We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Myogenic Regulatory Factors: Critical Determinants of Muscle Identity in Development, Growth and Regeneration

Dylan Sweetman

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/46873>

1. Introduction

The discovery of MyoD [1] was a landmark in our understanding of the processes leading to muscle cell differentiation. In this study a single cDNA was isolated which could induce conversion of fibroblasts into muscle cells [2]. This striking finding remains one of the clearest examples of a master regulator of cell fate and has made myogenesis an excellent paradigm for the understanding of how cell fate is induced and executed.

Other related genes were soon identified and three other closely related proteins have been isolated: Myf5 [3], Myogenin [4-6] and MRF-4 [7-9], which share the ability of MyoD to activate muscle gene expression. Together these are known as the Myogenic Regulatory Factors or MRFs. All of these genes are expressed during embryonic myogenesis exclusively in myogenic cells [10-14] although there are differences in the timing and stages of myogenesis, reflecting underlying differences in the roles of the MRFs in muscle cell commitment and differentiation [15, 16].

1.1. Muscle development

In vertebrate embryos muscle is derived from paraxial mesoderm which lies adjacent to the midline of the developing embryo [17]. In the head unsegmented mesoderm produces the branchial and extra-ocular muscles [18] while some of the neck muscles are derived from more lateral occipital mesoderm [19].

Grafting experiments in avian embryos, where quail mesoderm is grafted into chick embryo hosts, have demonstrated that all the muscles of the trunk are derived from somites, segmentally repeated epithelial structures that arise from the paraxial mesoderm [20, 21]. As

they differentiate somites produce the dermomyotome, a 'C' shaped epithelium containing proliferative muscle precursors (myoblasts) that express the transcription factor Pax3 [22].

Somites can be divided into two major domains: epaxial, located dorso-medially, and hypaxial, located ventrolaterally. Muscles arising from these domains correspond to the adult epaxial and hypaxial muscles which are innervated by the dorsal and ventral ramus of the spinal cord respectively. Cells from the dermomyotome migrate around the edges of the dermomyotome to form an underlying layer, the primary myotome [23, 24], where the MRFs are first expressed and muscles begin to differentiate.

The muscles of the limb are also derived from somites but are generated when myoblasts delaminate from the hypaxial dermomyotome and migrate into the forming limb bud [17, 25]. This process is regulated by production of HGF/SF from the lateral mesoderm at limb levels which induces migration of myoblasts, to maintain them in a proliferative state and to delay MRF expression [26-28]. The expression of MyoD in these different muscle groups during embryo development is shown in figure 1.

Figure 1. In situ hybridisation to show expression of MyoD in an HH stage 24 chicken embryo (approximately equivalent to mouse E11 or human Carnegie stage 16, around 40 days). Staining is seen in trunk muscle precursors in epaxial and hypaxial somites (ep, hyp), limb muscles of fore- and hindlimb buds (fl, hl), jaw and facial muscles in the pharyngeal arches (pa1, pa2), tongue muscle precursors in the hypoglossal chord (hgc) and the dorsal and ventral oblique extraocular muscles (do, vo)

Myogenesis in each of these different muscle groups, head, epaxial, hypaxial and limb, is regulated differently in the embryo [17]; however the MRFs play a key role in all of them and are part of a core transcriptional programme that operates in all skeletal muscles.

2. Regulation of the MRFs

Several signalling systems have been shown to affect MRF expression during development. It is notable that different sets of muscle precursors are regulated by separate sets of signals and, even with a single somite, there are distinct inductive pathways in hypaxial and epaxial

regions. In this section I will briefly review some of the molecular signals that have been shown to regulate MRF expression.

2.1. Signalling molecules regulating MRFs

2.1.1. *Wnt*

The signals induced by Wnts, the vertebrate homologues of the *Drosophila wingless* gene, are broadly divided into canonical and non-canonical types. Canonical signalling acts via β -catenin and the activation of TCF/LEF transcription factors [29] while non-canonical signalling acts via planar cell polarity or calcium dependant mechanisms [30] although these pathways are not always as clearly distinct as this division implies [31].

Explant culture of somites from chicken embryos demonstrated that signals from the neural tube and notochord are required for induction of MyoD [32] and Myf5 [33], an activity that can be recapitulated by the addition of purified Wnt-1 or Wnt-3 and low levels of Shh [34]. Mouse mesoderm explants exposed to Wnt-1 activate an epaxial, Myf5 dependant programme while exposure to Wnt-7a seems to induce a hypaxial, MyoD dependant myogenesis [35]. Wnt7 has also been implicated in the regulation of satellite cell activation via induction of MRF expression [36, 37].

In vivo Wnt1 and Wnt3a are secreted by the dorsal neural tube and are able to induce MyoD expression in the epaxial myotome, probably via β -catenin signalling [38]. In contrast, in limb muscles, Wnt-6, expressed in the limb ectoderm, has been shown to positively regulate Myf5 while downregulating MyoD [39]. In the limb induction of MRF expression is independent of β -catenin signalling although it is required for later myogenic differentiation [40].

2.1.2. *Shh*

The Sonic Hedgehog signalling pathway, which regulates the activity of the Gli family of transcription factors, is found in numerous inductive and patterning systems during development and plays a critical role in myogenesis [41, 42].

In somite explants induction of MyoD by Wnt is only observed in the presence of Shh [34]. This requirement for Shh signalling has been confirmed in vivo as loss of MyoD expression following notochord and floorplate removal can be rescued by grafting a Shh soaked bead into the excised region [43]. Analysis of mouse embryos lacking Shh shows this signal also controls expression of both Myf5 and MyoD in the epaxial somite [44].

Shh expression in the developing limb has been primarily analysed in its central role in patterning the anterior-posterior axis [45]. However, in contrast to its role as a positive inducer of MRF expression in epaxial somites, in limb muscles ectopic Shh expression delays MRF expression and maintains proliferative myoblasts, ultimately leading to muscle hypertrophy [46, 47].

2.1.3. BMPs

Bone morphogenetic proteins, members of the TGF- β family [48], are well characterised repressors of myogenic differentiation. BMP4 from the lateral mesoderm regulates formation of the hypaxial somite and represses MyoD expression [49]. In the epaxial myotome BMP signals must be inhibited for myogenesis to proceed and Wnt-1, from the dorsal neural tube, induces expression of noggin, an inhibitor of BMP signalling [50, 51]. The inhibitory effects of Shh in limb myogenesis are also mediated, at least in part, by induction of BMP expression [46].

2.1.4. Notch

Notch signalling can have either positive or negative effects on MRF expression, depending on context. Neural crest cells expressing the Notch ligand Delta migrate past the epaxial somite where they activate Notch in the myoblasts of the dorso-medial lip. This then induces expression of Myf5 and the beginning of myogenic differentiation [52]. In limb muscles Notch signalling does not affect Myf5 expression but does inhibit MyoD induction [53].

