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Review

# Signaling to the chromatin during skeletal myogenesis: Novel targets for pharmacological modulation of gene expression

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**Abstract**

Cellular differentiation entails an extensive reprogramming of the genome toward the expression of discrete subsets of genes, which establish the tissue-specific phenotype. This program is achieved by epigenetic marks of the chromatin at particular loci, and is regulated by environmental cues, such as soluble factors and cell-to-cell interactions. How the intracellular cascades convert the myriad of external stimuli into the nuclear information necessary to reprogram the genome toward specific responses is a question of biological and medical interest. The elucidation of the signaling converting cues from outside the cells into chromatin modifications at individual promoters holds the promise to unveil the targets for selective pharmacological interventions to modulate gene expression for therapeutic purposes.

Enhancing muscle regeneration and preventing muscle breakdown are important goals in the therapy of muscular diseases, cancer-associated cachexia and aging-associated sarcopenia. We will summarize the recent progress of our knowledge of the regulation of gene expression by intracellular cascades elicited by external cues during skeletal myogenesis. And will illustrate the potential importance of targeting the chromatin signaling in regenerative medicine—e.g. to boost muscle regeneration.


*Keywords:* Chromatin; Signal transduction; p38; Transcription; Muscle regeneration

**Contents**



The ultimate goal of pharmacological strategies in regenerative medicine is to achieve the desired effect—e.g. organ regeneration—through highly selective interventions. As the expression of particular subsets of genes determines the final outcome of any cellular process, it is obvious that deciphering the mechanisms that regulate gene transcription is an issue of critical importance. Understanding how the external cues impart the epigenetic marks that change the chromatin

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structure at specific loci during lineage commitment, how these modifications are maintained during mitosis, and how do they promote the differentiation program, are questions of critical importance in biology, with obvious implications in molecular medicine. Epigenetic marks are generated by chromatin-bound protein complexes, which are endowed with an enzymatic activity toward histones and DNA [1,2]. The unique combination of different post-translational modifications of histones—including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation—at specific loci, defines the chromatin configuration, either repressive or permissive for gene expression [3]. The engagement of chromatin-bound complexes on discrete sequences of the genome is governed, at least in part, by external cues. In this regard, there is an evident gap of knowledge on the mechanism by which intra-cellular signalling pathways convert environmental cues into specific chromatin modifications. The elucidation of the molecular basis by which the information transmitted by signalling cascades is deciphered by chromatin-binding proteins and broadcasted to other components of the transcription machinery will help to further clarify the molecular pathogenesis of human diseases and will possibly reveal new targets for pharmacological interventions aimed at selectively modulating gene expression.

In the following sections we will summarize and critically discuss the current knowledge on the signalling that regulates chromatin structure and function during skeletal myogenesis, with a particular emphasis on muscle regeneration and the potential therapeutic opportunities derived from this information.

## 1. The concept of signal delivery to the chromatin and selectivity of pharmacological interference

As the environmental cues determine the extent of nuclear reprogramming during cellular differentiation, pharmacological interference with the signaling pathways, which deliver external cues to the chromatin, can be exploited to modulate the differentiation program.

An emerging concept that should be anticipated in this review is that the cellular level of intervention is likely to provide the extent of selectivity in the pharmacological modulation of gene expression. For instance, blockade of the membrane receptor or downstream cytoplasmic cascades that transmit to the nucleus the information from outside the cell often affects the expression of a broad range of genes, since receptor-activated pathways usually spread to a variety of different promoters [4]. The selectivity of the interference would progressively increase by targeting events at the interface between the signaling cascades and the transcription machinery (Fig. 1). Thus, deciphering the unique "chromatin signature" generated by signaling cascades at individual promoters might provide important insight on the selectivity of

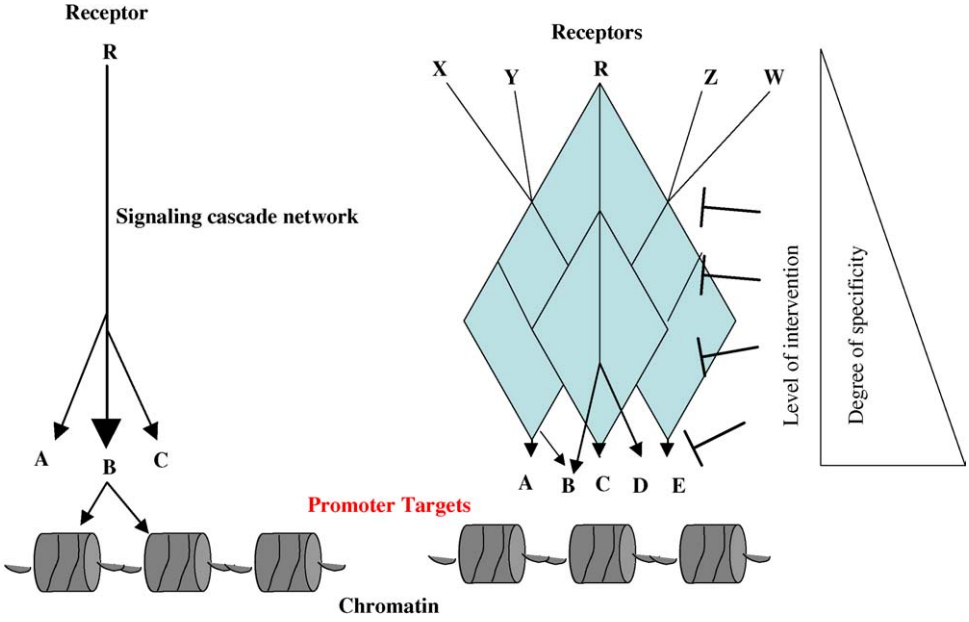


Fig. 1. Selective modulation of gene expression by pharmacological interventions on receptor-activated chromatin signaling. Receptor-activated intracellular cascades deliver external cues to the chromatin via cytosolic signaling networks, which spread the signal to a broad range of promoters to coordinate the expression of the genes involved in the cellular response. In this cartoon, two different models of intracellular signaling are envisioned. Left model: receptor (R) can trigger a linear signaling, which spread in the proximity to the nucleus—e.g. by targeting either different nuclear proteins (A, B and C) or one nuclear protein (B) recruited to different promoters. Right model: receptor (R) is engaged together with other receptors (X, Y, Z and W). Cross talk between the activated cascades establishes a complex signaling network that spreads the information to a variety of promoters, by targeting several downstream nuclear proteins (A, B, C, D and E). In both cases, selective modulation of either one gene or a restricted subset of genes can be achieved only by interfering with the events in proximity to the promoter(s) of the gene(s) of interest. For instance, in both models models it is important to identify the event(s) (e.g. particular phosphorylation pattern or promoter-specific chromatin-bound protein) that impart to B the information for recruitment to individual promoters.

signal transduction pathways toward gene expression. Recent reports have begun to shed light on the signaling that regulate the assembly of chromatin-modifying complexes at particular promoter/enhancer regions during different cellular responses.

