ELSEVIER

Mechanisms underlying the transcriptional regulation of skeletal myogenesis

Vittorio Sartorelli and Giuseppina Caretti

During skeletal myogenesis, chromatin-modifying enzymes are engaged at discrete genomic regions by transcription factors that recognize sequence-specific DNA motifs located at muscle gene regulatory regions. The composition of the chromatin-bound protein complexes and their temporally and spatially regulated recruitment influence gene expression. Recent findings are consistent with the concept that chromatin modifiers play an important role in regulating skeletal muscle gene expression and cellular differentiation.

Addresses
Muscle Gene Expression Group, Laboratory of Muscle Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, 50 South Drive, Room 1146, Bethesda, MD 20892-8024, USA

Corresponding author: Sartorelli, Vittorio (sartorev@mail.nih.gov)

Current Opinion in Genetics & Development 2005, **15**:1–8

This review comes from a themed issue on
Differentiation and gene regulation
Edited by Tony Kouzarides and Andrew J Bannister

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2005.04.015

Introduction

The specification, proliferation and terminal differentiation of skeletal muscle cells is controlled by the combinatorial activities of several transcription factors. Pivotal in the biology of skeletal muscle cells are the myogenic basic helix–loop–helix (bHLH) MyoD, Myf5, myogenin and Mrf4 proteins. After interaction of these proteins with the ubiquitously expressed bHLH E proteins, the resulting myogenic bHLH–E heterodimers bind to and regulate expression from the E-box, a specific DNA motif present at muscle gene enhancers and/or promoters. Additional activators such as members of the MEF2 and SRF families of MADS-box transcription factors co-regulate muscle gene transcription.

The interactions of transcription factors with enzymes that modify the structure of the nucleosome — the basic unit of chromatin — enable temporally regulated formation and recruitment of specific protein complexes at the chromatin of discrete muscle gene loci. Post-translational modifications (i.e. phosphorylation, acetylation, methylation, sumoylation [1], ubiquitination, ADP-ribosylation and possibly others) induced by the transcription factor-bound enzymes on nucleosomal and linker histones, and on transcription factors themselves have profound effects on gene expression. The binding of specific transcription factors to chromatin, and the composition and enzymatic activities of chromatin-bound protein complexes determine whether a specific gene will be transcribed or not.

In this review, we focus on recent advances in our understanding of the role of chromatin modifications as they pertain to skeletal muscle gene transcription

Absence of gene activation and/or transcriptional repression in skeletal muscle cells

MyoD, Myf5 and E proteins are expressed in undifferentiated myoblasts yet, in this cellular context, they do not activate transcription. Once extracellular cues are interpreted by the undifferentiated myoblasts as pro-differentiation signals, MyoD and Myf5 become transcriptionally competent and activate the skeletal myogenic program. It appears that multiple and distinct mechanisms ensure that transcription is not prematurely activated in undifferentiated muscle cells.

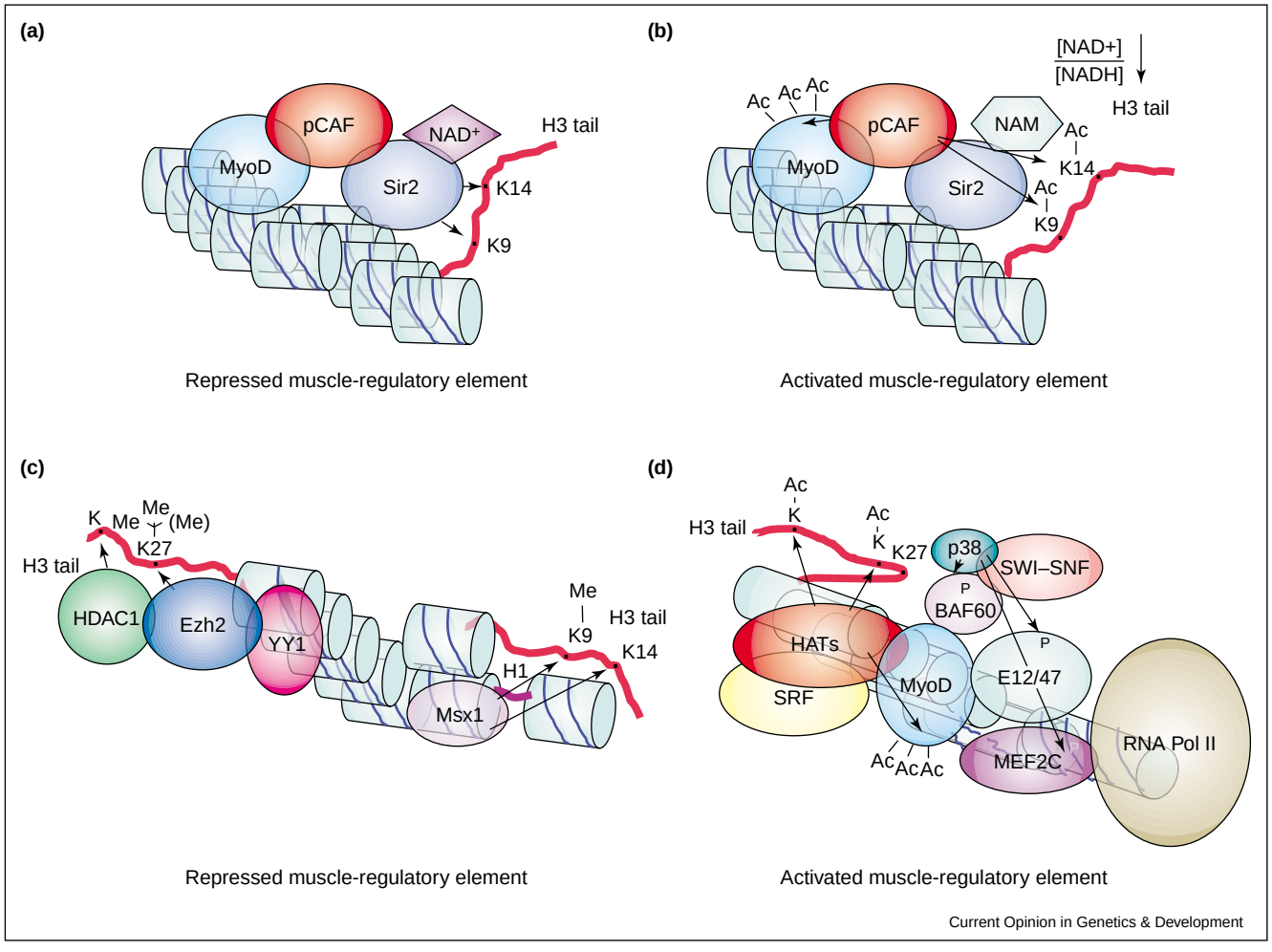
Recruitment of actively suppressing protein complexes on the chromatin of muscle gene regulatory regions