2.1.5. FGFs

In vertebrates there are 22 members of the fibroblast growth factor (FGF) family which act via four receptor tyrosine kinases, the FGF receptors [54]. Grafting of FGF4 or FGF8 beads adjacent to somites leads to the loss of expression of MyoD and other myogenic markers [55, 56] but induces the expression of the tendon marker scleraxis. However later in somite development FGF from the myotome induces epithelial to mesenchymal transition and translocation of dermomyotomal cells into the central region of the myotome [57], a process known to contribute to the satellite cells of the adult [58]. In limbs FGF4 beads have been reported to downregulate MyoD expression [59] although the receptor through which it is thought to signal, FGFR4, is required for limb muscle cell differentiation [60] as expression of a dominant negative form of the receptor leads to decreased MyoD expression.

As is often the case in development the response to signalling events is context dependent and it is becoming clear that there are many variant myogenic programmes which are activated in different muscle groups; uncovering these distinct regulatory mechanisms remains an exciting area of muscle biology.

2.2. Molecular and genomic regulation

The ability of the MRFs to induce muscle specific gene expression means that they, in turn, are tightly regulated as inappropriate expression of MRFs could lead to production of ectopic muscles.

To determine the genomic elements controlling the highly specific expression of Myf5 a series of mice have been generated where reporters, such as LacZ, are expressed under the control of specific regions of the surrounding genome. These have revealed a system of remarkable

complexity where *Myf5* is controlled by a combination of promoter and enhancer elements that span 150kb of chromosome. The overall expression pattern of *Myf5* is made up of numerous smaller patterns, each with a specific enhancer driving *Myf5* expression in a particular subset of muscle precursor cells [61-66]. One particularly striking example of the convergence of mouse genetics and experimental approaches is the finding that in one of these regions, the early epaxial enhancer, binding sites for both *Lef* and *Gli* have been identified [67]; these are the molecules responsible for transducing Wnt and Shh signals that had been previously implicated in MRF induction in somite explant experiments [34, 35]

One intriguing exception to the muscle specific expression of *Myf5* is found in some regions of the mouse CNS [68]. The other MRFs are not expressed here and, as there is no muscle present, the role of this neural expression was unclear. More recently it has become clear that a genomic reorganisation in evolution is responsible for this inappropriate expression. However to prevent the activation of muscle specific genes in the nervous system the mRNA transcribed here is not translated and endogenous microRNAs are able to repress the production of *Myf5* protein [69].

Together this provides both positive and negative mechanisms for the regulation of *Myf5*. Once *Myf5* is expressed it can then induce expression of the other MRFs which also regulate each other. The exception to this is *Myf5* which is not induced either by itself or the other MRFs [70-72]. Because of this the other MRFs do not seem to require such complex regulatory regions and have rather simpler genomic control mechanisms.

MyoD expression is largely regulated by two enhancers, the core enhancer located at -20kb, and the distal regulatory region (DRR) located at -4kb. These have been extensively analysed by generating enhancer reporter fusions and mutational analysis in mice [73-79] and birds [80] which have shown that the core enhancer is required for the onset of *MyoD* expression while the DRR has a more important role in later differentiation. Several factors have been identified which are required for *MyoD* transcription including *Pax3* which acts in concert with *DNMRT* and *Myf5* [81] *Six1* and *Six4* [82, 83], *Pitx2* [84], *Sim2* [85] and *Foxo3* [86]. Although *Myf5* can activate *MyoD* it is not required in all cases and *MyoD* can be induced independently by this array of transcription factors [87].

Myogenin expression can be largely recapitulated with a reporter containing 4kb of upstream sequence [11]. *Myogenin* expression is regulated, at least in part, by *MyoD* along other factors, such as *NFAT* [88], which recruit chromatin remodelling complexes to the *myogenin* locus [89, 90].

3. Biochemical activity of the MRFs

The MRFs are basic-helix-loop-helix (bHLH) proteins, members of a widespread family of transcription factors found throughout eukaryotes [91]. An alignment of the protein sequences of the four MRFs is shown in Figure 2 with important functional domains highlighted. bHLH proteins are well characterised regulators of differentiation and have been implicated in many developmental systems including ear [92], cardiac [93] and neural

differentiation [94]. bHLH proteins bind specific DNA motifs, known as E boxes, normally as heterodimers in combination with the ubiquitously expressed E12 and E57 proteins [95].

Figure 2. Clustal alignment of human MRF sequences. Basic domain is highlighted in blue, helix-loop-helix domain in green. MyoD methylation / acetylation sites shown in red, acetylation sites in grey, ubiquitination site in purple and phosphorylation site in orange.

Although they have similar biochemical activities in vitro and can bind E boxes in DNA it is clear that there are distinct biochemical activities and functions for the individual MRFs. MyoD and myogenin have been directly compared in their ability to bind to and activate transcription from several muscle specific promoters, such as the chicken myosin light chain, [96, 97] as well as in more global genome binding analysis [98]; it is clear that they bind distinct subsets of promoters and have different sets of target genes. Similar experiments have shown different DNA binding activity of MRF4, MyoD and Mgn [99] while comparison of Myf5 and MyoD activity has mapped part of this differential transcriptional activity to the N and C terminal regions of MyoD which co-operate to give increased transcriptional activation of specific genes which are not activated by Myf5 [100].

An interesting question is how MRF binding to DNA is able to specifically activate muscle gene expression. Recruitment of MyoD to E boxes can be enhanced by the presence of DNA quadruplex structures in promoters [101]; however E boxes are widespread throughout the genome and global analysis of MyoD binding suggests it is able to interact with a large number of these even though they are not associated with muscle specific genes and so do not result in transcriptional activation [102]. Part of the answer to this is that while MRF binding is required for muscle gene expression it is not sufficient and other transcriptional

activators, such as the Six [82, 83] and Pbx proteins [103, 104] are also required at muscle gene promoters to drive expression. However the widespread binding of MyoD may have a broader function and it has been suggested that this can lead to generalised remodelling of the genome in preparation for myogenic differentiation [102]. A similar role has been proposed where MyoD binding is first required at distal enhancers of repressed myogenic genes which have promoter elements inaccessible to transcription factor binding due to their chromatin structure. Interactions between these distal enhancers and more proximal promoters leads to chromatin remodelling at that locus. This opens the promoter and makes it available for MRF binding [105]. It is tempting to speculate that this may be the reason for the pulse of Myf5 expression in paraxial mesoderm prior to somite formation and that this is preparing cells for subsequent inductive events and thus enabling myogenesis.