## 2. Signal-dependent nuclear reprogramming during muscle differentiation

During skeletal myogenesis, precursors cells committed toward the myogenic lineage proliferate as undifferentiated, mononucleated, myoblasts, in the presence of mitogens or other anti-differentiation cues, and differentiate into multinucleated myotubes upon the exposure to pro-myogenic signals, such as cell confluence, mitogen withdrawal and secreted molecules [5,6].

Chromatin modifications generated by external cues can either be transient, to permit the transition throughout intermediate stages of lineage commitment, or permanent, when an irreversible phenotype is established—e.g. terminal differentiation. For instance, during muscle regeneration, the progression from muscle precursors to terminally differentiated cells entails sequential changes of expression of different subsets of genes in response to environmental cues [7]. Satellite cells define a population of quiescent, reserve cells, which are activated in response to muscle damage, and are deputed to repair injured myofibers [8,9]. These cells are exposed to locally released growth factors, hormones and cytokines, which coordinate the progressive stages of regeneration, from the first rounds of proliferation to the fusion into myofibers [7,10,11]. Extrinsic cues elicit a number of intracellular signaling pathways, which are evolutionary conserved and ubiquitous to almost all cell types [12]. The combinatorial activity of these pathways ultimately selects the genes to be expressed at each stage of muscle regeneration. Understanding how these pathways reprogram the genome in a cell-type specific fashion is essential to devise strategies aimed at modulating gene expression during regeneration.

The importance, and at the same time the complexity, of the environmental signals in the regulation of gene expression during skeletal myogenesis is well exemplified by the impact of inflammatory cues on muscle regeneration. In many muscular diseases, myofiber degeneration is either caused or accompanied by an inflammatory response [13]. Locally released inflammatory cytokines elicit intracellular pathways, which can either block or promote the myogenic program. For instance, activation of NFκB- and JNK-pathways by inflammatory cytokines represses the myogenic program, whereas calcineurin and p38 signaling promote muscle differentiation [14–22]. Several inflammatory cytokines, like tumor necrosis factor alpha (TNFα), and interleukin 1 (IL-1), counter myotube formation and post-mitotic growth [23–25], while IL-4 promotes myoblast fusion into myofibers [26]. The role of IL-6 in myogenesis remains unclear [7,27]. It is likely that the final effect of inflammation depends on the combination of pathways activated in response to locally released inflammatory substances, on their timing of activation and on the cross-talk between cytokine-induced intracellular cascades and additional pathways stimulated by growth factors [7]. These kinase pathways are propagated inside the cells by phosphorylation cascades, which converge to the nucleus and are integrated at the chromatin level to select the genes to be expressed.

Tissue-restricted proteins contribute to confer the cell-type specificity to extracellular-signal activated cascades. Basic helix–loop–helix (bHLH) proteins of the MyoD-family are exclusively expressed in skeletal muscle cells [28], and likely mark the genomic regions where the signaling pathways converge. However, additional chromatin-associated proteins, which are preferentially expressed in muscles, could also contribute to determine the cell-type specificity of the signaling elicited by environmental cues. Epigenetic chromatin modifications are generated by protein complexes assembled on gene-regulatory elements [29]. Sequence-specific transcriptional activators recruit to their target elements several co-regulatory factors, which are endowed with enzymatic activity toward both histones and transcription factors, and are generally defined as to chromatin-modifying complexes [30]. Two families of transcription factors—the muscle regulatory factors (MRFs) MyoD, Myf5, myogenin, MRF4, and the MEF2 family members, MEF2A to D, control the expression of muscle-specific genes [31]. Their stage-specific association with different transcriptional co-regulators ultimately dictates the expression of genes implicated in the regulation of skeletal myogenesis [32]. The interactions between MRFs, MEF2 proteins and co-regulators are imparted by external cues. In general, during myoblast proliferation, mitogens promote the association with transcriptional co-repressors to prevent the premature expression of muscle genes. Upon the exposure to differentiation cues, co-repressors dissociate from MRFs and MEF2 proteins and are replaced by transcriptional co-activators [32]. Recent studies have attempted to elucidate the mechanism by which extracellular-signal activated pathways control gene expression during skeletal myogenesis, by regulating interactions between MRFs, MEF2 proteins and chromatin-modifying complexes.

## 3. Chromatin signaling inducing lineage commitment in myoblasts

The expression of either MyoD or Myf5 establishes the myogenic lineage during developmental and adult myogenesis [33,34]. Consistently, ectopic expression of either protein converts several cell types into myogenic cells [35,36]. The modality of induction of MyoD expression in muscle precursor cells has not completely been clarified. DNA methylation at the regulatory sequences of MyoD gene precludes its expression in non-myogenic cell lines, as the demethylating agent 5-aza-2′-deoxycytidine can induce expression of MyoD in fibroblasts and convert them into myogenic cells

[37]. The regulatory elements of MyoD include the core enhancer region (CER), driving cell-type and time-restricted gene expression, the distal regulatory regions (DRR), required for stable MyoD expression, and the proximal regulatory region (PRR), which contains the core promoter [38–40]. Recent evidence indicates that the homeoprotein Msx1 represses MyoD gene expression by recruiting the linker histone H1b to CER, thereby generating heterochromatin at this region and inhibiting transcription [41]. Msx1 was previously shown to repress MyoD expression, and is highly expressed in immature myogenic precursors during development and adult myogenesis [7,42]. Thus, Msx1, in collaboration with histone H1b, determines the temporal pattern of the myogenic identity by regulating MyoD expression.

The signaling controlling Msx1/H1b physical and functional interaction in muscle progenitors is unknown to date. The expression of MyoD and Myf5 is controlled by the Wnt/Pax3–7 axis during developmental and adult myogenesis [43]. Different combinations of MyoD and Pax7 expression define the final fate of satellite cells. Satellite cells that downregulate Pax7 and maintain MyoD expression are able to differentiate, while those cells that downregulate MyoD and maintain Pax7 expression do not differentiate, and are destined to replenish the satellite cell pool available for further regenerative responses [11,44]. The Wnt family of proteins includes secreted molecules, which establish a signaling network regulating muscle development by binding to G-protein coupled-receptor related Frizzled proteins, and control the transcription of target genes, by promoting β-catenin nuclear activity [45,46]. Recent studies have identified CREB as a novel nuclear target of the Wnt pathway [47]. They reported that mice deficient for either the activity or the expression of CREB show an impaired expression of Pax3, MyoD and Myf5. CREB-responsive elements are located on the regulatory sequences of Myf5 [47], and the acetyltransferase activity of the CREB-binding protein p300 is required for the myogenic lineage commitment, as embryonic stem (ES) cells homozygous for a p300 acetyltransferase mutant or a p300 null fail to activate Myf5 and MyoD transcription efficiently, despite the presence of the upstream activator Pax3 [48]. MyoD is also induced in myoblasts, via serum response factor (SRF), by the activation of non-canonical Wnt pathways, such as the GTPase RhoA [49]—a master regulator in the decision to commit mesenchymal cells toward the myogenic rather than the adipogenic lineage in response insulin and IGF1 [50]. Insulin activates CREB in several cell types [51,52]. Interestingly, insulin-activated signaling and Wnt1 cooperate to induce MyoD expression and activity in cultured muscle reserve cells, via phosphorylation-dependent GSK-3 inhibition [53]. It will be interesting to elucidate the individual contribution, if any, of the insulin/IGF1, Wnt/CREB and Rho signaling in reversing MSX1-dependent inhibition of MyoD expression and activating p300 acetyltransferase in satellite cells.

