understanding of nucleosome array structures consisting of a dozen or more nucleosomes is still somewhat limited [8]. Ultimately, however, these arrays form interphase chromatin containing loops and other higher–order structures that define the physical state of the genome. When genes are activated, chromatin structure at the locus is altered and generally becomes less compacted, originally documented by relative increases in accessibility to nuclease digestion [9,10]. Consistent with such findings, genes associated with skeletal muscle differentiation were shown to acquire nuclease sensitivity upon differentiation [11]. Subsequent genome-wide probing documented skeletal muscle differentiation-dependent changes in nuclease sensitivity that led to defined changes in nucleosome positioning [12,13]. How the chromatin structural changes represented by these increases in nuclease sensitivity are achieved at differentiation-specific genes is the focus of this review. We will address the changes in chromatin composition and structure caused by enzymes that incorporate variant histones, that modify histones post-translational, that alter chromatin structure via ATP hydrolysis, and that contribute to higher order chromatin structure. We will cover these topics in a manner following temporal events at these loci, first describing the repressed state, then moving toward changes immediately preceding or concurrent with differentiation signaling, and then progressing to activated chromatin at transcriptionally active myogenic genes. Some chromatin modifications and structural changes are functionally understood; others are not but can be consistently correlated with transcriptionally activated, poised, or inactive chromatin and/or with other regions of the genome. The review will concentrate on terminal differentiation of myoblasts; activation of gene expression in quiescent satellite cells, the adult stem cells responsible for post-natal growth and recovery from injury, is a topic of great interest that has been reviewed extensively in recent years [14–18] and will not be covered in depth here.

2. The chromatin state of repressed myogenic loci

2.1. Histone modifiers and modifications prior to myoblast differentiation

Silencing of myogenic genes associated with terminal differentiation in proliferating precursor cells is mediated through a combination of chromatin modifying enzymes that generate well-known histone marks associated with gene repression. Among these are preclusion of histone acetylation and deposition of lysine methylation at H3K9 and H3K27.

Type I and type II histone deacetylases (HDACs), which are distinguished from each other based on relative sequence homology [19], maintain hypoacetylated chromatin at the promoters of myogenic genes and also prevent acetylation of MyoD and other non-histone components of the transcriptional machinery. The type I HDACs, HDAC1 and HDAC2, associate with MyoD [20,21] and are recruited to myogenic promoters by MyoD and as well as by the ubiquitous transcription factor YY1 [22,23]. More recent work implicates the repressor Snail as an additional factor that recruits type I HDACs as a mechanism to prevent MyoD binding [24]. Type II HDACs typically associate with Mef2 proteins and repress Mef2-based transcriptional activity [25], at least in part through association with a corepressor complex called NCoR/SMRT [26]. Members of a third type of HDAC class are related to the NAD+ dependent Sir2 deacetylase protein of yeast. These HDACs associate with MyoD and the acetyltransferase PCAF to prevent PCAF activity and MyoD acetylation and may act as a redox sensor to regulate chromatin structure in response to changing physiological conditions [27].

The Polycomb group (PcG) proteins were defined genetically in Drosophila as repressors of developmentally regulated transcription [28,29]. Subsequent characterization revealed that PcG proteins form two distinct complexes, PRC1 and PRC2 [30,31]. The latter of which contains the lysine methylase Ezh2, which catalyzes trimethylation on H3K27 [32–35] Targeting of PRC2 to myogenic genes occurs through YY1 [22], thereby giving YY1 a central role in two gene silencing mechanisms. A distinct repression mechanism, catalyzed by the Suv39H1 methyltransferase, results in methylation of H3K9 [36], providing a binding site for heterochromatin protein 1 [37,38] which interacts with MyoD and represses its transcriptional activity [23,39]. Another methyltransferase, G9a, dimethylates H3K9 and MyoD [40] repressing the transcriptional activity of the transcription factor as well as promoting a repressive chromatin environment. However, knockout of G9a in mouse skeletal muscle has no phenotype despite a global reduction in dimethylated H3K9 [41], possibly suggesting redundant function of another methyltransferase or a contributory but not essential role. A schematic compilation of the enzymes implicated in these post-translational modifications is presented in Fig. 1 (top).