A recent comparison has also shed light on the specificity of target gene activation by MyoD. Comparison of MyoD binding with a neuronal bHLH protein, NeuroD2, has identified both common and specific E box sequences that these proteins can bind. MyoD specific E boxes are linked to transcription of muscle specific genes while binding to the common E boxes results in broader epigenetic modifications [106].

The activity of MyoD is also regulated by several biochemical modifications and interactions. MyoD is regulated by ubiquitination at its N terminal which targets it for degradation [107, 108]. MyoD is also negatively regulated by methylation which impairs its ability to induce differentiation [109]. MyoD is also acetylated [110] and phosphorylated [111], with both events seeming to enhance MyoD activity. Many of the residues modified in MyoD are conserved across the other MRFs (see figure 2) and it is possible that they are also regulated in this way.

As well as interaction with the E proteins required for transcriptional activity MyoD has also been reported to interact with a wide range of other proteins including c-jun [112], CTCF [113], BAF60c [114], CLP-1 and HDAC at the cyclin D promoter [115], TAZ at the Mgn promoter [116] and β -catenin [117]. MyoD can also interact with cell cycle regulators such as pRB [118] and cdk4 [119] to induce cell cycle withdrawal directly during myogenic differentiation.

This range of interactions shows clearly that the control of MyoD activity is a carefully regulated process and subject to numerous levels of control.

3.1. Targets of MRFs

The biochemical differences in the MRFs contribute directly to their distinct functional roles. Myf5 is able to activate genes required for myogenic commitment while MyoD can also switch on differentiation genes [100]. Similarly MyoD and MRF4 have distinct sets of targets and differentially affect proliferation and differentiation [120].

Myogenin acts downstream of MyoD and is often only able to activate transcription from promoters which have already been bound by MyoD [98]. Myf5, MyoD and Myogenin binding of target sequences is also temporally regulated, providing another mechanism for specificity of target gene activation [121].

One of the best characterised MyoD targets is myogenin. MyoD can bind the myogenin promoter along with Mef2 (another transcription factor and MyoD target gene) [122, 123]. Myogenin, MyoD and Mef2 then co-operate with other transcriptional regulators, such as Six proteins, to activate muscle specific genes such as muscle myosins [96] or muscle specific microRNAs [71, 124] via demethylation of promoter elements [125].

4. Animal models of MRF function

Probably the most widely used animal models to study MRF function are transgenic mice. However knockout animals have shown surprisingly mild effects and mice lacking MyoD [126], Myf5 [127] and MRF4 [128, 129] are all able to develop apparently normal muscle although delays in myogenesis do occur in the limbs of MyoD [130] and somites of Myf5 [127] mutant animals. In contrast mice lacking myogenin have severe muscle defects and die soon after birth [131, 132].

Double knockouts of MyoD and Myf5 were originally reported to lack muscle [133] although subsequently it appears that these mice also lacked MRF4 expression as the targeting of Myf5 had also affected the closely linked MRF4 locus. In MyoD / Myf5 null animals which retain functional MRF4 this gene is able to compensate and initiate myogenesis [134]. Knockout mice have shown that the relationship between the different MRFs is complex and one probable explanation for the functional redundancy of these proteins is that in the absence of one another will be upregulated to substitute for it [126, 128]. The exception to this is myogenin which has a unique, non-redundant function [135] which cannot be compensated for by the other MRFs. Overlapping roles for MRFs are also demonstrated in mice lacking MyoD and MRF4 which have severe muscle defects [136]. It is also apparent that Myf5 alone is not sufficient to support myogenic differentiation as in mice lacking the other MRFs myogenesis is initiated but not maintained [137]. This specificity of individual MRF function has also been demonstrated in other animal models such as *Xenopus* [138], zebrafish [139] and chickens [70, 71] although it is striking that some specific functions of MRFs have changed during evolution. An example of this is the regulation of the muscle specific microRNA miR-206 which appears to have different requirements for MRF expression in mice, chickens and fish [70, 71, 140].

The role of Myf5 has been further examined by the production of transgenic mice which express diphtheria toxin under the control of Myf5, thus ablating all Myf5 expressing cells in the embryo. Fascinatingly these mice develop morphologically normal muscle [141, 142], suggesting that a Myf5 independent population of myoblasts are present and can expand to fill the niche left by loss of Myf5 expressing cells. This correlates well with data showing that distinct regulation of MyoD and Myf5 defines different subsets of cells based on reporter gene expression [16].

5. Summary

Although great strides have been made in understanding the MRFs at biochemical, genomic and whole animal levels there remain significant unanswered questions. Among these is

issue of what are target genes of each MRF in vivo and how do they differ in their activity in different muscle types. Understanding the answers to these questions will provide key insights which will directly influence both basic science and regenerative medicine.

Author details

Dylan Sweetman

Division of Animal Science, School of Biosciences, University of Nottingham, UK

6. References

- [1] Lassar, A., (2012) Finding MyoD with a little help from my friends. *Nat Cell Biol.* 14(2): 116-116.
- [2] Davis, R.L., H. Weintraub, and A.B. Lassar, (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell.* 51(6): 987-1000.
- [3] Braun, T., G. Buschhausen-Denker, E. Bober, E. Tannich, and H.H. Arnold, (1989) A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10T1/2 fibroblasts. *Embo J.* 8(3): 701-9.
- [4] Edmondson, D.G. and E.N. Olson, (1989) A gene with homology to the myc similarity region of MyoD1 is expressed during myogenesis and is sufficient to activate the muscle differentiation program. *Genes & Development.* 3(5): 628-640.
- [5] Fujisawa-Sehara, A., Y. Nabeshima, Y. Hosoda, and T. Obinata, (1990) Myogenin contains two domains conserved among myogenic factors. *J Biol Chem.* 265(25): 15219-23.
- [6] Wright, W.E., D.A. Sassoon, and V.K. Lin, (1989) Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. *Cell.* 56(4): 607-17.
- [7] Braun, T., E. Bober, B. Winter, N. Rosenthal, and H.H. Arnold, (1990) Myf-6, a new member of the human gene family of myogenic determination factors: evidence for a gene cluster on chromosome 12. *Embo J.* 9(3): 821-31.
- [8] Miner, J.H. and B. Wold, (1990) Herculin, a fourth member of the MyoD family of myogenic regulatory genes. *Proceedings of the National Academy of Sciences of the United States of America.* 87(3): 1089-1093.
- [9] Rhodes, S.J. and S.F. Konieczny, (1989) Identification of MRF4: a new member of the muscle regulatory factor gene family. *Genes & Development.* 3(12b): 2050-2061.
- [10] Bober, E., G.E. Lyons, T. Braun, G. Cossu, M. Buckingham, and H.H. Arnold, (1991) The muscle regulatory gene, Myf-6, has a biphasic pattern of expression during early mouse development. *The Journal of Cell Biology.* 113(6): 1255-1265.
- [11] Fujisawa-Sehara, A., K. Hanaoka, M. Hayasaka, T. Hiromasa-Yagami, and Y. Nabeshima, (1993) Upstream region of the myogenin gene confers transcriptional activation in muscle cell lineages during mouse embryogenesis. *Biochem Biophys Res Commun.* 191(2): 351-6.