In addition to the mechanisms — such as formation of E–Id1 protein complexes, and possibly MyoD hypoacetylation — that prevent binding of MyoD to its DNA targets, it is of interest to consider those that repress transcription through the recruitment of actively suppressing protein complexes on the chromatin.

The silent information regulator 2 (Sir2; the mouse homolog of human SIRT1) belongs to the histone deacetylase (HDAC) III family of enzymes. The activity of HDAC III enzymes is stimulated by the cofactor nicotinamide adenine dinucleotide (NAD^+) and repressed by nicotinamide (NAM) [2]. Sir2 controls several important biological processes, including transcriptional silencing, aging, DNA recombination and repair, and apoptosis. Some — if not all — of these functions require that Sir2 be recruited on the chromatin. Given that Sir2 does not bind specific DNA elements, how is it directed to specific chromatin domains? In some instances, Sir2 directly interacts with transcription factors such as p53; in others, Sir2 is 'piggy-backed' to chromatin through its interaction with histone-modifying enzymes, which, in turn, interact with specific DNA-binding proteins. In cell

extracts derived from undifferentiated myoblasts, Sir2 is found in a protein complex containing the acetyltransferase pCAF (p300/CBP-associated factor) and MyoD. This protein complex might associate with the chromatin of selected muscle gene enhancer and/or promoters that are bound by MyoD in undifferentiated myoblasts (Figure 1a and see also 'Myogenic bHLH binding not followed by gene activation'). The ability of Sir2 to deacetylate H3K9 and H3K14 correlates with repression of muscle gene expression and cell differentiation. Indeed, whereas overexpression of Sir2 retards muscle differentiation, a Sir2 mutant devoid of deacetylase activity no longer contrasts myotube formation [3]. Sir2 can also be detected, in conjunction with pCAF and MyoD, on the chromatin of actively transcribed muscle gene-specific promoters and/or enhancers. Given that the [NAD^+]/[NADH] ratio decreases as muscle cells differentiate, the deacetylase activity of the chromatin-bound Sir2 might decline during this process. Alternatively, high levels of NAM might inhibit Sir2 activity in differentiated myotubes (Figure 1b). The acetyltransferase activities of pCAF [4] and p300 [5] are stimulated by autoacetylation and

Figure 1



The presence of specific protein complexes at the chromatin of muscle loci correlates with defined histone modifications and transcriptional activity. **(a)** In undifferentiated myoblasts, a protein complex containing MyoD, pCAF and Sir2 is detected at transcriptionally inactive muscle regulatory regions. Lysines 9 and 14 of histone H3 are hypoacetylated. Interaction of NAD^+ with Sir2 is required to stimulate Sir2 deacetylase activity. **(b)** Upon induction of muscle differentiation, the protein complex is still detected on the chromatin, but lysines 9 and 14 become hyperacetylated and transcription is initiated. The [NAD^+]/[NADH] ratio is reduced and nicotinamide (NAM) might inhibit Sir2. **(c)** The transcription factor YY1 recruits the Polycomb Ezh2 methyltransferase and HDAC1 deacetylase at specific inactive muscle loci. Lysine 27 of histone H3 is methylated by Ezh2, and several other lysines are hypoacetylated. The transcription factor Msx1 interacts with the linker histone H1 in a region where lysines 9 and 14 of histone H3 are hypoacetylated and lysine 9 is methylated. **(d)** Transcriptional activation is associated with loss of the YY1–Ezh2–HDAC1 complex and recruitment of the transcription factors SRF, MyoD and associated chromatin-remodeling SWI–SNF complex and histone acetyltransferases (HATs). The p38 kinase interacts with and phosphorylates the BAF60 subunit of SWI–SNF and phosphorylates E12/E47 and MEF2C, which, in turn, recruits the RNA polymerase II complex. Formation of the activating protein complex coincides with lysine hyperacetylation, lysine 27 demethylation and MyoD acetylation.

are required to activate muscle gene expression. The ability of Sir2 to deacetylate both pCAF [3] and p300 [6], and the physical proximity of these proteins on muscle regulatory regions might offer a finely tunable mechanism for the rapid and reversible adjustment of muscle gene expression in response to changing metabolic muscle demands that occur during development and in the adult life.