2.2. Chromatin remodeling and histone variants prior to myoblast differentiation

Variants of three of the four core histones (H2A, H2B, H3) exist and play specialized roles in different biological processes. To date, there is no evidence for a role for H2B variants in myogenesis [42]. Replacement of canonical histones with histone variants of H2A
Proliferating myoblasts

repessed myogenic genes

Differentiating myoblasts

activated myogenic genes

Fig. 1. Chromatin regulators of proliferating and differentiating myoblasts. (Top) In proliferating myoblasts, expression of differentiation-specific genes in the skeletal muscle lineage is repressed. MyoD is associated with several transcription factors, including YY1, Pbx1, and Snail. Histone modifying enzymes, including the PRC2 complex, Suv39H1, G9a and Type I/II HDACs, establish repressive histone marks. Two components of distinct ATP-dependent chromatin remodeling enzymes, Baf60c and Chd2, are also present. (Bottom) During differentiation, muscle-specific genes are transcriptionally activated via chromatin remodeling by the SWI/SNF remodeling complex. Repressive histone marks are erased and active histone marks are established by the histone demethylases UTX and KDM4a, p300/CBP acetyltransferases, and the histone methyltransferases MLL, Prmt5 and Prmt4/Carm1. Variant histone, represented by the orange nucleosomes, are present in both conditions but are present at elevated levels in differentiating cells. See text for relevant citations.

and H3 contributes to the regulation of gene expression during skeletal muscle differentiation. The histone H2A variant macroH2A (mH2A) is characterized by the presence of a ~25 kDa evolutionarily conserved carboxyl-terminal domain called the macro domain, which makes the protein nearly three times larger than its canonical counterpart. Evidence suggests the genome-wide distribution of mH2A is similar in myoblasts and in differentiated myotubes and is predominantly associated with regions of active transcription [43]. Regions of co-localization of mH2A with trimethylated H3K27 in myoblasts was also reported, but knockdown of mH2A has no effect on this modification and no major effect on gene expression, suggesting that it is not required or is redundant for repression of gene expression in the undifferentiated state [43]. This finding is consistent with reports from other systems [44]. However, mH2A is necessary for the recruitment of the homeodomain transcription factor Pbx1 to some myogenic promoters and enhancers, which then contributes to the recruitment of MyoD and expression of differentiation-specific gene expression [43,45–47]. In addition, mH2A promotes inclusion of an activating histone mark, acetylated H3K27, at myogenic enhancers and promoters [48]. How mH2A is deposited and its exact role in myoblast chromatin remain to be determined.

H3.3 is a H3 variant that, unlike the canonical H3 isoforms, H3.1 and H3.2, is deposited in chromatin in a replication-independent manner. Deposition is widespread throughout the genome; it occurs at both active and inactive genes, and in other genomic regions, including enhancers, telomeres, and retroviral elements. Multiple H3.3 chaperones exist, including a SNF2 family enzyme called ATRX, which deposits H3.3 into heterochromatin with a partner protein called DAXX, a multi-functional protein that interacts with H3.3/H4 dimers [49]. Though ATRX and DAXX can be localized to heterochromatin in differentiating myoblasts [50], to date no connection between ATRX and H3.3 deposition in proliferating or differentiating myoblasts has been reported.

A general question about the association of H3.3 with actively transcribing genes was whether or not this modification was a cause or consequence of transcription. Considerable insight was gained from experiments probing transcriptional memory using Xenopus somatic cell nuclear transfer. Nuclei expressing MyoD were transferred into enucleated eggs and underwent up to 24 rounds of cell division prior to the acquisition of transcriptional competence. MyoD expression was observed when transcriptional competence was established, despite the large number of intervening cell divisions. Overexpression of either wildtype or mutant H3.3 increased H3.3 deposition at the MyoD as well as at the Myf5 locus, but the mutant H3.3 could not support transfer (memory) of the expressed state. In other words, the presence of the wildtype H3.3 was required for the recipient embryo to be able to express MyoD. Because of the extended period of cell division in the absence of transcriptional competence, these experiments strongly suggest

[References]
that H3.3 deposition is a precursor for subsequent transcription [51].