The presence of the bHLH proteins MyoD and/or Myf5 in the nucleus of myoblasts poses a fundamental issue: how do MyoD and Myf5 confer and maintain the myogenic identity to these proliferating muscle precursors, without activating the differentiation program? A simplistic, former model of inactivation of myogenic bHLH proteins in myoblasts relies on serum-induced expression of the anti-myogenic Id proteins, which sequestrate the heterodimeric partners of MyoD—the products of the E2A gene, E12 and E47 [54,55]. This model assumes that in myoblasts MyoD is unable to bind its DNA-recognition sequences—the Ebox sites—on the regulatory regions of muscle genes. However, early studies could not address two critical issues relative to the DNA binding activity of MyoD in myoblasts: whether MyoD–MyoD homodimers can be transiently recruited to the DNA during myoblast proliferation, and whether this transient interaction is restricted to the promoter/enhancer elements of muscle-specific loci, or might extend to other genes. The recent introduction of the Chromatin Immunoprecipitation (ChIP) technique provided a powerful tool to address these issues. ChIP studies have demonstrated the absence of MyoD on the regulatory regions of most differentiation-related muscle genes in myoblasts [56–59]. However, Mal and Harter have shown that MyoD can occupy the myogenin promoter in myoblasts [60]. This discrepancy might in principle result from different conditions of myoblast culture. We have noticed that culturing myoblasts at low confluence is strictly required to obtain a homogeneous population of undifferentiated myoblasts. By contrast, cellular confluence triggers pro-myogenic signals (e.g. p38 activation) that promote myogenin expression, despite the presence of serum and without morphological features of differentiation; ChIP of myogenin promoter in these conditions shows that MyoD occupies myogenin promoter (SF and PLP unpublished). Alternatively, association of MyoD with class I histone deacetylase HDAC1 [61,62] could weaken MyoD binding to DNA, since acetylation increases the affinity of MyoD for Ebox sites [63]. Therefore, MyoD–HDAC1 complex can be detected by ChIP on myogenin promoter in low-stringency conditions of immunoprecipiation.

Chromatin recruitment would explain the function of MyoD in the nuclei of undifferentiated myoblasts, as the determinant of the myogenic lineage. In this respect, the overall assumption is that transient recruitment of MyoD to specific loci somehow marks discrete chromatin spots to prime myoblasts for the differentiation-associated nuclear reprogramming. Alternatively, or in addition, chromatin recruitment of MyoD in myoblasts, in association with HDAC1, could generate transcriptionally silent regions—heterocromatin—to restrict the repertoire of transcribed genes to those specific to the myogenic lineage.

Recent studies have begun to shed light on this issue. Blais et al. recently reported a number of MyoD target genes in myoblasts by exploiting a combination of ChIP on ChIP and micro-array analysis [64]. In this study, some of the genes bound by MyoD in myoblasts were also bound in myotubes. However, it is unknown whether these genes are actually activated by MyoD at both stages. Very few genes occupied by

MyoD in myoblasts were found downregulated [64]. As such, it is possible that MyoD operates in myoblasts both by marking the promoters for subsequent activation upon exposure to proper cues and by inducing the expression of factors, which in turn collaborate with MyoD to convert "marked" promoters from a "poised" to an active state.

Some of the MyoD targets identified by Blais et al. in myoblasts are genes with unanticipated function in myogenesis. As expected, they did not include any of those muscle genes typically expressed upon induction of differentiation; rather, a number of transcription factors were found to be MyoD targets in myoblasts. These genes might collaborate with MyoD, and other MRFs, to amplify the myogenic signal, as differentiation proceed. In a previous study, Wyzykowski et al., using conditional expression of MyoD, showed that the Id3 and NP1 (neuronal pentraxin 1) genes become transcriptionally active following MyoD induction in undifferentiated myoblasts [65]. Activation of Id3 and NP1 represents a stable, heritable event that does not rely on continued MyoD activity and is not subject to negative regulation by activation of mitogen-activated pathways [65].

It remains unclear how MyoD can induce transcription of target genes regulated by conventional Ebox sites in myoblasts, considering that at this stage the inability of MyoD to form hetero-dimers with E12/47 precludes the binding to canonical DNA Ebox sites and that MyoD can form homodimers in vitro. It is possible that in myoblasts MyoD can form homodimers and activate transcription of different clusters of genes than in myotubes, through unconventional, unknown mechanism. For instance, particular chromatin conformation could be conferred to the regulatory regions of MyoD target genes in myoblasts by specific combinations of DNA-binding proteins on sequences flanking the Ebox sites, thereby allowing the promoter accessibility of MyoD homo-dimers. Recently, Berkes et al. provided an interesting model of MyoD recruitment to the chromatin of target genes (e.g. the myogenin promoter), via interactions with the homeodomain proteins Pbx, which is constitutively bound to this site [66]. According to this model, Pbx would penetrate repressive chromatin at the myogenin promoter and mark specific genes for activation by MyoD [67]. An indirect recruitment of MyoD on promoters of genes induced during muscle differentiation was also proposed by Magenta et al., who reported MyoD recruitment on pRb promoter through CREB-responsive elements (CRE) [68]. Thus, specific combinations of proteins recruited at the regulatory regions of MyoD-target genes in myoblasts could promote transcription of MyoD downstream transcription factors, which will in turn collaborate with MyoD–E12/47 hetero-dimers to activate the expression of differentiation-specific muscle genes at subsequent stages of differentiation. These MyoD "downstream collaborator genes" are induced in differentiation-committed myoblasts, prior to their phenotypic differentiation, and could persist along the whole process of differentiation. This model could explain the overlapping class of MyoD target genes in myoblasts and myotubes found by Blais et al. [64]. MEF2 proteins are typical genes induced by MyoD in myoblasts, and collaborate with MyoD at the stage of myotube formation [69,70]. MyoD targets in differentiating muscle cells are muscle-specific genes, often regulated by both Ebox and MEF2 sites [31]. Recent results from Tapscott lab provide an interesting model in which MyoD, MEF2 and p38 establish a feed-forward circuit to promote differentiation [70]. Other examples of MyoD target genes in myoblasts that cooperate with MyoD (and other MRFs) during myotube formation, are Six1, SRF and nuclear receptors [64]. It remains to be clarified the sequence and molecular modality of MyoD-dependent gene transcription at different myoblast stages. None of the studies reported so far have discriminated the network of gene expression in actively proliferation myoblasts vs myoblasts committed to differentiate. It will also be important to elucidate the signaling cascades that regulate MyoD-dependent induction of target genes at different stages of myoblast progression from proliferation to differentiation, and in particular to understand if the activity of MyoD homodimers and MyoD–E12/47 hetero-dimers is controlled by different signaling cascades.