An additional inhibitory complex found at muscle gene regulatory regions contains the Polycomb group protein Enhancer of zeste 2 (Ezh2), a histone lysine methyltransferase (HKMT) that promotes transcriptional repression. Overexpression of Ezh2 in either established or primary skeletal muscle cells contrasts muscle gene expression and cell differentiation, a phenomenon that is dependent on the HKMT activity of Ezh2 [7••]. A protein complex comprising the transcription regulator YY1, Ezh2 and HDAC1 can be detected on the chromatin of selected muscle gene regulatory regions when their correspondent genes are inactive and lysine 27 of histone H3 (H3K27) is hypermethylated (Figure 1c). Transcriptional activation that accompanies skeletal muscle differentiation is characterized by loss of YY1, Ezh2 and HDAC1, and recruitment of the transcriptional activators MyoD and SRF (Figure 1d). Chromatin engagement of Ezh2 relies on YY1, because reducing the levels of YY1 by RNA interference results in a lack of Ezh2 recruitment. Intriguingly, PRC4 (Polycomb repressor complex 4) contains both Ezh2 and SIRT1 [8], and Sir2 is required for Polycomb-mediated silencing [9]. These observations suggest the possibility that SIRT1 might be recruited on the chromatin by both MyoD-dependent (mediated by the MyoD–pCAF–SIRT1 complex) and MyoD-independent (YY1–Ezh2–SIRT1) pathways, and that Sir2 and Ezh2 might cooperate in repressing muscle gene expression. The Ezh2 complex might not regulate expression of every muscle-specific gene. For instance, myogenin expression does not seem to be influenced by Ezh2 [7••]. The HKMT Suv39H1 and the associated HP-1, HDAC4 and HDAC5 proteins might negatively regulate myogenin expression [10], because its promoter is methylated at H3K9 [11] — a hallmark of Suv39H1 activity — and is hypoacetylated in undifferentiated myoblasts. Consistently, Suv39H1 expression plunges in differentiated myotubes [12]. Nonetheless, using RNA interference to reduce the levels of SUV39H1 in myoblasts prevents their differentiation, perhaps through an indirect mechanism related to the ability of Suv39H1 to silence S-phase genes [12].

Why would multiple HDACs and HKMTs be required to repress muscle gene expression? Muscle gene activation is a temporally regulated phenomenon, with some genes being activated earlier than others. The overall pattern of methylation and acetylation established by a given combination of HKMTs and HDACs might determine whether histones will assume a conformation that is easily 'permissive' to transcription factors — in the case of genes activated at earlier stages — or more difficult for transcriptional machinery to penetrate — in the case of genes activated at later stages of myogenesis.

In addition to nucleosomal core histones, linker histones also participate in muscle gene regulation. Overexpression of either the homeoprotein Msx1 or the linker histone H1b represses muscle gene expression. Furthermore, overexpressed Msx1 interacts with histone H1b, and both proteins are found at the enhancer region of MyoD, where they increase H3K9 methylation, reduce acetylation of H3K9 and H3K14, and decrease phosphorylation of H3S10 (Figure 1c) [13••]. Co-expression of Msx1 and H1b in *Xenopus* results in synergistic repression of MyoD expression. These findings predict that when muscle-specific transcription is activated H1b and Msx1 will be either removed from the chromatin or inactivated. Whether association of endogenous Msx1 and H1b with the MyoD enhancer and regulatory elements of other genes is developmentally regulated in muscle cells remains to be determined.

Myogenic bHLH binding not followed by gene activation

Chromatin immunoprecipitation (ChIP) coupled with mouse promoter DNA microarray hybridization (ChIP-chip) has enabled the identification of approximately 200 genes bound by MyoD and/or myogenin and MEF2 [14•]. Of its approximately 100 target genes, MyoD bound to half in undifferentiated myoblasts and the other half in differentiated myotubes. Several targets bound by MyoD were activated neither in myoblasts nor in myotubes. Others were activated only in either myoblasts or myotubes. These findings are intriguing and raise several questions: which are the mechanisms that impede transcriptional activation of some of the target genes bound by MyoD in myoblasts? How is it that other targets are recognized and activated by MyoD in myoblasts? Finally, how does MyoD discriminate, in terms of binding, between different targets? Several — not mutually exclusive — hypotheses can be formulated to explain these observations: (i) in myoblasts, MyoD might form transcriptionally futile homodimers that can bind some targets but are unable to promote transcription; (ii) MyoD-mediated recruitment of chromatin-remodeling machines and/or the acetyltransferase p300 and pCAF might be ineffective in myoblasts; (iii) the presence of suppressive protein complexes, such as the PcG Ezh2, on the neighboring chromatin might counteract the activity of chromatin-bound MyoD complexes; (iv) MyoD might engage HDACs such as HDAC1 and/or Sir2 on the chromatin and prevent gene activation; (v) differences in the composition of DNA modules present in the regulatory regions and subtle variations in the MyoD DNA-binding sites might influence the affinity of MyoD for different targets and/or affect the ability to recruit

additional factors, such as MEF2 and SRF, and distinct co-activators and/or co-repressors.