Subsequent work directly addressed this issue at differentiation-specific genes in proliferating and differentiating myoblasts [52]. Chd2, an ATP-dependent chromatin remodeling enzyme from the SNF2 family, interacts with MyoD and binds to differentiation-specific genes in both proliferating and differentiating myoblasts (Fig. 1—top panel). Chd2 knockdown inhibits myogenic gene expression and differentiation, and remarkably, prevents incorporation of H3.3 into differentiation-specific genes prior to gene activation, thereby demonstrating that H3.3 incorporation precedes gene activation during myogenic differentiation. Chd2 interacts with H3.3, suggesting that the remodeling enzyme may act as an H3.3 chaperone [52]. Conversely, knockdown of H3.3 or ectopic expression of H3.1, one of the canonical H3 proteins, in myoblasts increases incorporation of H3.1 at the expense of H3.3 incorporation, increases trimethylation of H3K27 while decreasing methylation of H3K4, and inhibits myoblast differentiation, revealing how the balance of H3 isoforms in myoblasts regulates differentiation potential [53].

In mammals, H3.3 differs from the canonical H3.1 and H3.2 histones by only five (Ser31, Ala87, Ile89, Gly90, Ser96) or four amino acids (Ser31, Ala87, Ile89, Gly90), respectively [54]. Amino acids 87–90 determine variations in deposition timing and localization that correspond to the interactions of preferred chaperones for the H3.1/H3.2 and H3.3 variants [55–57]. Recent work showed that Ser31 was necessary for H3.3 to promote myogenic gene expression [53]. Additional details describing the mechanism by which this seemingly very specific requirement results in such significant changes in myoblast chromatin structure await further study. It is possible that there are receptor proteins that specifically bind to H3.3 or that recognize known histone modifications in the context of H3.3. One example is the zinc finger MYND-domain containing protein 11 (ZMYND11), which is a receiver protein for H3.3-specific trimethylation of K36 [58]. In other systems, ZMYND11 regulates transcriptional elongation of RNA Polymerase II via its recognition of trimethylated H3K36 on H3.3. There is only one report of H3K36 trimethylation in myoblasts, but this work did not distinguish between H3 isoforms [59]. Whether this or any other histone modification relates to the functionality of H3.3 remains to be determined. Fig. 2 (top) is a schematic diagram showing post-translational modifications and variant histones present at myogenic loci prior to differentiation.

3. The chromatin state at active myogenic loci

3.1. Changes in histone modifiers and modifications upon myoblast differentiation

The transition between repression and activation of differentiation-specific genes requires both the removal of repressive marks and the deposition of histone marks associated with gene activation. At the earliest stages of myoblast differentiation, promoters controlling the expression of differentiation-specific genes become hyperacetylated in a MyoD- or Myf5-dependent manner [46,60,61]. This is accomplished by downregulation of Snail, HDACs and Ezh2 levels and the alleviation of repression by acetyltransferase activity [22,24,62,63]. In addition, repression mediated through trimethylation of H3K27 is relieved via the activity of the lysine demethylase UTX. Removal of H3K27 methyl marks facilitates targeting of the Ash2L complex via the Me2D and Six1 transcription factors [6,64]. The Ash2L complex trimethylates H3K4 via enzymatic subunits of the MLL family [65], thereby assisting in a chromatin transition from repressive H3K27 methylation to permissive H3K4 trimethylation. Interestingly, demethylation of H3K27 needs to precede trimethylation of H3K4 because methylation of H3K27 precludes binding of the Ash2L methyltransferase complex [66]. The histone acetyltransferases p300/CBP also antagonize H3K27 methylation activities by acetylating this amino acid [67]. Removal of H3K9 methylation is not as well understood. One report indicates that a novel isoform of the KDM4A demethylase that is upregulated upon differentiation signaling mediates H3K9 demethylation and is required for myogenic differentiation [68]. KDM4A also demethylates MyoD protein, which results in an increase in its stability [69]. Additional investigation is warranted. In particular, information on the temporal relationships and inter-dependencies, if any, between the enzymes that relieve hypoacetylation and that remove H3K27 and H3K9 trimethylation may reveal mechanistic details that explain the integration of each of these enzymatic reactions.