The importance of the presence and continuous activity of MyoD in the nuclei of undifferentiated muscle cells might explain some biological differences (e.g. the different substes of muscle genes inhibited in response to p38 inhibition) observed between cultured myoblasts and fibroblasts converted by MyoD fused to the estrogen-receptor (MyoD-ER) [17–19,56]. In these latter cells, MyoD is confined to the cytoplasm of fibroblasts until estrogens are added to the medium [71]. This system provides an excellent model of synchronization of MyoD-dependent transcription, suitable to study the temporal pattern of MyoD-mediated activation of muscle genes in repressive chromatin of non-muscle cells. However, it minimizes the impact of MyoD activity in the nuclei of undifferentiated myoblasts, and the possible proliferation-associated changes in chromatin structure at loci that will be activated during differentiation.

In keeping with the possibility that in myoblasts MyoD prepares the chromatin for subsequent re-programming, but does not activate muscle-gene transcription, one would predict that mitogens, which promote myoblast proliferation, inhibit MyoD-dependent activation of differentiation genes, while tolerating MyoD binding to the chromatin. A number of redundant mechanisms of MyoD inactivation by mitogenic cascades in myoblasts have been described [72–74]. Mitogen-activated signaling pathways, such as the Ras/Raf/MEK1/ERK signaling and Src-activated pathway repress MyoD ability to activate muscle gene expression in myoblasts without altering its DNA binding activity [75–78]. During proliferation MyoD stability in myoblasts is regulated by mitogen-induced cyclins/cdk1 and 2, via direct phosphorylation of serine 200, which prevents accumulation of MyoD before mitosis [79–81]. Although an ubiquitin-dependent degradation of MyoD has been described [82],

the biochemical relationship between serum-dependent phosphorylation, ubiquitination and cell cycle turnover of MyoD has not been definitely established. Likewise, Myf5 stability is regulated by cdk-dependent phosphorylation, although at different cell cycle phases than MyoD [83]. Thus, regulation of MyoD and Myf5 expression at the protein level ensures cell cycle-dependent fluctuations of these proteins in myoblasts, allowing their proliferation, while maintaining the myogenic identity.

Stress- and inflammation-induced cascades, such as NFκB and JNK signaling pathways also contribute to silence MyoD in myoblasts [14,15]. Activation of NFκB by TNF is sufficient to promote MyoD RNA degradation [14]. Elimination of MyoD in myoblasts can potentially erase the myogenic lineage. Thus, the regulation of MyoD levels in satellite cells exposed to inflammation cues might have important implications in the maintenance of the myogenic lineage of muscle progenitors and affect the extent of regeneration [84–86].

## 4. Silencing premature transcription of muscle genes in myoblasts

Despite the presence of MyoD and Myf5, and their activity in lineage determination and maintenance, the expression of muscle genes typical of the differentiated phenotype is silenced in myoblasts. Moreover, transcription of differentiation-specific muscle genes is temporally regulated, with a sub-set of early genes being transcribed before clusters of late-muscle genes [56]. Interestingly, even after the differentiation program is initiated, MyoD binds to the promoters of muscle genes that have not been expressed yet (e.g. late genes), suggesting that MyoD-dependent silencing can regulate temporal regulation of muscle-gene transcription.

In myoblasts, muscle-gene expression is silenced by interaction between MRFs and MEF2 proteins with nuclear deacetylases (HDACs) [32,61,62,87–90]. On myogenin promoter, MEF2 proteins associate with class II HDACs4, 5, 7 and 9, leading to chromatin condensation, via histone deacetylation and recruitment of co-repressory complexes, such as heterocromatin protein 1 (HP1) and associated methyltransferases, which promote H3 lysine 9 methylation [90]. Furthermore, class II HDACs potentiate SUMO2- and 3-dependent sumoylation at the C-terminal activation domain of MEF2D and MEF2C, leading to the inhibition of transcription [91]. MyoD associates with class I HDAC1 [61,62]. While the signaling responsible for HDAC dissociation from MyoD and MEF2 proteins has been at least in part elucidated (see section below), the intracellular pathways promoting interactions among these proteins in myoblasts are unknown. It is predictable that anti-myogenic factors, such as mitogens, somehow induce these interactions. Class I HDACs can be phosphorylated and sumoylated, suggesting that these post-translational modifications can regulate their association with MRFs [32]. An indirect action of mitogen-activated cyclin-cdks can be envisioned via hyperphosphorylation of pRb, which prevents interactions with class I HDACs, thereby favoring MyoD–HDAC1 association in myoblasts [62]. As hypoacetylated MyoD and MEF2 proteins have impaired ability to bind their recognition sites [63,92], it is still unclear if HDAC-containing complexes can stably bind the DNA at specific binding sites.

A recent report by Caretti et al. begun to shed new light on muscle gene repression [58]. They showed that in myoblasts the chromatin of several muscle genes adopts a repressive configuration for transcription, via the recruitment of the histone lysine methyltransferase Ezh2, a component of the Polycomb PRC2 and PRC3 complexes, which silences transcription by di- and tri-methylation of lysine 27 of histone 3 (H3-K27) [93]. Interestingly, Ezh2 is recruited to the chromatin of muscle regulatory regions via interaction with YY1, which recognizes CarG-box motifs presented in promoter regions of muscle genes. Further association with HDAC1 forms a repressive complex, which ensures repression of transcription and prevents MyoD binding (Fig. 2A). At the onset of differentiation, the simultaneous down-regulation of Ezh2 and HDAC1 proteins, and the replacement of YY1 with SRF [58], allows the binding of MyoD–E12/47 and the recruitment of the positive co-activators, to form an active myogenic transcriptosome. The intracellular signaling that governs these interactions on muscle-gene regulatory sequences remains unknown to date. It is likely that pathways converging on post-translational modifications of YY1, SRF and on the regulatory sequences of HDAC1 and Ezh2 regulate the chromatin switch from repressive to permissive for transcription, at muscle loci.

It is interesting to note that in myoblasts lysine 9 methylation and class II HDACs were detected on myogenin promoter [60,90], but not on muscle creatine kinase (MCK) and myosin heavy chain (MHCIIb) promoters/enhancer sequences [58], whereas lysine 27 di- and tri-methylation and HDAC1 were only detected on MCK enhancer and MHCIIb promoter [58]. Lysine 9 and 27 methylation creates docking sites for recruitment of different co-repressory complexes—e.g. HP1 and Polycomb Repressive Complex 1, respectively, to ensure gene silencing. The distinct pattern of HDAC distribution and lysine methylation at regulatory sequences of early (myogenin) versus late (MCK and MHCIIb) muscle genes suggests that discrete pathways regulate the timing of gene repression/de-repression during the myogenic program.

Inhibition of MyoD-dependent transcription can also be exerted by anti-myogenic proteins, which are abundant in proliferating myoblasts (e.g. EID I and 2) [94–96], or are induced by certain growth factors (e.g. Twist) [97], via inactivation of p300 and PCAF acetyltransferases [95,98]. Finally, muscle-gene transcription can also be silenced by DNA damage-activated signaling, to ensure that DNA lesions are repaired before differentiation proceeds [99], thereby providing a “differentiation checkpoint” that avoids the formation of genetically unstable myofibers [100].

## 5. Chromatin signaling to reprogram myoblast nuclei toward terminal differentiation

The dramatic changes in chromatin structure occurring at the onset of differentiation reflect the fluctuation of extracellular cues that regulate myoblast to myotube transition [101].