Activation of muscle gene expression

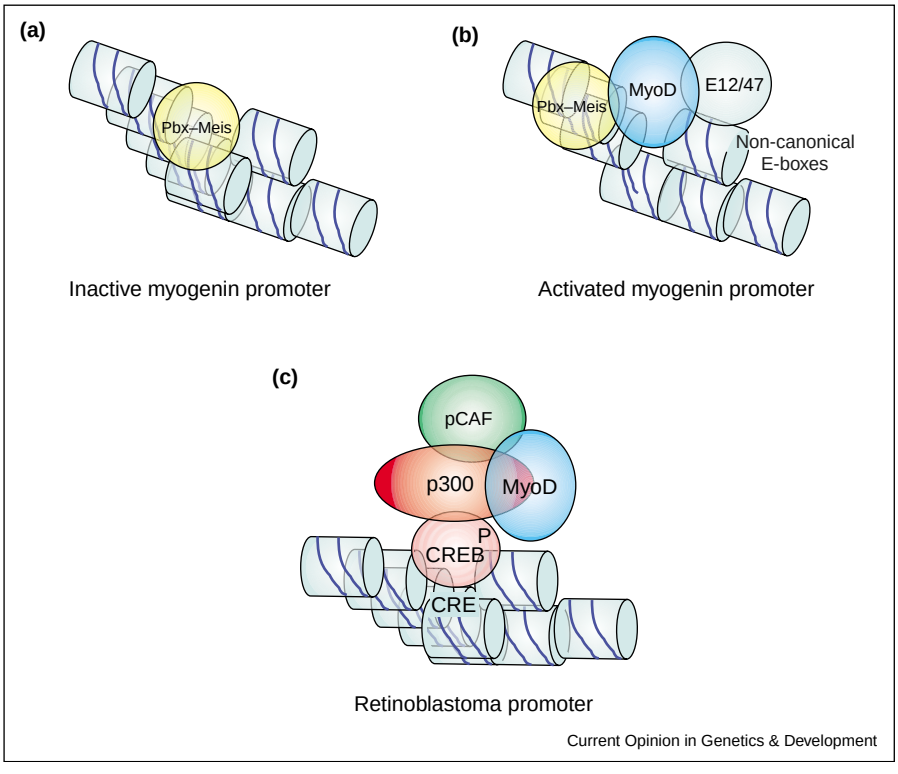
MyoD–E protein heterodimer formation

Formation of myogenic–E bHLH heterodimers is required for productive DNA-binding and transcriptional activation. CDO (cAMP response element decoy oligonucleotide), a cell surface receptor of the Ig superfamily that is expressed in muscle precursor cells and other cell types, favors muscle differentiation, and *Cdo*$^{-/-}$ mice show delayed myogenesis. CDO increases phosphorylation of the E proteins and enhances formation of MyoD–E protein heterodimers [15]. Similarly, the p38 kinase stimulates formation of MyoD–E protein heterodimers [16]. Given that CDO does not seem to have kinase activity on its own, it is possible that its influence on MyoD–E heterodimer formation is mediated by p38 kinase.

MyoD binding to chromatin targets — direct and indirect binding modalities

The binding of MyoD to selected regulatory regions is followed by chromatin remodeling. Two domains of MyoD — a histidine- and cysteine-rich region (H/C domain) at the N terminus, and a C-terminal region forming an amphipathic α helix (helix III domain) — are required to mediate chromatin modification. Interestingly, different regions of MyoD mediate activation of distinct subset of genes during myogenesis. Activation of the myogenin locus requires the H/C and helix III domains of MyoD. These two domains of MyoD enable it to bind stably to the myogenin promoter through a non-canonical E-box — by protein–protein interaction with an adjacent complex containing the homeodomain proteins Pbx and Meis (Figure 2a,b) [17••]. Given that the Pbx–Meis complex is bound to the myogenin promoter before transcriptional activation of the gene (Figure 2a), it might serve as a mark for recruitment of MyoD and subsequent gene activation. The H/C and helix III domains of MyoD are not conserved in myogenin, suggesting the possibility that genes marked by Pbx–Meis in skeletal muscle cells might be selective targets of MyoD. In a separate instance, MyoD has been reported to be recruited on the retinoblastoma promoter through interaction with the cyclic AMP-responsive element CRE in a protein complex containing CREB, p300 and pCAF (Figure 2c) [18]. Therefore, MyoD can interact directly with the E-box,

Figure 2



Indirect recruitment of MyoD at specific muscle promoters. **(a)** The myogenin promoter is constitutively occupied by a protein complex containing the homeodomain protein Pbx in undifferentiated myoblasts. **(b)** Interaction of MyoD–E12 heterodimers with non-canonical E-boxes of the myogenin promoter is stabilized through interaction with the Pbx complex, and coincides with transcriptional activation. **(c)** MyoD is indirectly recruited on the promoter of the retinoblastoma gene through interaction with a p300–pCAF complex associated with phosphorylated CREB bound to a DNA CRE element.

with non-canonical E-boxes or with alternative DNA binding sites, provided that it is assisted by other transcription factors.