- [12] Ott, M.O., E. Bober, G. Lyons, H. Arnold, and M. Buckingham, (1991) Early expression of the myogenic regulatory gene, *myf-5*, in precursor cells of skeletal muscle in the mouse embryo. *Development*. 111(4): 1097-107.
- [13] Sassoon, D., G. Lyons, W.E. Wright, V. Lin, A. Lassar, H. Weintraub, and M. Buckingham, (1989) Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis. *Nature*. 341(6240): 303-7.
- [14] Summerbell, D., C. Halai, and P.W. Rigby, (2002) Expression of the myogenic regulatory factor *Mrf4* precedes or is contemporaneous with that of *Myf5* in the somitic bud. *Mech Dev*. 117(1-2): 331-5.
- [15] Della Gaspera, B., A.-S. Armand, I. Sequeira, A. Chesneau, A. Mazabraud, S. Lécolle, F. Charbonnier, and C. Chanoine, (2012) Myogenic waves and myogenic programs during *Xenopus* embryonic myogenesis. *Developmental Dynamics*. 241(5): 995-1007.
- [16] Kablar, B., K. Krastel, S. Tajbakhsh, and M.A. Rudnicki, (2003) *Myf5* and *MyoD* activation define independent myogenic compartments during embryonic development. *Dev Biol*. 258(2): 307-18.
- [17] Mok, G.F. and D. Sweetman, (2011) Many routes to the same destination: lessons from skeletal muscle development. *Reproduction*. 141(3): 301-312.
- [18] Noden, D.M., R. Marcucio, A.G. Borycki, and C.P. Emerson, (1999) Differentiation of avian craniofacial muscles: I. Patterns of early regulatory gene expression and myosin heavy chain synthesis. *Developmental Dynamics*. 216(2): 96-112.
- [19] Theis, S., K. Patel, P. Valasek, A. Otto, Q. Pu, I. Harel, E. Tzahor, S. Tajbakhsh, B. Christ, and R. Huang, (2010) The occipital lateral plate mesoderm is a novel source for vertebrate neck musculature. *Development*. 137(17): 2961-2971.
- [20] Brent, A.E. and C.J. Tabin, (2002) Developmental regulation of somite derivatives: muscle, cartilage and tendon. *Curr Opin Genet Dev*. 12(5): 548-57.
- [21] Christ, B., R. Huang, and M. Scaal, (2007) Amniote somite derivatives. *Dev Dyn*. 236(9): 2382-96.
- [22] Buckingham, M. and F. Relaix, (2007) The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annu Rev Cell Dev Biol*. 23(645-73).
- [23] Gros, J., M. Scaal, and C. Marcelle, (2004) A two-step mechanism for myotome formation in chick. *Dev Cell*. 6(6): 875-82.
- [24] Kahane, N., Y. Cinnamon, and C. Kalcheim, (1998) The origin and fate of pioneer myotomal cells in the avian embryo. *Mech Dev*. 74(1-2): 59-73.
- [25] Christ, B. and B. Brand-Saberi, (2002) Limb muscle development. *Int J Dev Biol*. 46(7): 905-14.
- [26] Dietrich, S., F. Abou-Rebyeh, H. Brohmann, F. Bladt, E. Sonnenberg-Riethmacher, T. Yamaai, A. Lumsden, B. Brand-Saberi, and C. Birchmeier, (1999) The role of SF/HGF and c-Met in the development of skeletal muscle. *Development*. 126(8): 1621-9.
- [27] Scaal, M., A. Bonafede, V. Dathe, M. Sachs, G. Cann, B. Christ, and B. Brand-Saberi, (1999) SF/HGF is a mediator between limb patterning and muscle development. *Development*. 126(21): 4885-93.

- [28] Brand-Saberi, B., T.S. Muller, J. Wilting, B. Christ, and C. Birchmeier, (1996) Scatter factor/hepatocyte growth factor (SF/HGF) induces emigration of myogenic cells at interlimb level in vivo. *Dev Biol.* 179(1): 303-8.
- [29] Archbold, H.C., Y.X. Yang, L. Chen, and K.M. Cadigan, (2012) How do they do Wnt they do?: regulation of transcription by the Wnt/beta-catenin pathway. *Acta Physiol (Oxf)*. 204(1): 74-109.
- [30] Sugimura, R. and L. Li, (2010) Noncanonical Wnt signaling in vertebrate development, stem cells, and diseases. *Birth Defects Research Part C: Embryo Today: Reviews*. 90(4): 243-256.
- [31] van Amerongen, R. and R. Nusse, (2009) Towards an integrated view of Wnt signaling in development. *Development*. 136(19): 3205-3214.
- [32] Munsterberg, A.E. and A.B. Lassar, (1995) Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite. *Development*. 121(3): 651-60.
- [33] Cossu, G., R. Kelly, S. Tajbakhsh, S. Di Donna, E. Vivarelli, and M. Buckingham, (1996) Activation of different myogenic pathways: myf-5 is induced by the neural tube and MyoD by the dorsal ectoderm in mouse paraxial mesoderm. *Development*. 122(2): 429-37.
- [34] Munsterberg, A.E., J. Kitajewski, D.A. Bumcrot, A.P. McMahon, and A.B. Lassar, (1995) Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev*. 9(23): 2911-22.
- [35] Tajbakhsh, S., U. Borello, E. Vivarelli, R. Kelly, J. Papkoff, D. Duprez, M. Buckingham, and G. Cossu, (1998) Differential activation of Myf5 and MyoD by different Wnts in explants of mouse paraxial mesoderm and the later activation of myogenesis in the absence of Myf5. *Development*. 125(21): 4155-62.
- [36] Le Grand, F., A.E. Jones, V. Seale, A. Scime, and M.A. Rudnicki, (2009) Wnt7a Activates the Planar Cell Polarity Pathway to Drive the Symmetric Expansion of Satellite Stem Cells. *Cell Stem Cell*. 4(6): 535-547.
- [37] von Maltzahn, J., C.F. Bentzinger, and M.A. Rudnicki, (2012) Wnt7a-Fzd7 signalling directly activates the Akt/mTOR anabolic growth pathway in skeletal muscle. *Nat Cell Biol*. 14(2): 186-191.
- [38] Schmidt, M., M. Tanaka, and A. Munsterberg, (2000) Expression of (beta)-catenin in the developing chick myotome is regulated by myogenic signals. *Development*. 127(19): 4105-4113.
- [39] Geetha-Loganathan, P., S. Nimmagadda, F. Pr'ls, K. Patel, M. Scaal, R. Huang, and B. Christ, (2005) Ectodermal Wnt-6 promotes Myf5-dependent avian limb myogenesis. *Developmental Biology*. 288(1): 221-233.
- [40] Hutcheson, D.A., J. Zhao, A. Merrell, M. Haldar, and G. Kardon, (2009) Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for beta-catenin. *Genes Dev*. 23(8): 997-1013.
- [41] Ingham, P.W. and M. Placzek, (2006) Orchestrating ontogenesis: variations on a theme by sonic hedgehog. *Nat Rev Genet*. 7(11): 841-850.