The repressive conformation of the chromatin of muscle-gene promoter/enhancer regions, imposed by co-repressor complexes, implies that at least two critical events have to occur in order to initiate the myogenic program upon exposure to differentiation cues. The first consists in the displacement of negative regulators of transcription, such as HDACs and lysine methyltransferases, and the removal of repressory modifications on histone residues, such as lysine methylation; the second entails the recruitment of transcriptional co-activators (Fig. 2B). While it is still unknown whether these events are temporally separated or occur simultaneously, a number of studies have identified individual signaling cascades that govern these processes.

The bHLH proteins MyoD and Myf5 have the unique ability to initiate the myogenic program by promoting chromatin remodeling at previously silent loci [102]. The exposure to pro-myogenic cues favors the hetero-dimerization between

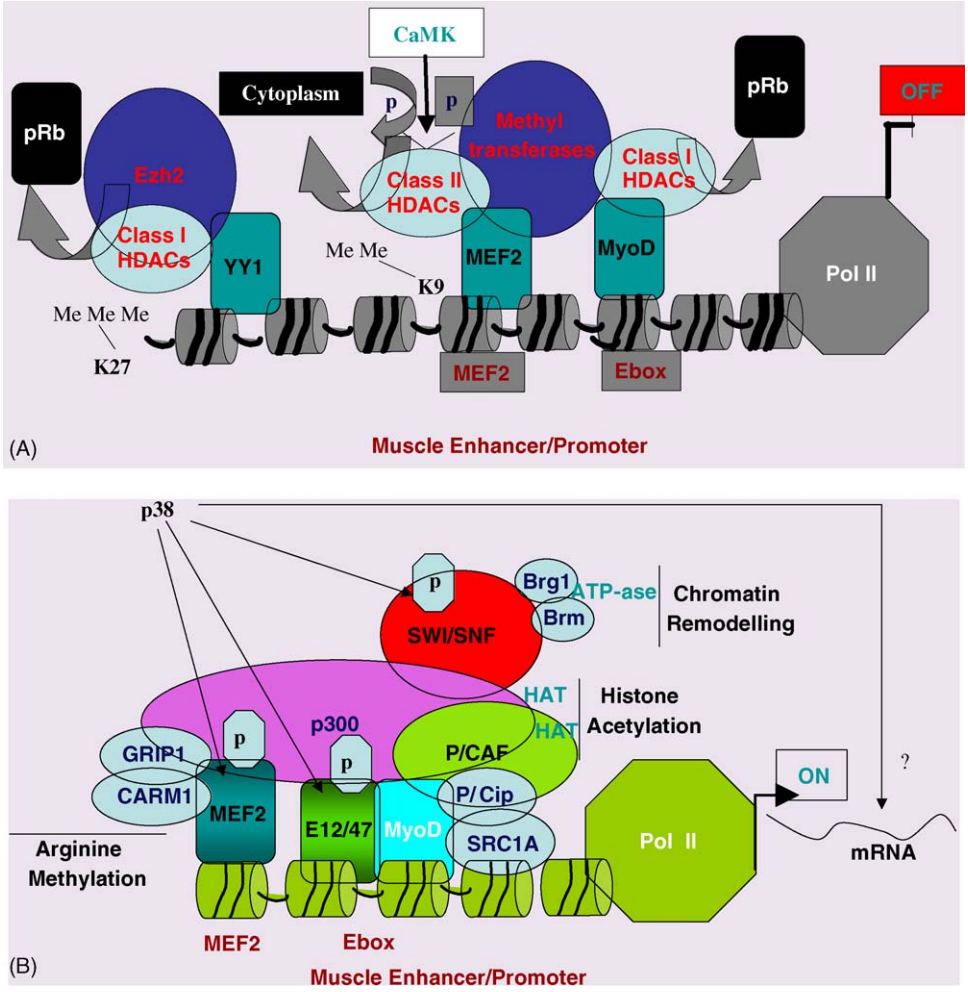


Fig. 2. Chromatin and chromatin-interacting proteins at muscle-regulatory genes in the repressed (A) or activated (B) conformation. (A) MyoD, MEF2 and YY1 recruit to the chromatin of silenced muscle genes co-repressory complexes containing nuclear deacetylases (HDACs) and methyltransferases (e.g. Ezh2), which prevents local hyperacetylation and promote di- tri-methylation of specific lysines (e.g. K9 and K27) to generate a chromatin conformation repressive transcription of target genes. Differentiation-activated CaMK pathway and increasing levels of unphosphorylated pRb (resulting from the mitogen withdrawal) displace deacetylases from the chromatin and allow hyperacetylation by acetyltransferases (see B). The differentiation-induced signal(s) responsible for the displacement of YY1 and methyltransferases, as well as for removal of lysine methylation is unknown. (B) After (or simultaneous to) the displacement of co-repressory complexes and chromatin marks, the assembly of the myogenic transcriptosome entails the recruitment of several complexes endowed with distinct enzymatic activities—acetyltransferases, ATP-dependent chromatin-remodeling complexes, arginine methyltransferases. Differentiation-activated p38 kinases regulate several steps of the transcriptosome assembly, by targeting transcription factors (MEF2), hetero-dimer partners (E47) and SWI/SNF components (BAF60) by direct phosphorylation. p38 kinases can also regulate the stability of the nascent RNA. Acetyltransferase recruitment to muscle-gene regulatory regions does not appear to be dependent of the p38 pathway, and is likely regulated by a differentiation-activated parallel cascade. Each of the components recruited into the myogenic transcriptosome is essential to confer the competence to activate transcription.

muscle-specific bHLH proteins and the ubiquitous HLH products of the E2A gene, E12 and E47 [29,54], and stimulates the dissociation of nuclear deacetylases from MRFs and MEF2 proteins [32]. A number of signaling pathways elicited during muscle differentiation contribute to the activation of the myogenic program by promoting muscle-gene expression. An important pathway that stimulates muscle differentiation is the calcium/calmodulin-dependent protein kinase (CaMK)-mediated pathway. Differentiation-activated CaMK I and IV phosphorylate class II HDAC members on conserved serine residues to stimulate interactions with the chaperon protein 14-3-3, thereby disrupting the association between HDACs and MEF2 proteins and exposing the nuclear export sequences in the C-terminal of HDACs [32,88,89]. As result, class II HDACs translocate to the cytoplasm, and MEF2 becomes competent to activate transcription. It is likely that additional kinases regulate class II HDAC interactions with 14-3-3 [32]. Moreover, CaMK signaling disrupts class II HDAC-HP1 binding independent of phosphorylation-mediated 14-3-3/HDAC interactions [90]. And CaMK and extracellular signal-regulated kinase 5 signaling pathways prevent MEF2 inactivation by sumoylation [91]. Recently, it has been reported that the interferon-related developmental regulator 1 (IFRD1) protein PC4 counters HDAC4-mediated inhibition of MEF2C, by displacing HDAC4 from MEF2C [103]. By contrast, HDAC1 repression of MyoD is relieved by an indirect mechanism. The concomitant decline of HDAC1 levels and the down-regulation of cyclin/cdk activity in response to the absence of mitogens, leads to the accumulation of hypo-phosphorylated pRb, which has higher affinity for HDAC1, and displaces it from MyoD [62]. The interplay between YY1-associated co-repressors and SRF-mediated recruitment of MyoD [58] has been described above. Importantly, along with the displacement of co-repressory enzymes, it is necessary to erase the epigenetic modification generated by these enzymes, in order to reset the chromatin for differentiation-related nuclear reprogramming. This is particularly true for lysine methylation; thus, differentiation-activated signaling is likely to direct the erasure of epigenetic silencing either via recruitment of de-methylases or by histone variant exchange [3].