Recruitment of chromatin-remodeling and HAT protein complexes

Binding of MyoD to DNA is required but not sufficient to promote transcription [14•]. In some instances, MyoD binding is not followed by transcriptional activation; in others, there is a significant delay between binding of MyoD and gene activation [19]. The distinct behavior of MyoD at different chromatin targets is probably dictated by the differential engagement of MyoD-associated factors. The chromatin-remodeling SWI–SNF factor interacts with and promotes MyoD activity. The SWI–SNF subunit BAF60 is phosphorylated by the p38 kinase, which can be found at myogenic loci (Figure 1d) [20••]. Indeed, forced activation of the p38 kinase pathway by a constitutively active form of MKK6 favors SWI–SNF chromatin recruitment, facilitates binding of MyoD and MEF2, and recruitment of RNA polymerase II (Figure 1d), and anticipates expression of late-activated genes at early stages of muscle differentiation [21•]. Pharmacological blockade of p38 prevents SWI–SNF recruitment and impedes muscle gene expression without affecting either binding of MyoD or recruitment of HATs. Given that the p38 kinase activity is necessary for the expression of a restricted subset of genes regulated by MyoD [19], other kinases might regulate recruitment of SWI–SNF at different myogenic loci. Alternatively, additional chromatin-remodeling complexes might be involved in modulating muscle gene expression. In addition to its recruitment of chromatin-remodeling complexes, MyoD also recruits p300 and pCAF. Both MyoD and histones are substrates of p300 and pCAF acetyltransferase activities. The development of a MyoD-dependent *in vitro* transcription system has enabled the clarification of the distinct roles played by these two acetyltransferases in muscle-specific transcription [22•]. Initially, p300 acetylates histones H3 and H4 and, subsequently, pCAF acetylates MyoD, with both proteins synergistically activating transcription of a nucleosomal MyoD-target template. Therefore, p300 and pCAF execute sequential and distinct but coherent functions. Members of the p160 family of co-regulators impose an additional level of control on MyoD function. Although both SRC1A and p/CIP interact with and co-activate MyoD, GRIP1 inhibits it. Interestingly, GRIP interacts with MyoD regions that are required to recruit SRC1A, pCIP and p300, suggesting the possibility that a competition between these cofactors might regulate MyoD activity [23]. An indirect mechanism converging on MyoD activation entails removal of the inhibition exerted by HDAC4 on MEF2C. The interferon-related developmental regulator PC4 associates with MEF2C and, in doing so, displaces HDAC4 from MEF2C, thereby indirectly promoting MyoD activity [24].

Role of non-muscle-restricted transcription factors and cofactors in skeletal myogenesis

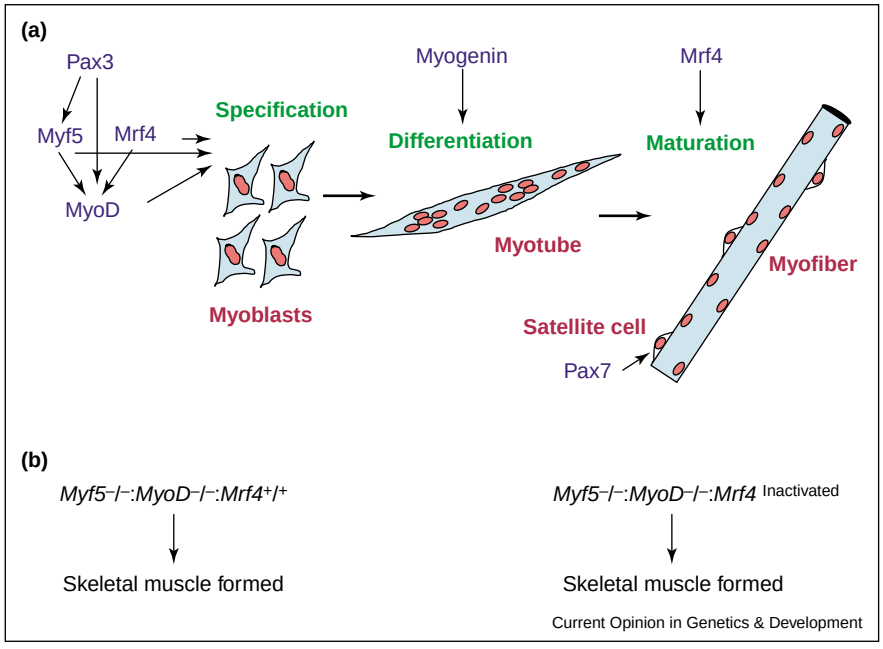
SRF regulates muscle gene expression through its interaction with a variety of co-regulatory proteins, including members of the myocardin family. To overcome the embryonic lethality of $Srf^{-/-}$ animals, *Srf* was specifically inactivated in skeletal muscle, using skeletal muscle-specific transgenes encoding Cre recombinase. Skeletal muscles derived from animals lacking *Srf* expression displayed smaller multinucleated muscle fibers, and the animals died during the perinatal period with severe skeletal muscle hypoplasia [25]. Skeletal and cardiac-α actin transcripts were reduced in these animals whereas other muscle-specific transcripts were not affected. A similar phenotype was obtained in transgenic animals overexpressing a dominant negative mutant form of a myocardin family member [25], thus suggesting that the effects of SRF on the muscle maturation might be ascribed to its ability to recruit myocardin.

MEF2C and MEF2D cooperate with myogenic bHLH to activate muscle gene expression. Association of class IIa HDACs with MEF2C and MEF2D promotes their sumoylation by SUMO2 and SUMO3 enzymes, respectively. Sumoylation reduces the transcriptional activity of the MEF2 proteins, and, accordingly, the SUMO protease SENP3 reverses sumoylation and augments the transcriptional and myogenic activities of MEF2 proteins. It remains to be determined if and when sumoylation and desumoylation of endogenous MEF2 proteins occur in skeletal muscle cells [26].