Additional coactivators involved in modifying chromatin structure in differentiating myoblasts include members of the arginine methyltransferase (PRMT) family. Arginine methylation yields monomethylation or either symmetric or asymmetric dimethylation [70,71]. Prmt5 is a symmetric arginine dimethylase of H3R8 and H4R3 that was originally characterized as a transcriptional repressor of cell proliferation [72–74]. There are reports of Prmt5 functioning as a co-repressor in some differentiation systems [75–77], but Prmt5 binding and symmetric dimethylation of H3R8 are induced at myogenic promoters after differentiation signaling, correlating with the onset of differentiation-specific gene expression [78,79]. Though Prmt5 binds to myogenin and several other gene promoters that are expressed later during myogenic differentiation, it is only required for myogenin expression, as ectopic expression of myogenin in Prmt5 knockdown cells rescues the expression of these late genes [78]. Remarkably, a different PRMT, Prmt4, also called Carm1, that asymmetrically dimethylates H3R17 and H3R26 [80], is not required for myogenin expression but binds to late gene promoters, locally dimethylates H3R17, acts as a cofactor for the Me2 transcription factor [81] and is required for expression of these genes [78]. Subsequent efforts determined that microRNAs induced during myogenic differentiation show the same differential requirement; Prmt5 is required for myogenin expression, which is required, along with Carm1, for microRNA expression [82]. A review on myogenic miRNAs by Munsterberg can be found elsewhere in this issue. Thus, there is a temporal relationship between the activities of different PRMTs in the activation of differentiation-specific genes during myoblast differentiation.

The roles of PRMTs in myogenesis appear to be more complex than just contributing to differentiation-specific gene expression. In vivo analysis of Prmt5 and Carm1 function in zebrafish also demonstrated a differential but combinatorial role for both of these PRMTs in overall skeletal muscle development [83]. In particular, it was reported that both Prmt5 and Carm1 contribute to myogenin expression, whereas the two enzymes differ in requirement for slow vs fast muscle fiber formation [83]. Later work used genetic depletion or depletion via electroporation of shRNA in mouse skeletal muscle to further probe function. Depletion of the Prmt5–associated factor Cop5 hinders regeneration of injured skeletal muscle [84], while genetic knockout of Prmt5 severely inhibits skeletal muscle regeneration after injury [85]. Interestingly, while genetic knockout studies confirm a role for Prmt5 in post-natal differentiation, they also show that Prmt5 is not required for embryonic skeletal muscle differentiation [85]. However, molecular evidence demonstrates both Prmt5 and dimethylated H3R8 on the myogenin promoter in isolated somites dissected from E8.25–E9.5 embryos [86], suggesting that Prmt5 and H3R8 dimethylation are likely contributing to embryonic gene activation even if not absolutely required.

Many differentiation-specific genes also have enhancer sequences contributing to their expression. Genome-wide pro-
filing also implicates MyoD as an organizer of active enhancers. Incorporation of monomethylated H3K4 via the Set7 methyltransferase and acetylated H3K27 by p300, along with binding of RNA polymerase II and the transcription factors Runx1 and c-jun, among others, mark these enhancers [87,88]. Myogenic enhancers are also a source of non-coding enhancer RNAs, short RNAs that promote MyoD-mediated gene activation in a manner that seems to involve increasing chromatin accessibility through an undefined mechanism [89,90].

In contrast to MyoD binding, which can be identified at many myogenic genes prior to and at the onset of differentiation signaling [60], the transition from repressed to active chromatin generally correlates with the onset of gene expression. This is facilitated, at least in part, by the continued association of MyoD with HDAC proteins at promoters that are not expressed until later in differentiation, even though other differentiation-specific genes are no longer bound by HDACs and co-repressors and are being actively transcribed [86,91,92]. The functionality of this interaction was revealed by experiments demonstrating that ectopic expression of myogenin and Me2iD prevented HDAC association with promoters expressed at later times and resulted in premature expression of these genes [91]. Thus the chromatin state of different genes can be different, with actively transcribing genes showing MyoD and coactivators bound, simultaneously existing with MyoD and HDAC bound, transcriptionally repressed genes. Triggers that stimulate the conversion of late expressed genes from an inactive conformation marked by repressive histone marks to the active state remain to be determined. Fig. 1 (bottom) shows a schematic diagram indicating the presence of enzymes implicating in transitioning chromatin at differentiation-specific genes from the repressed to the active state.