The sub-sequent recruitment of the acetyltransferases p300/CBP, PCAF, GRIP, p/CIP, SRC1A, the arginine-methyltransferase CARM1, and the ATPase-dependent SWI/SNF chromatin-remodeling complexes, endows the myogenic transcriptosome with the enzymatic repertoire necessary to modify the nucleosome structure and initiate the transcription of target genes [104–112]. The recruitment of these co-activators on the regulatory regions of muscle genes is mediated by distinct interactions with MRFs and MEF2 proteins. For instance, both MyoD and MEF2 proteins recruit the acetyltransferases p300/CBP and PCAF by direct physical interactions mapped on distinct regions [104–108]. Functional and genetic inactivation of p300 and PCAF is sufficient to block the formation of differentiated myotubes, although these studies reported different levels of interference with muscle-gene expression [48,108,113]. The acetyltransferases PCAF and p300 acetylate MyoD on three evolutionary conserved lysines (K99–K102 and K104), and this acetylation is a critical event to activate MyoD-dependent transcription of muscle genes [63,114,115]. MyoD acetylation increases during the transition from myoblasts to myotubes, and hyper-acetylated MyoD displays higher affinity for its DNA-binding consensus motif—the Ebox [63]—and for the bromodomain of p300 [116]. Recent studies from Dilworth et al., who exploited an "in vitro transcription system", demonstrate that the acetyltransferase activities of p300 and PCAF are not redundant, with p300-dependent acetylation of histones preceding promoter recruitment of PCAF; and PCAF-dependent acetylation of MyoD being necessary for transcription [117]. MEF2 proteins also interact with p300 [107], and their acetylation at multiple lysines is essential for DNA binding and transcriptional activity [92]. The recruitment of p160 proteins appears instead to be mediated by individual interactions of p/CIP and SRC1A with MyoD [111] and GRIP1 with MEF2 [109]. Likewise, CARM1 selectively interacts only with MEF2 proteins [110]. Finally, a chromatin-remodeling activity associated to the myogenic transcriptosome [102] is provided by the recruitment of the ATPase-dependent SWI/SNF chromatin-remodeling complexes [112].

How do intracellular pathways coordinate the assembly of the myogenic transcriptosome? Is each of the events leading to the transcriptosme formation controlled by distinct signaling cascades? Mitogen-activated cascades prevent MyoD–E12/47 heterodimerization via induction of Id or other mechanisms [29,54,55,118,119], and serum-induced cyclinD-cdk4 prevents the association between MEF2 and GRIP1 into punctate nuclear sub-domains [120]. It is likely that other extracellular-signal activated kinases regulate interactions between the components of the myogenic transcriptosome by direct phosphorylation. MRFs, MEF2 proteins and p300 are regulated by a variety of kinases, and their phosphorylation pattern changes along with the cell cycle and terminal differentiation [29,121–123]. However, the contribution of individual pro-myogenic cascades in regulating the interactions and the activity of these proteins has begun to be elucidated only in the last years. Two independent cascades, the IGF1-activated Pi3K/AKT signaling and the MKK6/p38 pathway, exert a critical role in promoting the activity of MRFs and MEF2 proteins [17–20,124,125]. Although these two pathways are activated by distinct stimuli and proceed as parallel cascades [19], they are not functionally redundant, as inhibition of either pathway is sufficient to prevent muscle-gene expression [17–20,126–128], suggesting that they converge on chromatin elements to regulate discrete steps of the transcriptosome assembly.

The identity of the proteins targeted by these two pathways is only partially defined. MEF2 are activated via direct phosphorylation by the p38 kinases, which trigger their transcriptional activity [18,19,70,129–133]. p38 $\alpha/\beta$ kinase activity is also required for SWI/SNF recruitment by MyoD and MEF2 proteins to the regulatory regions of muscle genes [57,134].

Interestingly, p38 blockade does not affect the DNA binding of MRFs and MEF2 and the recruitment of p300 and PCAF, as well as their enzymatic activity [57], suggesting that a parallel pathway regulates these events. Insulin- and IGF1-activated Pi3K pathway is a candidate regulator of the assembly of acetyltransferases with MyoD, as in neuronal cells the Pi3K downstream Akt1/2 kinases promote the interactions between the bHLH protein NeuroD and neurogenin with p300 and PCAF [135].

Dynamic interactions between chromatin-associated proteins at the regulatory sequences of muscle genes are not restricted to the initial stages of differentiation, but extend to later stages of myotube formation, to regulate chromatin structure and gene expression in response to metabolic and electrical stimuli. For instance, the NAD+-dependent histone deacetylase Sir2 forms a complex with PCAF and MyoD to inhibit muscle-gene expression in response to redox changes [136]. Radical oxygen intermediates are likely mediators of this pathway. Furthermore, Mejat et al. showed that muscle innervation by pre-synaptic neurons controls chromatin acetylation of the myogenin gene by regulating MEF2 interactions with HDAC9 and class I HDACs [137].

## 6. p38 signaling regulates multiple steps of muscle-gene expression

p38 kinases are the effectors of a master regulatory pathway of myogenic differentiation. Although it is assumed that MKK6 and MKK3 are the physiological activators of p38 kinases in response to differentiation cues, the upstream regulators of p38 during skeletal myogenesis have not precisely been identified. It is known that HMGB1, a chromatin component released by necrotic cells during inflammation [138], induces p38 signaling in myoblasts via RAGE-independent pathway [139]. Importantly, when p38 is induced in the context of differentiation-unrelated cellular responses (e.g. by certain inflammatory cytokines, stress, mitogens), it fails to promote differentiation or even inhibits the myogenic program (140, and PLP unpublished results). Functional blockade of p38 kinases α and β in myoblasts induced to differentiate, is sufficient to inhibit the transcription of most muscle genes and prevents myoblast fusion into myotubes [17–19,140]. Deliberate activation of p38 kinases by the constitutive active form of the upstream activators, MKK3 and MKK6, enforces premature differentiation in myoblasts cultured in the presence of serum mitogens [18,19,140]. This remarkable property the p38 pathway is unique among the other intracellular cascades, and implies that active MKK3/6 can initiate and sustain the whole differentiation program, including all the steps leading to the formation of the transcriptosome. It remains to be defined if this effect occurs by direct action of MKK3/6-activated p38 kinases on chromatin-binding proteins, or if the ectopic activation of this pathway in myoblasts triggers parallel, cytosolic pro-myogenic pathways [142]. p38 also participates to the regulation of cell cycle during muscle differentiation. Indeed, activation of p38 kinases by ectopic expression of MKK6EE causes cell cycle arrest in skeletal and cardiac myocytes [140,141]. And the role of p38 in the control of cell cycle arrest has been also reported in response to other stimuli, like stress and DNA damage [143,144].