Members of the Wnt family of secreted glycoproteins promote expression of *Pax3*, *MyoD* and *Myf5*, and formation of the trunk skeletal muscles. Recent studies have established that such induction is mediated by the cAMP-activated protein kinase A through the activity of the transcription factor CREB [27••]. In CREB-deficient mice, *Pax3*, *MyoD* and *Myf5* are not expressed, and myotome formation is defective. Furthermore, Wnt1 or Wnt7 can induce myogenesis in explants containing muscle precursor cells, and this phenomenon is associated with increased phosphorylation of CREB. Whether CREB directly or indirectly regulates expression of the myogenic bHLH and *Pax3* remains to be determined. Nonetheless, it is of interest to note that CREB phosphorylation enables recruitment of p300, and that mice lacking either the CREB protein [27••] or the acetyltransferase activity of p300 [28] have impaired expression of the myogenic bHLH, suggesting the possibility that CREB and p300, perhaps through direct interaction, might be required for activation of the myogenic program.

The retinoblastoma (Rb) tumor suppressor protein is involved in skeletal myogenesis by promoting the expression of late skeletal muscle differentiation markers. Results obtained using muscle-restricted and temporally

Figure 3



Hierarchical relationships of muscle regulatory factors. **(a)** Pax3, Myf5 and Mrf4 activate MyoD. Myf5, Mrf4 and MyoD can act as specification genes, whereas myogenin is required for differentiation of specified myoblasts into myotubes, and Mrf4 is involved in myofiber formation. Pax7 is required for satellite cell specification. **(b)** In the absence of both MyoD and Myf5, skeletal muscle is formed as long as expression of Mrf4 is not perturbed. Functional inactivation of Mrf4 — a consequence of genetic ablation of *Myf5* in $MyoD^{-/-}$ $Myf5^{-/-}$ double mutant mice — results in the lack of skeletal muscle.

regulated ablation of the *Rb* gene in mice indicate that Rb is required for the differentiation of myoblasts into myotubes but is dispensable for the maintenance of the terminally differentiated state of muscle cells [29]. Differentiation of *Rb*-null myotubes and expression of myosin-heavy chain were indistinguishable from those of *Rb*-positive myotubes. Although similar results have been reported when *Rb* was excised by adenoviral-mediated Cre recombinase in cultured myotubes [30], microarray assays conducted in this study showed that muscle gene expression was significantly reduced in $Rb^{-/-}$ myotubes, suggesting that the continuous presence of Rb is required for optimal muscle transcription.

Hierarchical organization of the myogenic basic helix–loop–helix proteins: a paradigm to be revised

Both MyoD and Myf5 are thought to specify the skeletal muscle phenotype, because double-mutant mice ($MyoD^{-/-}$ $Myf5^{-/-}$) completely lack skeletal muscle fibers and myoblasts, and have precursor cells that remain multipotent — with the ability to change their fate towards cell lineages other than muscle. Myogenin and Mrf4 are hierarchically downstream of MyoD and Myf5 and are involved in muscle differentiation and myofiber formation, respectively (Figure 3a). The results of a recent study challenge this paradigm by demonstrating that normal skeletal muscle develops in the absence of both MyoD and Myf5, as long as *Mrf4* expression is not compromised (Figure 3b) [31••]. Indeed, *Mrf4* expression can be detected, albeit transiently, in muscle progenitor cells before myotome formation and before *MyoD* expression, and might, therefore, have a role in instructing these cells to adopt the skeletal muscle phenotype. How can the discrepancies between the phenotype of $MyoD^{-/-}$ $Myf5^{-/-}$ previously described and that reported in this study [31••] be reconciled? *Mrf4* lies immediately upstream of *Myf5*, and, following insertion of foreign sequences, sequences regulating *Mrf4* expression and located in the vicinity of the *Myf5* locus might be affected [32].

Conclusions and future perspectives

Among several areas of interest, the following are worth considering for future investigation:

1. Characterization of the transcriptional mechanisms operating in satellite cells.
2. Re-evaluation of the hierarchical relationships among the myogenic bHLH proteins during development.
3. Clarification of the issues related to the phenotype of resident and non-resident adult muscle stem cells and their transcriptional circuitries.
4. Use of small molecules that modulate the activity of chromatin remodeling and HATs and/or HDACs to influence muscle gene expression, differentiation and regeneration.

The study of the transcriptional regulators governing myogenesis, and the possibility of pharmacologically modulating their activities will eventually lead to a detailed understanding of the mechanisms involved in establishing cell-lineage commitment, with potential therapeutic implications.

Update

Recently, cells originating from the central dermomyotome and expressing the paired box proteins Pax3 and Pax7 have been shown to constitute muscle progenitor cells giving rise to skeletal muscle cells of the trunk and limbs. Furthermore, $Pax3^{+}/Pax7^{+}$ progenitors adopt a satellite position from late fetal stages, suggesting that most, if not all, satellite cells are derived from the central dermomyotome [33••–35••].

Acknowledgements

We thank the members of the Sartorelli group for helpful discussions and comments on the manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Gill G, **Something about SUMO inhibits transcription**. *Curr Opin Genet Dev* 2005, **15**: in press.

2. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, Sinclair DA: **Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1**. *J Biol Chem* 2002, **277**:45099-45107.

3. Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, Hoffman E, Veech RL, Sartorelli V: **Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state**. *Mol Cell* 2003, **12**:51-62.