- [42] Murdoch, J.N. and A.J. Copp, (2010) The relationship between sonic Hedgehog signaling, cilia, and neural tube defects. *Birth Defects Res A Clin Mol Teratol.* 88(8): 633-52.
- [43] Borycki, A.G., L. Mendham, and C.P. Emerson, Jr., (1998) Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development.* 125(4): 777-90.
- [44] Borycki, A.G., B. Brunk, S. Tajbakhsh, M. Buckingham, C. Chiang, and C.P. Emerson, Jr., (1999) Sonic hedgehog controls epaxial muscle determination through Myf5 activation. *Development.* 126(18): 4053-63.
- [45] Tickle, C., (2006) Making digit patterns in the vertebrate limb. *Nat Rev Mol Cell Biol.* 7(1): 45-53.
- [46] Amthor, H., B. Christ, M. Weil, and K. Patel, (1998) The importance of timing differentiation during limb muscle development. *Curr Biol.* 8(11): 642-52.
- [47] Duprez, D., C. Fournier-Thibault, and N. Le Douarin, (1998) Sonic Hedgehog induces proliferation of committed skeletal muscle cells in the chick limb. *Development.* 125(3): 495-505.
- [48] Sieber, C., J. Kopf, C. Hiepen, and P. Knaus, (2009) Recent advances in BMP receptor signaling. *Cytokine Growth Factor Rev.* 20(5-6): 343-55.
- [49] Pourquie, O., C.M. Fan, M. Coltey, E. Hirsinger, Y. Watanabe, C. Breant, P. Francis-West, P. Brickell, M. Tessier-Lavigne, and N.M. Le Douarin, (1996) Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell.* 84(3): 461-71.
- [50] Hirsinger, E., D. Duprez, C. Jouve, P. Malapert, J. Cooke, and O. Pourquie, (1997) Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. *Development.* 124(22): 4605-4614.
- [51] Marcelle, C., M.R. Stark, and M. Bronner-Fraser, (1997) Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. *Development.* 124(20): 3955-3963.
- [52] Rios, A.C., O. Serralbo, D. Salgado, and C. Marcelle, (2011) Neural crest regulates myogenesis through the transient activation of NOTCH. *Nature.* 473(7348): 532-535.
- [53] Delfini, M.C., E. Hirsinger, O. Pourquie, and D. Duprez, (2000) Delta 1-activated notch inhibits muscle differentiation without affecting Myf5 and Pax3 expression in chick limb myogenesis. *Development.* 127(23): 5213-24.
- [54] Dorey, K. and E. Amaya, (2010) FGF signalling: diverse roles during early vertebrate embryogenesis. *Development.* 137(22): 3731-3742.
- [55] Smith, T.G., D. Sweetman, M. Patterson, S.M. Keyse, and A. Münsterberg, (2005) Feedback interactions between MKP3 and ERK MAP kinase control scleraxis expression and the specification of rib progenitors in the developing chick somite. *Development.* 132(6): 1305-14.
- [56] Sweetman, D., T. Rathjen, M. Jefferson, G. Wheeler, T.G. Smith, G.N. Wheeler, A. Münsterberg, and T. Dalmay, (2006) FGF-4 signaling is involved in mir-206 expression in developing somites of chicken embryos. *Dev Dyn.* 235(8): 2185-91.

- [57] Delfini, M.-C., M. De La Celle, J. Gros, O. Serralbo, I. Marics, M. Seux, M. Scaal, and C. Marcelle, (2009) The timing of emergence of muscle progenitors is controlled by an FGF/ERK/SNAIL1 pathway. *Developmental Biology*. 333(2): 229-237.
- [58] Gros, J., M. Manceau, V. Thome, and C. Marcelle, (2005) A common somitic origin for embryonic muscle progenitors and satellite cells. *Nature*. 435(7044): 954-8.
- [59] Edom-Vovard, F., M.-A. Bonnin, and D. Duprez, (2001) Misexpression of Fgf-4 in the Chick Limb Inhibits Myogenesis by Down-Regulating Fgf Expression. *Developmental Biology*. 233(1): 56-71.
- [60] Marics, I., F. Padilla, J.F. Guillemot, M. Scaal, and C. Marcelle, (2002) FGFR4 signaling is a necessary step in limb muscle differentiation. *Development*. 129(19): 4559-69.
- [61] Carvajal, J.J., D. Cox, D. Summerbell, and P.W. Rigby, (2001) A BAC transgenic analysis of the Mrf4/Myf5 locus reveals interdigitated elements that control activation and maintenance of gene expression during muscle development. *Development*. 128(10): 1857-68.
- [62] Carvajal, J.J., A. Keith, and P.W.J. Rigby, (2008) Global transcriptional regulation of the locus encoding the skeletal muscle determination genes Mrf4 and Myf5. *Genes & Development*. 22(2): 265-276.
- [63] Summerbell, D., P.R. Ashby, O. Coutelle, D. Cox, S. Yee, and P.W. Rigby, (2000) The expression of Myf5 in the developing mouse embryo is controlled by discrete and dispersed enhancers specific for particular populations of skeletal muscle precursors. *Development*. 127(17): 3745-57.
- [64] Tajbakhsh, S., E. Bober, C. Babinet, S. Pournin, H. Arnold, and M. Buckingham, (1996) Gene targeting the myf-5 locus with nlacZ reveals expression of this myogenic factor in mature skeletal muscle fibres as well as early embryonic muscle. *Dev Dyn*. 206(3): 291-300.
- [65] Buchberger, A., N. Nomokonova, and H.H. Arnold, (2003) Myf5 expression in somites and limb buds of mouse embryos is controlled by two distinct distal enhancer activities. *Development*. 130(14): 3297-307.
- [66] Buchberger, A., D. Freitag, and H.H. Arnold, (2007) A homeo-paired domain-binding motif directs Myf5 expression in progenitor cells of limb muscle. *Development*. 134(6): 1171-80.
- [67] Teboul, L., D. Summerbell, and P.W.J. Rigby, (2003) The initial somitic phase of Myf5 expression requires neither Shh signaling nor Gli regulation. *Genes & Development*. 17(23): 2870-2874.
- [68] Tajbakhsh, S. and M.E. Buckingham, (1995) Lineage restriction of the myogenic conversion factor myf-5 in the brain. *Development*. 121(12): 4077-4083.
- [69] Daubas, P., C.G. Crist, L. Bajard, F. Relaix, E. Pecnard, D. Rocancourt, and M. Buckingham, (2009) The regulatory mechanisms that underlie inappropriate transcription of the myogenic determination gene Myf5 in the central nervous system. *Dev Biol*. 327(1): 71-82.
- [70] Delfini, M.C. and D. Duprez, (2004) Ectopic Myf5 or MyoD prevents the neuronal differentiation program in addition to inducing skeletal muscle differentiation, in the chick neural tube. *Development*. 131(4): 713-23.