3.2. Chromatin remodeling and histone variants upon myoblast differentiation

Genetic ablation of MRF genes in the mouse demonstrated that MyoD and Myf5 are determinants of the skeletal muscle lineage while myogenin drives terminal differentiation. The Mrf4 factor likely contributes to both processes [2]. An initial investigation into the mechanisms of chromatin alteration at differentiation-specific genes during myogenesis compared the activities of MyoD and myogenin in inducing nuclear sensitivity, a proxy for local chromatin structural changes, at myogenic regulatory sequences. MyoD proved efficient at inducing changes in chromatin accessibility whereas myogenin was not [11]. Structure-function analysis identified regions of the MyoD protein conserved with Myf5 that were distinct from the known activation and DNA binding domains and that were necessary for mediating chromatin accessibility, and it was suggested that these domains mediated interaction with factors capable of directly altering chromatin structure [11].

The mammalian SWI/SNF chromatin remodeling enzyme complexes were identified and shown biochemically to alter nucleosome structure in an ATP-dependent manner in vitro [93,94]. The ATPases acting as the catalytic components of these complexes, called Brahma (Brg) and Brg1 (Brahma related gene 1) [95,96] belong to the SNF2 family of DNA-dependent ATPases that had been implicated via yeast genetic approaches as regulators of chromatin structure [97]. Expression of ATPase-deficient alleles of these enzymes resulted in a dominant negative effect, as enzyme complexes formed around the nonfunctional ATPases and interfered with gene expression and cellular function [95,96,98]. Using this approach, reprogramming to the skeletal muscle lineage by MyoD and other MRF family members was shown to be dependent on the SWI/SNF ATPases because chromatin remodeling at myogenic loci could not occur [99,100]. The requirement for energy-dependent chromatin alterations at differentiation-specific genes is consistent with a coordinated, genome-wide transition of the cohort of genes needed to promote a new cellular phenotype from a repressed, transcriptionally silent state to a transcriptionally competent configuration.

Initial genome-wide assessment of myogenic gene expression suggested that the requirement for SWI/SNF enzymes varied from modest to completely dependent at different loci and that chromatin remodeling followed histone acetylation [46]. The presence
of bromodomains, which promote binding to acetylated chromatin [101,102] in at least three subunits of SWI/SNF complexes, including the Brm and Brg1 ATPase subunits, suggests a mechanism that enables association of the SWI/SNF enzymes with chromatin. While likely not essential for every SWI/SNF-mediated cellular function [103–106], a bromodomain inhibitor specific for the three SWI/SNF bromodomain proteins nevertheless negatively impacts myoblast differentiation [107], suggesting a contributing role for bromodomain-mediated chromatin interactions. Similarly, knockdown of either the Prmt5 or Carm1 methyltransferases precludes SWI/SNF enzyme binding and function [78,79], indicating that dimethylation of histone arginines or other PRMT substrates and/or the interaction of a reader of dimethylated histone arginines is a necessary prerequisite for ATP-dependent remodeling of differentiation-specific loci. No formal relationship between the MLL enzymes mediating H3K4 methylation and SWI/SNF enzyme function during myogenic gene activation exists.

The different SWI/SNF enzyme ATPases appear to play distinct roles in differentiation. Brg1 is essential for driving proliferating myoblasts into differentiation [108] and via ChIP has been implicated as being present at myogenic gene regulatory sequences in developing somites and limb buds [86,92]. Mechanistically, the alteration of chromatin structure by SWI/SNF enzymes at differentiation-specific genes results in stable interaction of MyoD and Mef2 transcription factors, and likely facilitates the binding of additional transcription factors [46,92]. At some myogenic loci, the interaction of Brg1 and MyoD are facilitated by the Pbx1 homeodomain transcription factor [45–47], resulting in stabilization of MyoD binding. More specific roles for the BRM ATPase have recently been reported. BRM is required for cell cycle arrest prior to differentiation and seems to play a role in the activation of some differentiation-specific genes that are expressed late in the differentiation process [109]. In general, understanding of the physiological roles of the BRM ATPase has been hampered by a lack of consistently available antibodies and concerns about the existing knockout mouse model [110]. Distinguishing specific and cooperative functions of the SWI/SNF ATPases remains a general challenge; it is likely that the interplay between the two enzymes is complex and highly relevant to understanding chromatin structural changes during myogenesis.