4. Santos-Rosa H, Valls E, Kouzarides T, Martinez-Balbas M: **Mechanisms of P/CAF auto-acetylation**. *Nucleic Acids Res* 2003, **31**:4285-4292.

5. Thompson PR, Wang D, Wang L, Fulco M, Pediconi N, Zhang D, An W, Ge Q, Roeder RG, Wong J *et al.*: **Regulation of the p300 HAT domain via a novel activation loop**. *Nat Struct Mol Biol* 2004, **11**:308-315.

6. Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, Guarente L: **Mammalian SIRT1 represses forkhead transcription factors**. *Cell* 2004, **116**:551-563.

7. •• Caretti G, Di Padova M, Micales B, Lyons GE, Sartorelli V: **The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation**. *Genes Dev* 2004, **18**:2627-2638.

The Polycomb methyltransferase Ezh2 protein is reported to be involved in promoting specific histone modifications that are associated with repression of selected muscle regulatory regions.

8. Kuzmichev A, Margueron R, Vaquero A, Preissner TS, Scher M, Kirmizis A, Ouyang X, Brockdorff N, Abate-Shen C, Farnham P *et al.*: **Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation**. *Proc Natl Acad Sci USA* 2005, **102**:1859-1864.

9. Furuyama T, Banerjee R, Breen TR, Harte PJ: **SIR2 is required for polycomb silencing and is associated with an E(Z) histone methyltransferase complex**. *Curr Biol* 2004, **14**:1812-1821.

10. Zhang CL, McKinsey TA, Olson EN: **Association of class II histone deacetylases with heterochromatin protein 1: potential role for histone methylation in control of muscle differentiation**. *Mol Cell Biol* 2002, **22**:7302-7312.

11. Mal A, Harter ML: **MyoD is functionally linked to the silencing of a muscle-specific regulatory gene prior to skeletal myogenesis**. *Proc Natl Acad Sci USA* 2003, **100**:1735-1739.

12. Ait-Si-Ali S, Guasconi V, Fritsch L, Yahi H, Sekhri R, Naguibneva I, Robin P, Cabon F, Polesskaya A, Harel-Bellan A: **A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells**. *EMBO J* 2004, **23**:605-615.

13. •• Lee H, Habas R, Abate-Shen C: **MSX1 cooperates with histone H1b for inhibition of transcription and myogenesis**. *Science* 2004, **304**:1675-1678.

The authors report that Msx1 interacts with histone H1 and that this interaction is associated with increased methylation of H3 lysines 9 and 14, and decreased phosphorylation of H3 serine 10. This report indicates that linker histones are involved, as are their nucleosomal counterparts, in regulating tissue-specific gene expression.

14. • Blais A, Tsikitis M, Acosta-Alvear D, Sharan R, Kluger Y, Dynlacht BD: **An initial blueprint for myogenic differentiation**. *Genes Dev* 2005, **19**:553-569.

The authors describe a genome-wide identification of binding sites for myogenic bHLH and MEF2 coupled to expression-profiling of skeletal muscle cells, and they identify novel target genes for the muscle regulatory factors.

15. Cole F, Zhang W, Geyra A, Kang JS, Krauss RS: **Positive regulation of myogenic bHLH factors and skeletal muscle development by the cell surface receptor CDO**. *Dev Cell* 2004, **7**:843-854.

16. Lluis F, Ballestar E, Suelves M, Esteller M, Munoz-Canoves P: **E47 phosphorylation by p38 MAPK promotes MyoD/E47 association and muscle-specific gene transcription**. *EMBO J* 2005, **24**:974-984.

17. •• Berkes CA, Bergstrom DA, Penn BH, Seaver KJ, Knoepfler PS, Tapscott SJ: **Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential**. *Mol Cell* 2004, **14**:465-477.

The authors explain that two domains of MyoD involved in chromatin remodeling are required for binding of MyoD to selected promoter regions by interaction with the constitutively chromatin-bound homeodomain protein Pbx. These findings suggest the existence of a mechanism through which MyoD might penetrate transcriptionally silent chromatin regions.

18. Magenta A, Cenciarelli C, De Santa F, Fuschi P, Martelli F, Caruso M, Felsani A: **MyoD stimulates *RB* promoter activity via the CREB/p300 nuclear transduction pathway**. *Mol Cell Biol* 2003, **23**:2893-2906.

19. Bergstrom DA, Penn BH, Strand A, Perry RL, Rudnicki MA, Tapscott SJ: **Promoter-specific regulation of MyoD binding and signal transduction cooperate to pattern gene expression**. *Mol Cell* 2002, **9**:587-600.

20. •• Simone C, Forcales SV, Hill DA, Imbalzano AN, Latella L, Puri PL: **p38 pathway targets SWI–SNF chromatin-remodeling complex to muscle-specific loci**. *Nat Genet* 2004, **36**:738-743.

In this publication, the authors report that the p38 kinase targets the SWI–SNF chromatin-remodeling complex to activate muscle-specific loci, thereby establishing a mechanistic link — between pro-myogenic extracellular cues and chromatin modifications — that results in muscle gene activation.

21. • Penn BH, Bergstrom DA, Dilworth FJ, Bengal E, Tapscott SJ: **A MyoD-generated feed-forward circuit temporally patterns gene expression during skeletal muscle differentiation**. *Genes Dev* 2004, **18**:2348-2353.