- [71] Sweetman, D., K. Goljanek, T. Rathjen, S. Oustanina, T. Braun, T. Dalmay, and A. Munsterberg, (2008) Specific requirements of MRFs for the expression of muscle specific microRNAs, miR-1, miR-206 and miR-133. *Dev Biol.* 321(2): 491-9.
- [72] Braun, T., E. Bober, G. Buschhausen-Denker, S. Kohtz, K.H. Grzeschik, and H.H. Arnold, (1989) Differential expression of myogenic determination genes in muscle cells: possible autoactivation by the Myf gene products. *Embo J.* 8(12): 3617-25.
- [73] Asakura, A., G.E. Lyons, and S.J. Tapscott, (1995) The regulation of MyoD gene expression: conserved elements mediate expression in embryonic axial muscle. *Dev Biol.* 171(2): 386-98.
- [74] Chen, J.C. and D.J. Goldhamer, (2004) The core enhancer is essential for proper timing of MyoD activation in limb buds and branchial arches. *Dev Biol.* 265(2): 502-12.
- [75] Chen, J.C., C.M. Love, and D.J. Goldhamer, (2001) Two upstream enhancers collaborate to regulate the spatial patterning and timing of MyoD transcription during mouse development. *Dev Dyn.* 221(3): 274-88.
- [76] Chen, J.C., R. Ramachandran, and D.J. Goldhamer, (2002) Essential and redundant functions of the MyoD distal regulatory region revealed by targeted mutagenesis. *Dev Biol.* 245(1): 213-23.
- [77] Faerman, A., D.J. Goldhamer, R. Puzis, C.P. Emerson, Jr., and M. Shani, (1995) The distal human myoD enhancer sequences direct unique muscle-specific patterns of lacZ expression during mouse development. *Dev Biol.* 171(1): 27-38.
- [78] Goldhamer, D.J., A. Faerman, M. Shani, and C.P. Emerson, Jr., (1992) Regulatory elements that control the lineage-specific expression of myoD. *Science.* 256(5056): 538-42.
- [79] Tapscott, S.J., A.B. Lassar, and H. Weintraub, (1992) A novel myoblast enhancer element mediates MyoD transcription. *Mol Cell Biol.* 12(11): 4994-5003.
- [80] Pinney, D.F., F.C. de la Brousse, A. Faerman, M. Shani, K. Maruyama, and C.P. Emerson, Jr., (1995) Quail myoD is regulated by a complex array of cis-acting control sequences. *Dev Biol.* 170(1): 21-38.
- [81] Sato, T., D. Rocancourt, L. Marques, S. Thorsteinsdóttir, and M. Buckingham, (2010) A Pax3/Dmrt2/Myf5 Regulatory Cascade Functions at the Onset of Myogenesis. *PLoS Genet.* 6(4): e1000897.
- [82] Grifone, R., J. Demignon, C. Houbbron, E. Souil, C. Niro, M.J. Seller, G. Hamard, and P. Maire, (2005) Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. *Development.* 132(9): 2235-49.
- [83] Spitz, F., J. Demignon, A. Porteu, A. Kahn, J.P. Concordet, D. Daegelen, and P. Maire, (1998) Expression of myogenin during embryogenesis is controlled by Six/sine oculis homeoproteins through a conserved MEF3 binding site. *Proc Natl Acad Sci U S A.* 95(24): 14220-5.
- [84] Abu-Elmagd, M., L. Robson, D. Sweetman, J. Hadley, P. Francis-West, and A. Münsterberg, (2010) Wnt/Lef1 signaling acts via Pitx2 to regulate somite myogenesis. *Developmental Biology.* 337(2): 211-219.
- [85] Havis, E., P. Coumailleau, A. Bonnet, K. Bismuth, M.A. Bonnin, R. Johnson, C.M. Fan, F. Relaix, D.L. Shi, and D. Duprez, (2012) Sim2 prevents entry into the myogenic program by repressing MyoD transcription during limb embryonic myogenesis. *Development.*

- [86] Hu, P., K.G. Geles, J.H. Paik, R.A. DePinho, and R. Tjian, (2008) Codependent activators direct myoblast-specific MyoD transcription. *Dev Cell*. 15(4): 534-46.
- [87] Kablar, B., K. Krastel, C. Ying, S.J. Tapscott, D.J. Goldhamer, and M.A. Rudnicki, (1999) Myogenic determination occurs independently in somites and limb buds. *Dev Biol*. 206(2): 219-31.
- [88] Armand, A.S., M. Bourajjaj, S. Martinez-Martinez, H.E. Azzouzi, P.A. da Costa Martins, P. Hatzis, T. Seidler, J.M. Redondo, and L.J. De Windt, (2008) Cooperative Synergy between NFAT and MyoD Regulates Myogenin Expression and Myogenesis. *J Biol Chem*. 283(43): 29004-29010.
- [89] de la Serna, I.L., Y. Ohkawa, C.A. Berkes, D.A. Bergstrom, C.S. Dacwag, S.J. Tapscott, and A.N. Imbalzano, (2005) MyoD targets chromatin remodeling complexes to the myogenin locus prior to forming a stable DNA-bound complex. *Mol Cell Biol*. 25(10): 3997-4009.
- [90] Deato, M.D., M.T. Marr, T. Sottero, C. Inouye, P. Hu, and R. Tjian, (2008) MyoD targets TAF3/TRF3 to activate myogenin transcription. *Mol Cell*. 32(1): 96-105.
- [91] Skinner, M.K., A. Rawls, J. Wilson-Rawls, and E.H. Roalson, (2010) Basic helix-loop-helix transcription factor gene family phylogenetics and nomenclature. *Differentiation*. 80(1): 1-8.
- [92] Fritsch, B., D.F. Eberl, and K.W. Beisel, (2010) The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis. *Cell Mol Life Sci*. 67(18): 3089-99.
- [93] Conway, S.J., B. Firulli, and A.B. Firulli, (2010) A bHLH code for cardiac morphogenesis. *Pediatr Cardiol*. 31(3): 318-24.
- [94] Powell, L.M. and A.P. Jarman, (2008) Context dependence of proneural bHLH proteins. *Curr Opin Genet Dev*. 18(5): 411-7.
- [95] Berkes, C.A. and S.J. Tapscott, (2005) MyoD and the transcriptional control of myogenesis. *Semin Cell Dev Biol*. 16(4-5): 585-95.
- [96] Asakura, A., A. Fujisawa-Sehara, T. Komiya, and Y. Nabeshima, (1993) MyoD and myogenin act on the chicken myosin light-chain 1 gene as distinct transcriptional factors. *Mol Cell Biol*. 13(11): 7153-62.
- [97] Czernik, P.J., C.A. Peterson, and B.K. Hurlburt, (1996) Preferential Binding of MyoD-E12 versus Myogenin-E12 to the Murine Sarcoma Virus Enhancer in Vitro. *Journal of Biological Chemistry*. 271(15): 9141-9149.
- [98] Cao, Y., R.M. Kumar, B.H. Penn, C.A. Berkes, C. Kooperberg, L.A. Boyer, R.A. Young, and S.J. Tapscott, (2006) Global and gene-specific analyses show distinct roles for Myod and Myog at a common set of promoters. *Embo J*. 25(3): 502-11.
- [99] Fujisawa-Sehara, A., Y. Nabeshima, T. Komiya, T. Uetsuki, and A. Asakura, (1992) Differential trans-activation of muscle-specific regulatory elements including the myosin light chain box by chicken MyoD, myogenin, and MRF4. *J Biol Chem*. 267(14): 10031-8.
- [100] Ishibashi, J., R.L. Perry, A. Asakura, and M.A. Rudnicki, (2005) MyoD induces myogenic differentiation through cooperation of its NH2- and COOH-terminal regions. *The Journal of Cell Biology*. 171(3): 471-482.