Several lines of evidence indicate that p38 targets multiple components of the myogenic transcriptosome. p38 kinases directly regulate MEF2 function by phosphorylation of all four members [129–133], and indirectly promote MyoD-mediated transcription by stimulating MyoD heterodimerization with E47, via phosphorylation of this latter [59]. Interestingly, p38-dependent phosphorylation of E47 has different effects on MyoD function depending on the stage of cellular differentiation and the environmental context. In myoblasts, p38-mediated phosphorylation of E47 is stimulated by serum activated Raf-MEEK1, and results in the inhibition of E47-dependent transcription [145]. This inhibition might have a possible function in restricting the myogenic lineage by impairing E12/47-dependent transcription of genes specific of the B cell lineage [146]. Since in myoblasts MyoD binding to E12/E47 is inhibited [55], it can be assumed that MyoD is spared by the control of p38 kinases at the myoblast stage. Conversely, when p38 is activated by differentiation-related cues—that is in the absence of mitogens—p38-mediated phosphorylation of E47, at serine 140, occurs in conditions permissive for MyoD/E47 hetero-dimerization, and further stimulates this process [59]. It remains unclear from these studies whether E47 phosphorylation occurs on the same residues in myoblasts vs myotubes. Interestingly, CDO, an Ig superfamily member activated by cell-to-cell contact, promotes hetero-dimer formation between MyoD and E12/47, most likely by inducing hyperphosphorylation of E-proteins [147]. It will be interesting to know whether CDO mediates the activation of p38 pathway in response to cellular confluence. As myogenic bHLH and MEF2 proteins synergistically activate muscle-gene expression, it is conceivable that p38 promotes the transcription of muscle genes also by targeting a common regulator of these proteins. The demonstration that p38 α and β kinases direct the recruitment of the SWI/SNF complex on muscle-gene regulatory regions [57] has provided a direct link between extra-cellular signal activated kinases and chromatin remodeling [148]. p38-dependent recruitment of SWI/SNF correlates with the engagement of hyper-phosphorylated, active PolII holoenzyme to muscle-gene promoters [57]. As polymerase II holoenzyme contains SWI/SNF components, p38-mediated recruitment of PolII could be either a consequence of SWI/SNF phosphorylation or an independent event. The ability of p38 kinases to recruit chromatin-modifying complexes, such as SWI/SNF, to their target promoters in response to environmental cues is not unprecedented. In yeast, the p38 functional homologous, Hog1 kinase, activates ATF/CREB-dependent transcription, in response to osmotic stress, by favoring the recruitment of SWI/SNF to osmotic-inducible promoters [149]. Interestingly, both p38 and Hog1

have been detected on the chromatin of target promoters, associated with PolII [57,70,149]. And physical interactions have been described between Hog1 and p38 with PolII and general components of the transcription machinery [150].

One mechanism by which p38 recruits SWI/SNF to target promoters can rely on direct phosphorylation of the structural SWI/SNF sub-unit BAF60 [57]. BAF proteins provide the surface for interactions between SWI/SNF and sequence-specific transcription factor [151–154]. The heterogeneity and cell-type specific distribution of SWI/SNF structural BAF sub-units can account for the specificity of the SWI/SNF recruitment at discrete loci in response to external signals in different cell lineages. For instance, there are three BAF60 isoforms (a, b and c) described to date [154], with BAF60c abundantly expressed in cardiac and skeletal muscles [153]. Genetic ablation of BAF60c in mice selectively impairs cardiac and skeletal muscle differentiation during embryogenesis [155], suggesting that BAF60c is a key molecule in the activation of differentiation genes of the myogenic lineage. We have observed that p38 α and β kinases preferentially phosphorylate BAF60c in vitro (SVF and PLP unpublished results). Thus, an important function of BAF60c could be to receive the information transmitted by cytoplasmic cascades (e.g. the p38 signaling) and broadcast it to other SWI/SNF members and chromatin-bound proteins, thereby allowing the myogenic transcriptosome to adopt the conformation permissive for transcription. In this regard, BAF60c could be an interesting target for interventions aimed at selectively modulating muscle-gene expression.

SWI/SNF is also implicated in the regulation of cell cycle arrest, when recruited on promoters of proliferation genes (e.g. cyclins), via interaction with pRb [156,157]. In this context, SWI/SNF provides an inhibitory function on transcription of target genes, as part of the pRb-associated complex, which establishes the cell cycle withdrawal during terminal differentiation [158]. Given the cytostatic activity of the p38 pathway in differentiating myoblasts [140,141], it will be interesting to determine whether p38 kinases also regulate SWI/SNF recruitment into the pRb-associated co-repressory complex.

A careful analysis of the interactions reported between MRFs, MEF2 and chromatin-modifying enzymes, and their regulation by p38 kinases, leads to the formulation of a stepwise model of assembly of the myogenic transcriptosome through distinct interactions. MyoD binding to p300, SCR1a or p/Cip occurs in vitro, in the absence of E12/47 and MEF2 proteins [104,105,107,111], and MyoD/acetyltransferase interactions are detectable already in myoblasts [105,107,108,111], suggesting that MyoD homo-dimers can bind acetyltransferases. The N-terminal of MyoD appears to accommodate simultaneous interactions with p300, PCAF, SRC and p/CIP [107,108,111]. As p38 blockade prevents MyoD–E47 interaction, but is permissve for MyoD binding to the DNA [57,59], it is likely that in the absence of p38 signaling, MyoD homo-dimers form interactions with acetyltransferases on target promoters. In keeping with the notion that MyoD–E47 interactions only occur upon activation of the p38 pathway, it is possible that MyoD–E12/47 hetero-dimer formation is required for interaction with SWI/SNF. MEF2 also interact with SWI/SNF [59,134], and this binding could take place independent of MRFs, and could be regulated by p38-dependent phosphorylation of both SWI/SNF and MEF2 proteins.

The p38 pathway regulates expression of muscle genes also by direct phosphorylation of additional regulatory proteins. p38-mediated phosphorylation of the p160 myb-binding protein (p160-MBP), a repressor of the PPAR γ coactivator 1 α (PGC-1 α), disrupts p160/PGC-1 α interaction, leading to PGC-1 α-mediated transcription of genes involved in the regulation of metabolic processes, (e.g. mitochondrial biogenesis and respiration) in response to cytokines or β-adrenergic signaling [159]. Finally, a role of p38 in the control of post-transcriptional events is suggested by the ability of p38 kinases to attenuate the decay of mRNA containing AU-rich elements (ARE) [160]. As several muscle transcripts contains ARE, it is possible that p38 regulates the stability of nascent transcripts. Given the ability of 38 kinases to affects multiple events of muscle gene transcription, from transcriptosome assembly to mRNA stabilization, they can be defined the “master-regulators of the muscle-gene factory”.