Experiments reported in this paper indicate that the p38 kinase promotes binding of MyoD and MEF2 to a subset of late-activated muscle promoters, and that MEF2D recruits RNA polymerase II. Precocious activation of the p38 pathway results in anticipated expression of genes that are normally expressed at later stages of muscle differentiation.

22. • Dilworth FJ, Seaver KJ, Fishburn AL, Htet SL, Tapscott SJ: ***In vitro* transcription system delineates the distinct roles of the coactivators pCAF and p300 during MyoD/E47-dependent transactivation**. *Proc Natl Acad Sci USA* 2004, **101**:11593-11598.

The authors report the establishment of a muscle-specific *in vitro* transcription system that uses nucleosomal DNA templates. The system is employed to successfully dissect and clarify the distinct roles of the acetyltransferases pCAF and p300 during MyoD–E47-dependent transactivation.

23. Wu HY, Hamamori Y, Xu J, Chang SC, Saluna T, Chang MF, O'Malley BW, Kedes L: **Nuclear hormone receptor coregulator GRIP1 suppresses, whereas SRC1A and p/CIP coactivate, by domain-specific binding of MyoD**. *J Biol Chem* 2005, **280**:3129-3137.

24. Micheli L, Leonardi L, Conti F, Buanne P, Canu N, Caruso M, Tirone F: **PC4 coactivates MyoD by relieving the histone deacetylase 4-mediated inhibition of myocyte enhancer factor 2C**. *Mol Cell Biol* 2005, **25**:2242-2259.

25. Li S, Czubryt MP, McAnally J, Bassel-Duby R, Richardson JA, Wiebel FF, Nordheim A, Olson EN: **Requirement for serum response factor for skeletal muscle growth and maturation revealed by tissue-specific gene deletion in mice**. *Proc Natl Acad Sci USA* 2005, **102**:1082-1087.

26. Gregoire S, Yang X-J: **Association with class IIa histone deacetylases upregulates the sumoylation of MEF2 transcription factors**. *Mol Cell Biol* 2005, **25**:2273-2287.

27. •• Chen AE, Ginty DD, Fan CM: **Protein kinase A signalling via CREB controls myogenesis induced by Wnt proteins**. *Nature* 2005, **433**:317-322.
In this study, Wnt-induced myogenesis was shown to be mediated by the protein kinase A through CREB. This is the first report indicating that the cyclic adenosine monophosphate (cAMP) pathway acts downstream of the Wnts.

28. Roth JF, Shikama N, Henzen C, Desbaillets I, Lutz W, Marino S, Wittwer J, Schorle H, Gassmann M, Eckner R: **Differential role of p300 and CBP acetyltransferase during myogenesis: p300 acts upstream of MyoD and Myf5**. *EMBO J* 2003, **22**:5186-5196.

29. Huh MS, Parker MH, Scime A, Parks R, Rudnicki MA: **Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation**. *J Cell Biol* 2004, **166**:865-876.

30. Camarda G, Siepi F, Pajalunga D, Bernardini C, Rossi R, Montecucco A, Meccia E, Crescenzi M: **A pRb-independent mechanism preserves the postmitotic state in terminally differentiated skeletal muscle cells**. *J Cell Biol* 2004, **167**:417-423.

31. •• Kassar-Duchossoy L, Gayraud-Morel B, Gomes D, Rocancourt D, Buckingham M, Shinin V, Tajbakhsh S: **Mrf4 determines skeletal muscle identity in *Myf5:Myod* double-mutant mice**. *Nature* 2004, **431**:466-471.
This study challenges the dogma that *Myf5* and *MyoD* are the sole muscle specification genes by showing that Mrf4 can, in the absence of both Myf5 and MyoD, induce skeletal myogenesis in mice.

32. Olson EN, Arnold HH, Rigby PW, Wold BJ: **Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene *Mrf4***. *Cell* 1996, **85**:1-4.

33. •• Relaix F, Rocancourt D, Mansouri A, Buckingham M: **A Pax3/Pax7-dependent population of skeletal muscle progenitor cells**. *Nature* 2005, **435**:948-953.
The authors report that a Pax3/Pax7-dependent progenitor-cell population, negative for myogenic markers, is a source of myogenic cells that participate to the formation of trunk and limb skeletal muscle of the mouse. Furthermore, the Pax3/Pax7 cells derived from the central portion of the dermomyotome migrate to adopt a position characteristic of satellite cells.

34. •• Gros J, Manceu M, Thome' V, Marcelle C: **A common somatic origin for embryonic muscle progenitors and satellite cells**. *Nature* 2005, **435**:954-958.
Using electroporation of green fluorescent proteins, video confocal microscopy, and quail-chick grafting experiments, the authors have identified a population of muscle progenitor cells that, initially located in the dorsal compartment of the chick dermomyotome, contribute to the growth of trunk muscles during embryonic and fetal life. Long-term analyses indicate that satellite cells derive from the same dermomyotome cell population.

35. •• Kassar-Duchossoy L, Giacone E, Gayraud-Morel B, Jory A, Gomes D, Tajbakhsh S: **Pax3/Pax7 mark a novel population of primitive myogenic cells during development**. *Genes Dev* 2005, **19**:1426-1431.
In their study, the authors identified a population of resident $Pax3^+/Pax7^+$ muscle marker-negative cells that give rise to muscle progenitors. At late stages of fetal development, expression of Pax3 is down-regulated, whereas Pax7 expression persists, and these progenitor cells are located underneath a basal lamina, outside of the muscle fiber — a characteristic location of satellite cells.