- [101] Shklover, J., P. Weisman-Shomer, A. Yafe, and M. Fry, (2010) Quadruplex structures of muscle gene promoter sequences enhance in vivo MyoD-dependent gene expression. *Nucleic Acids Res.* 38(7): 2369-77.
- [102] Cao, Y., Z. Yao, D. Sarkar, M. Lawrence, G.J. Sanchez, M.H. Parker, K.L. MacQuarrie, J. Davison, M.T. Morgan, W.L. Ruzzo, R.C. Gentleman, and S.J. Tapscott, (2010) Genome-wide MyoD Binding in Skeletal Muscle Cells: A Potential for Broad Cellular Reprogramming. *Developmental Cell.* 18(4): 662-674.
- [103] Berkes, C.A., D.A. Bergstrom, B.H. Penn, K.J. Seaver, P.S. Knoepfler, and S.J. Tapscott, (2004) Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential. *Mol Cell.* 14(4): 465-77.
- [104] Maves, L., A.J. Waskiewicz, B. Paul, Y. Cao, A. Tyler, C.B. Moens, and S.J. Tapscott, (2007) Pbx homeodomain proteins direct Myod activity to promote fast-muscle differentiation. *Development.* 134(18): 3371-82.
- [105] Taberlay, P.C., T.K. Kelly, C.-C. Liu, J.S. You, D.D. DeCarvalho, T.B. Miranda, X.J. Zhou, G. Liang, and P.A. Jones, (2011) Polycomb-Repressed Genes Have Permissive Enhancers that Initiate Reprogramming. *Cell.* 147(6): 1283-1294.
- [106] Fong, A.P., Z. Yao, J.W. Zhong, Y. Cao, W.L. Ruzzo, R.C. Gentleman, and S.J. Tapscott, (2012) Genetic and Epigenetic Determinants of Neurogenesis and Myogenesis. *Developmental Cell.* 22(4): 721-735.
- [107] Breitschopf, K., E. Bengal, T. Ziv, A. Admon, and A. Ciechanover, (1998) A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. *Embo J.* 17(20): 5964-73.
- [108] Noy, T., O. Suad, D. Taglicht, and A. Ciechanover, (2012) HUWE1 ubiquitinates MyoD and targets it for proteasomal degradation. *Biochemical and Biophysical Research Communications.* 418(2): 408-413.
- [109] Ling, B.M.T., N. Bharathy, T.-K. Chung, W.K. Kok, S. Li, Y.H. Tan, V.K. Rao, S. Gopinadhan, V. Sartorelli, M.J. Walsh, and R. Taneja, (2012) Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. *Proceedings of the National Academy of Sciences.* 109(3): 841-846.
- [110] Sartorelli, V., P.L. Puri, Y. Hamamori, V. Ogryzko, G. Chung, Y. Nakatani, J.Y. Wang, and L. Kedes, (1999) Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol Cell.* 4(5): 725-34.
- [111] Jo, C., S.J. Cho, and S.A. Jo, (2011) Mitogen-activated protein kinase kinase 1 (MEK1) stabilizes MyoD through direct phosphorylation at tyrosine 156 during myogenic differentiation. *J Biol Chem.* 286(21): 18903-13.
- [112] Bengal, E., L. Ransone, R. Scharfmann, V.J. Dwarki, S.J. Tapscott, H. Weintraub, and I.M. Verma, (1992) Functional antagonism between c-Jun and MyoD proteins: a direct physical association. *Cell.* 68(3): 507-19.
- [113] Delgado-Olguín, P., K. Brand-Arzamendi, I.C. Scott, B. Jungblut, D.Y. Stainier, B.G. Bruneau, and F. Recillas-Targa, (2011) CTCF Promotes Muscle Differentiation by Modulating the Activity of Myogenic Regulatory Factors. *Journal of Biological Chemistry.* 286(14): 12483-12494.