The interaction of the SWI/SNF enzymes with myogenic genes is also facilitated by regulated phosphorylation and dephosphorylation of specific subunits. Most, if not all SWI/SNF subunits are heavily phosphorylated [111], and regulated phosphorylation of the Brg1 and Brm ATPases is required for cell proliferation [112]. The Bafl60c subunit interacts with MyoD and with myogenic gene promoters prior to differentiation signaling. Bafl60c is phosphorylated by the p38α/β mitogen activated protein (MAP) kinase upon differentiation signaling, which permits the association of the ATPase and other enzyme subunits and subsequent chromatin remodeling of local chromatin structure [113,114]. SWI/SNF enzyme binding to chromatin and function is also regulated by the phosphorylation state of the Brg1 ATPase, which is controlled by opposing, calcium-dependent enzymes. In proliferating myoblasts, Brg1 is phosphorylated by PKCβ1. Shortly after the onset of differentiation, calcineurin dephosphorylates Brg1 at PKCβ1 dependent sites, permitting association with myogenic regulatory sequences and chromatin remodeling function [115]. Surprisingly, both PKCβ1 and calcineurin can be localized to myogenic gene promoters in association with Brg1, but in a mutually exclusive, temporally separable manner [115]. Recent evidence shows additional regulation of Brg1-mediated chromatin remodeling in proliferating myoblasts by casein kinase 2 [116]. Regulation of chromatin remodeling by post-translational modification of subunit proteins indicates that simply targeting remodeling enzymes via interactions with transcription factors is likely an insufficient mechanism to regulate chromatin remodeling activity. It also raises the possibility of additional modes of regulation through other post-translational modifications of the subunit proteins.

Other ATP-dependent remodeling enzymes exist and in different contexts work in cooperation or opposition to SWI/SNF enzymes [117]. To date, enzymes from other SNF2 ATPase families have been studied in more limited detail during the activation of skeletal muscle differentiation. An ATPase called Chd4, which is part of a remodeling enzyme called NuRD [118], causes inappropriate expression of cardiac muscle–specific genes when depleted in mouse skeletal muscle [119], perhaps suggesting a represive role for the NuRD complex at cardiac and perhaps other genes to prevent their induction during skeletal muscle differentiation. SRCAP (SNF2-related CBP activator protein) is one of two mammalian enzymes that modulates chromatin structure by exchanging canonical H2A for the H2AZ variant [120–122]. The H2AZ variant was originally characterized in yeast as a positive regulator of transcription using approaches combining genetics with molecular analyses of chromatin structure and composition [123,124], but multiple later reports using global analyses of histone incorporation and gene expression showed it can be found at both active and repressed genes with apparent roles in both activation and repression [125–130]. During myoblast differentiation, the SRCAP subunit called p18\textsuperscript{lan} is upregulated in a manner dependent on p38 kinase signaling. Though a basal level of H2AZ can be found in myogenic chromatin in proliferating myoblasts, both p18\textsuperscript{lan} binding and H2AZ incorporation are induced at myogenic promoters early after the onset of differentiation, and knockdown of the p18\textsuperscript{lan} subunit inhibited differentiation-specific gene expression and myoblast differentiation [131]. It should be noted that a recent report indicates H2AZ is also present at the MyoD locus in myoblasts and that its ability to be acetylated is critical for MyoD expression, thereby providing an indirect mechanism for a role for deposition of H2AZ in myoblasts impacting differentiation [132]. Whether or not deposition of H2AZ at myogenic sequences prior to differentiation requires SRCAP, p400, or a different cooperating enzyme is unknown.

Finally, though deposition of the H3.3 variant at differentiation-specific genes was shown to occur in proliferating myoblasts in a manner dependent on the Chd2 ATP-dependent chromatin remodeling enzyme, incorporation of H3.3 at the MyoD locus was reported to happen as differentiation proceeded [133]. Deposition was mediated by the histone chaperones Hira (histone cell cycle regulator) and Asf1A (Anti-silencing function protein 1 homolog A). Knockdown of Hira compromised MyoD expression, which blocked activation of differentiation-specific genes, thereby preventing differentiation [133]. Later work confirmed that knockdown of Chd2 had no effect on H3.3 incorporation at the MyoD locus [52]. It is unclear why the MyoD locus requires different H3.3 chaperones and why the kinetics of H3.3 deposition are different from those of differentiation-specific genes. The data reflect a further complexity in orchestrating the activation of gene expression during myoblast differentiation. Fig. 2 (bottom panel) schematically shows post-translational modifications and variant histones present at myogenic loci upon differentiation.