While the role of the p38 pathway in promoting differentiation of muscle cell cultures is well established, the biological impact of the p38 pathway should be considered within the context of biological systems, such as somitogenesis and muscle regeneration, which are regulated by a more complex environmental network—e.g. lateral cues from other tissues. In this respect, it is worthy to note that one in vivo experiment of p38 blockade, by its soluble inhibitor SB, performed in limb buds, produced a surprising increase in myotube formation—an effect reproduced by co-culturing myoblasts with limb mesenchimal cells [161]. In contrast, de Angelis et al. reported that transplacental injection of the p38 inhibitor SB203580 resulted in the inhibition of myogenic differentiation in somite cultures and in embryos in vivo [162]. The same study also shows that the commitment to the myogenic lineage is not appreciably affected by p38 inhibition, since the activation of an early marker of myogenic commitment (Myf5) occurs normally when p38 signaling is inhibited. Collectively, the results reported above underscore the importance of the microenvironment in directing the final effect of the p38 pathway in physiological contexts and indicates that the p38 pathway can provide an ambiguous signal for muscle differentiation. The timing of p38 activation, the signal-dependent upstream p38 regulators, and the parallel pathways activated along with p38 kinases, all contribute to determine the final impact of the p38 signaling on the myogenic program. Consistent with such a context-dependent versatility of the p38 pathway, p38-mediated phosphorylation can inhibit the function of MRF4 [163] and E47 [145]. And DNA damage-activated p38 promotes p300 degradation, leading to down-regulation of muscle gene expression in cardiomyocytes [164]. Cross-talk with other cytoplasmic

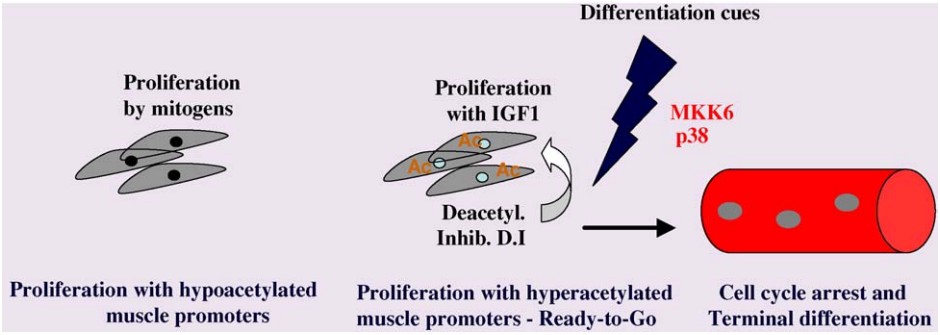


Fig. 3. Different stages of myoblast proliferation, with or without hyperacetylated promoters. In myoblasts exposed to mitogens the presence of deacetylase on muscle regulatory regions inhibit local hyperacetylation. Differentiation is induced by mitogen withdrawal and exposure to IGF or insulin. In the absence of p38 activity (e.g. by experimental p38 blockade with SB), IGF- or insulin-mediated signaling is sufficient to promote the hyperacetylation at muscle regulatory regions; however, cell cycle arrest and gene transcription are not induced until the p38 pathway is induced (or is resumed upon SB removal). Thus, the simultaneous interference with p38 signaling to the chromatin of muscle genes and exposure to IGF1 can expand of population of proliferating myoblasts, which are "primed" for differentiation by hyperacetylation at muscle loci. An equivalent effect can be obtained by exposing myoblasts to deacetylase inhibitors. This model can explain the enhanced differentiation potential of IGF- or TSA-treated muscle cells, and can inspire pharmacological strategies to enhance the efficiency of muscle regeneration.

cascades also contributes to determine the biological outcome of the p38 signaling. The mutually exclusive pattern of activation of ERK and p38 pathways during myoblast-to-myotube transition [19] suggests that these two cascades can regulate each other's activity. Indeed, p38 pathway can inhibit the Ras pathway [165], and MKK6/3-dependent activated p38 kinases inhibit ERK signaling [166]. On the other hand, in myoblasts, mitogen-activated Ras signaling can down-modulate or re-direct the p38 pathway toward other functions [167]. Furthermore, differentiation-activated p38 induces NFκB-activity in differentiating myoblasts [27].

By contrast, p38 and IGF1/Pi3K pathways proceed as parallel pathways in the cytoplasm of differentiating myoblasts [19,127]. As these two pathways appear to converge in the nucleus, their functional interdependence could be envisioned at the chromatin level. In this respect, it is interesting to note that p38 blockade in myoblasts exposed to IGF1-activated signaling, without the presence of mitogens, allows the partial formation of a myogenic transcriptosome, containing muscle-transcription factors and acetyltransferases, and leading to the hyperacetylation of muscle promoters in proliferating myoblasts [57]. This evidence suggests that the ability of IGF1 to stimulate both proliferation and differentiation of muscle cells [168] is strictly dependent on the timing of p38 activation. In the absence of p38 signaling, IGF1 stimulates proliferation of myoblasts, while promoting hyperacetylation at muscle-gene regulatory elements. Upon p38 activation, SWI/SNF chromatin recruitment imparts to the myogenic transcriptosome the competence to activate gene transcription, and promotes cell cycle arrest. Thus, the activation of the p38 pathway during myoblast differentiation can convert the IGF1- from a mitogenic signaling into a pro-myogenic pathway. Interestingly, induction of p38 kinases is detectable in activated satellite cells [169], suggesting that this sequence of events can determine the efficiency of satellite-mediated muscle regeneration. The timing of activation of the IGF1 signaling and p38 pathway during regeneration should be defined by future studies, as it could provide an interesting target for pharmacological interventions aimed at expanding a large population of myoblasts "primed" by IGF to differentiate upon the activation of p38 kinases (Fig. 3). It is obvious that chromatin targets of the p38 pathway, such as BAF60, are interesting candidates for screening directed toward the identification of agents that can modulate the efficiency of muscle regeneration.

Finally, it will be interesting to establish the relationship between signal transduction pathways and novel regulators of gene expression, such as micro-RNA, and how the nuclear architecture changes in response to environmental cues.

## 7. Conclusion

The results summarized in this review indicate the potential importance of transcription modulation in regenerative medicine. Deacetylase inhibitors provide an example of pharmacological interference on chromatin events (e.g. deacetylation) exploitable for therapeutic purposes. Exposure of myoblasts to deacetylase inhibitors leads to the anticipated hyperacetylation of both MyoD and the histones surrounding MyoD-binding sites, and results in the earlier transcription of muscle genes and in the formation of hypernucleated myotubes with an increased size [170]. A relevant target of deacetylase inhibitors in muscle cells is the follistatin gene [171]. Follistatin is the physiological antagonist of myostatin—a negative regulator of muscle mass and regeneration [172,173]. Muscles exposed to deacetylase inhibitors express high levels of follistatin and form myofibers larger than normal [171]. Deacetylase inhibitors are used in the clinical practice [174,175], and can be therefore exploitable for pharmacological modulation of muscle mass. Studies on mouse models of neuromuscular diseases showed that

myostatin blockade counters the dystrophic phenotype in MDX mice [176,177]. Future studies will determine the suitability of deacetylase inhibitors as a potential pharmacological agent to increase muscle mass in the treatment of muscular disorders via follistatin-mediated blockade of myostatin.

It should be emphasized that targeting cellular deacetylases generally affects gene expression, hence can generate several side effects. Therefore, the identification of promoter-specific targets of signaling pathways is imperative to increase the selectivity of pharmacological interventions.

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