- [114] Forcales, S.V., S. Albin, L. Giordani, B. Malecova, L. Cignolo, A. Chernov, P. Coutinho, V. Saccone, S. Consalvi, R. Williams, K. Wang, Z. Wu, S. Baranovskaya, A. Miller, F.J. Dilworth, and P.L. Puri, (2012) Signal-dependent incorporation of MyoD-BAF60c into Brg1-based SWI/SNF chromatin-remodelling complex. *Embo J.* 31(2): 301-316.
- [115] Galatioto, J., E. Mascareno, and M.A. Siddiqui, (2010) CLP-1 associates with MyoD and HDAC to restore skeletal muscle cell regeneration. *J Cell Sci.* 123(Pt 21): 3789-95.
- [116] Jeong, H., S. Bae, S.Y. An, M.R. Byun, J.-H. Hwang, M.B. Yaffe, J.-H. Hong, and E.S. Hwang, (2010) TAZ as a novel enhancer of MyoD-mediated myogenic differentiation. *FASEB J.* fj.09-151324.
- [117] Kim, C.H., H. Neiswender, E.J. Baik, W.C. Xiong, and L. Mei, (2008) Beta-catenin interacts with MyoD and regulates its transcription activity. *Mol Cell Biol.* 28(9): 2941-51.
- [118] Gu, W., J.W. Schneider, G. Condorelli, S. Kaushal, V. Mahdavi, and B. Nadal-Ginard, (1993) Interaction of myogenic factors and the retinoblastoma protein mediates muscle cell commitment and differentiation. *Cell.* 72(3): 309-324.
- [119] Zhang, J.-M., X. Zhao, Q. Wei, and B.M. Paterson, (1999) Direct inhibition of G1 cdk kinase activity by MyoD promotes myoblast cell cycle withdrawal and terminal differentiation. *Embo J.* 18(24): 6983-6993.
- [120] Jin, X., J.G. Kim, M.J. Oh, H.Y. Oh, Y.W. Sohn, X. Pian, J.L. Yin, S. Beck, N. Lee, J. Son, H. Kim, C. Yan, J.H. Wang, Y.J. Choi, and K.Y. Whang, (2007) Opposite roles of MRF4 and MyoD in cell proliferation and myogenic differentiation. *Biochem Biophys Res Commun.* 364(3): 476-82.
- [121] Londhe, P. and J.K. Davie, (2011) Sequential association of myogenic regulatory factors and E proteins at muscle-specific genes. *Skelet Muscle.* 1(1): 14.
- [122] Dodou, E., S.M. Xu, and B.L. Black, (2003) *mef2c* is activated directly by myogenic basic helix-loop-helix proteins during skeletal muscle development in vivo. *Mech Dev.* 120(9): 1021-32.
- [123] Potthoff, M.J. and E.N. Olson, (2007) MEF2: a central regulator of diverse developmental programs. *Development.* 134(23): 4131-40.
- [124] Rao, P.K., R.M. Kumar, M. Farkhondeh, S. Baskerville, and H.F. Lodish, (2006) Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc Natl Acad Sci U S A.* 103(23): 8721-6.
- [125] Palacios, D., D. Summerbell, P.W. Rigby, and J. Boyes, (2010) Interplay between DNA methylation and transcription factor availability: implications for developmental activation of the mouse Myogenin gene. *Mol Cell Biol.* 30(15): 3805-15.
- [126] Rudnicki, M.A., T. Braun, S. Hinuma, and R. Jaenisch, (1992) Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene *Myf-5* and results in apparently normal muscle development. *Cell.* 71(3): 383-90.
- [127] Braun, T., M.A. Rudnicki, H.H. Arnold, and R. Jaenisch, (1992) Targeted inactivation of the muscle regulatory gene *Myf-5* results in abnormal rib development and perinatal death. *Cell.* 71(3): 369-82.
- [128] Zhang, W., R.R. Behringer, and E.N. Olson, (1995) Inactivation of the myogenic bHLH gene MRF4 results in up-regulation of myogenin and rib anomalies. *Genes & Development.* 9(11): 1388-1399.

- [129] Patapoutian, A., J.K. Yoon, J.H. Miner, S. Wang, K. Stark, and B. Wold, (1995) Disruption of the mouse MRF4 gene identifies multiple waves of myogenesis in the myotome. *Development*. 121(10): 3347-3358.
- [130] Kablar, B., K. Krastel, C. Ying, A. Asakura, S.J. Tapscott, and M.A. Rudnicki, (1997) MyoD and Myf-5 differentially regulate the development of limb versus trunk skeletal muscle. *Development*. 124(23): 4729-38.
- [131] Hasty, P., A. Bradley, J.H. Morris, D.G. Edmondson, J.M. Venuti, E.N. Olson, and W.H. Klein, (1993) Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene. *Nature*. 364(6437): 501-6.
- [132] Nabeshima, Y., K. Hanaoka, M. Hayasaka, E. Esumi, S. Li, and I. Nonaka, (1993) Myogenin gene disruption results in perinatal lethality because of severe muscle defect. *Nature*. 364(6437): 532-5.
- [133] Rudnicki, M.A., P.N. Schnegelsberg, R.H. Stead, T. Braun, H.H. Arnold, and R. Jaenisch, (1993) MyoD or Myf-5 is required for the formation of skeletal muscle. *Cell*. 75(7): 1351-9.
- [134] Kassar-Duchossoy, L., B. Gayraud-Morel, D. Gomes, D. Rocancourt, M. Buckingham, V. Shinin, and S. Tajbakhsh, (2004) Mrf4 determines skeletal muscle identity in Myf5:Myod double-mutant mice. *Nature*. 431(7007): 466-71.
- [135] Rawls, A., J.H. Morris, M. Rudnicki, T. Braun, H.H. Arnold, W.H. Klein, and E.N. Olson, (1995) Myogenin's functions do not overlap with those of MyoD or Myf-5 during mouse embryogenesis. *Dev Biol*. 172(1): 37-50.
- [136] Rawls, A., M.R. Valdez, W. Zhang, J. Richardson, W.H. Klein, and E.N. Olson, (1998) Overlapping functions of the myogenic bHLH genes MRF4 and MyoD revealed in double mutant mice. *Development*. 125(13): 2349-58.
- [137] Valdez, M.R., J.A. Richardson, W.H. Klein, and E.N. Olson, (2000) Failure of Myf5 to support myogenic differentiation without myogenin, MyoD, and MRF4. *Dev Biol*. 219(2): 287-98.
- [138] Chanoine, C., B. Della Gaspera, and F. Charbonnier, (2004) Myogenic regulatory factors: redundant or specific functions? Lessons from *Xenopus*. *Dev Dyn*. 231(4): 662-70.
- [139] Hinitz, Y., D.P.S. Osborn, and S.M. Hughes, (2009) Differential requirements for myogenic regulatory factors distinguish medial and lateral somitic, cranial and fin muscle fibre populations. *Development*. 136(3): 403-414.
- [140] Hinitz, Y., V.C. Williams, D. Sweetman, T.M. Donn, T.P. Ma, C.B. Moens, and S.M. Hughes, (2011) Defective cranial skeletal development, larval lethality and haploinsufficiency in Myod mutant zebrafish. *Developmental Biology*. 358(1): 102-112.
- [141] Gensch, N., T. Borchardt, A. Schneider, D. Riethmacher, and T. Braun, (2008) Different autonomous myogenic cell populations revealed by ablation of Myf5-expressing cells during mouse embryogenesis. *Development*. 135(9): 1597-604.
- [142] Halder, M., G. Karan, P. Tvrdik, and M.R. Capecchi, (2008) Two cell lineages, myf5 and myf5-independent, participate in mouse skeletal myogenesis. *Dev Cell*. 14(3): 437-45.