3.3. Changes in higher order chromatin organization during myoblast differentiation

An increasingly apparent concept is that higher order chromatin organization is dynamic and changes in response to myriad signaling events [134]. Chromosome conformation capture methodology has provided direct physical evidence for promoter-enhancer looping changes that increase or decrease as a result of myogenic differentiation [135–138]. A recent low-resolution (400 kb) genome-wide analysis of chromatin interactions in myoblasts...
pre- and post-differentiation showed overall similarity in genome organization despite the change in differentiation state. The resolution achieved precluded identifying gene-specific interactions but nevertheless showed that differentiation did induce some compartment switching, indicating interconversion of specific regions of hetero- and euchromatin in myoblasts and myotubes [139].

Detailed mechanistic studies about myogenic higher order chromatin organization are limited. A candidate gene approach using chromosome capture methods in cell lines and primary tissue reported the trans-interaction of promoter sequences of different genes expressed late in differentiation, despite being located on different chromosomes. Interactions between the implicated loci were confirmed by FISH [91]. No interactions between these “late” genes and an early gene, myogenin, or between these genes and cellular housekeeping genes, were detected. These interactions occur at the onset of differentiation prior to gene expression and are not detected once gene expression was initiated, thereby correlating with repression of the “late” genes at early times. Transcriptionally active genes are concentrated on the genome into what are known as RNA Polymerase II foci or “specialized transcription factories” [140,141]. It appears the physical convergence of these myogenic genes represents the converse situation: a concentration of genes that are not expressed but are poised to be activated later in the differentiation cascade. Formation of the interactions is dependent on MyoD, and by implication, the HDAC associated with MyoD at early times of differentiation, and on the Brg1 SWI/SNF ATPase [91]. Brg1 was already known as a required element in organizing local higher-order chromatin structures at complex loci such as α-globin, β-globin, TH2, and CHIT [142–145], and a recent genome-wide assessment of intra- and inter-chromosomal interactions in a different cell type suggests a global role for Brg1 in higher-order chromatin organization [146]. Collectively, the data suggest that chromatin remodeling by Brg1 and SWI/SNF enzymes very likely is an essential component in the regulation of genome-wide chromatin structure both prior to and during myogenic differentiation.

A point of future research will be to determine how the chromatin remodeling enzyme contributes to overall genome organization. It is known to locally remodel nucleosome structure at regulatory sequences to facilitate factor binding and utilization of the DNA for transcription as well as replication, recombination, and repair. It is possible that this function is sufficient to translate to regulation of genome organization, but it may be that there is a separate chromatin remodeling function to support factors specifically involved in regulating chromatin looping and compaction and or factors involved in maintaining nuclear integrity. A speculative model discussing this hypothesis has been published [147].

4. Conclusions

The number of possible changes to chromatin structure due to the incorporation of different histone variants, the incorporation of different histone modifications, and the function of chromatin remodeling enzymes seems almost limitless. Many different cofactors play a role in establishing a repressive chromatin state at differentiation-specific genes in myoblasts, yet it is interesting that some of the cofactors and transcription factors that are present prior to gene expression are also central players in the transition from a repressed to an activated chromatin conformation. By necessity, the discussion here is oversimplified. Different studies cited utilized different cell types and different approaches, with some providing readout on a single gene, some presenting several candidate genes, and others providing genome-wide data. It is unlikely that every factor and every modification or variant is present at every differentiation-specific gene that is activated during myogenesis; there are almost certainly subsets of genes that have shared mechanisms, but there are likely to be significant differences as well. Nevertheless, as illustrated in the schematic figures, significant progress has been made in identifying the major players and mechanisms that mediate both repressive and activated chromatin at myogenic genes. Better understanding of temporal regulation of these mechanisms will likely increase general understanding of the transition of chromatin states at myogenic genes. Single cell analysis is one possible approach for accomplishing this goal. Rapidly improving methodologies in this arena should make possible integrated analyses of changes in the transcriptome and epigenome during skeletal muscle differentiation and will likely generate a more precise, genome- and population-wide picture of differentiation-dependent alterations in chromatin structure during myogenic differentiation.

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A. Harada et al. / Seminars in Cell & Developmental Biology 72 (2017) 77–86


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