Myogenic regulatory factors: The orchestrators of myogenesis after 30 years of discovery

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Impact statement

Myogenic regulatory factors (MRFs) are key players in the process of myogenesis. Despite a considerable amount of literature regarding these factors, their exact mechanisms of actions are still incompletely understood with several overlapped functions. Herein, we revised what has hitherto been reported in the literature regarding MRF structures, molecular pathways that regulate their activities, and their roles during pre- and post-natal myogenesis. The work submitted in this review article is considered of great importance for researchers in the field of skeletal muscle formation and regeneration, as it provides a comprehensive summary of all the biological aspects of MRFs and advances a better understanding of the cellular and molecular mechanisms regulating myogenesis. Indeed, attaining a better understanding of MRFs could be utilized in developing novel therapeutic protocols for multiple myopathies.

Abstract

Prenatal and postnatal myogenesis share many cellular and molecular aspects. Myogenic regulatory factors are basic Helix-Loop-Helix transcription factors that indispensably regulate both processes. These factors (Myf5, MyoD, Myogenin, and MRF4) function as an orchestrating cascade, with some overlapped actions. Prenatally, myogenic regulatory factors are restrictedly expressed in somite-derived myogenic progenitor cells and their derived myoblasts. Postnatally, myogenic regulatory factors are important in regulating the myogenesis process via satellite cells. Many positive and negative regulatory mechanisms exist either between myogenic regulatory factors themselves or between myogenic regulatory factors and other proteins. Upstream factors and signals are also involved in the control of myogenic regulatory factors expression within different prenatal and postnatal myogenic cells. Here, the authors have conducted a thorough and an up-to-date review of the myogenic regulatory factors since their discovery 30 years ago. This review discusses the myogenic regulatory factors structure, mechanism of action, and roles and regulations during prenatal and postnatal myogenesis.

Keywords: MRF4, Myf5, MyoD, myogenic determination, myogenin, myoblasts, satellite cells

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Historical view

Since the seminal work of identifying the myogenic stem cells, satellite cells (SCs), in rats and frogs respectively by Alexander Mauro and Bernard Katz in 1961,[1] many extensive studies have been conducted to further investigate the underlying mechanisms of skeletal muscle development and regeneration. It has become established that growth of postnatal skeletal muscle fibers is substantially dependent on SCs.[2,3] However, the exact molecular mechanisms of myogenic program regulation are still incompletely understood.[4]

SCs are mononuclear cells located between the basal lamina and plasmalemma of the skeletal muscle fiber.[3,5] These cells induce muscle growth either by fusing to preexisting myofibers (hypertrophy) or, less commonly, by fusing together to form new myofibers (hyperplasia).[5–7]

Surprisingly, studies on non-SCs were the first to reveal the molecular mechanisms of the myogenic program. At first, it was noticed that fraction of fibroblast cell line (C3H 10T1/2) was transformed into myogenic lineage cells after being treated with an anti-neoplastic nucleoside analogue, 5-Azacytidine.[8] It was hence determined that demethylation action of 5-Azacytidine induces the whole myogenic machinery cascade.[9] Thereafter, myogenic transformation was also achieved through transfection of a single gene locus.[10] These preliminary steps opened the door for the identification of gene loci of four orchestrated

transcription factors that regulate the process of myogenesis. These factors, which have some structural homologies and overlapped functional potentials, were appealingly designated as myogenic regulatory factors (MRFs).

Myogenic determination factor 1 (MyoD) was the first factor to be identified as a result of transfection of C3H 10T1/2 cells with cDNA containing MyoD gene loci.[11,12] The other three factors were then independently identified based on the original concept of gene loci transfection. These were myogenic factor 5 (Myf5),[13] myogenin[14] and herculin (MRF4).[15]

Structure of MRFs

Despite variation in length and amino acid sequence, each MRF protein contains three structural domains that are almost homologous with that of other MRFs. The first one is a basic domain linked to a helix-loop-helix region, collectively called basic helix-loop-helix (bHLH) domain. The second one is a cysteine/histidine domain that lies on the N-terminal side adjacent to the bHLH. The third one is a serine/threonine-rich domain, which is located near the carboxyl terminal (Figure 1).[16] It is, however, the bHLH domain that is considered the main contributor to myogenesis activation.[17] The bHLH domain has been found in MRFs of many different species, such as humans, rodents, avian, and xenopus.[16]

In general, there are seven classes of HLH proteins. MRFs are considered class II proteins as their expression is restricted to a single tissue type (skeletal muscle).[18] In addition, they have more tendency to heterodimerize, with proteins of other HLH classes, notably E2A proteins, before their binding to DNA.[19] The E2A proteins (E12 and E47) belong to class I of HLH superfamily which is characterized by the nonspecific distribution in tissues.[18] In addition, other proteins called inhibitor of DNA binding (ID) proteins can heterodimerize with either E2A or MRF proteins. These are HLH proteins belong to class V as they are characterized by their HLH regions which are uniquely not linked to basic domains.[18]

MRFs mechanism of action

The MRFs, via their bHLH domains, can bind specific sequence in DNA called E-box (CANNTG) that is ubiquitously found in promoter and enhancer regions of different downstream muscle and non-muscle specific genes.[20] Before DNA binding, each MRF has to adopt either heterodimerization or homodimerization.[19] Nonetheless, as they belong to class II of HLH proteins, MRFs have much more tendency to heterodimerize specifically with E2A proteins to proceed for myogenesis.[18] However, ID proteins can act as potential competitors as they can heterodimerize with either E2A proteins or MRFs, reducing their E-box binding capacity and consequently downstream gene transcription.[21]

The collective action of MRFs appears highly concerted and cumulative where some factors are able to induce others, while others can further regulate their own level of expression (Figure 2).[22] For example, MyoD and Myogenin have been long known to have both auto-regulatory and cross-regulatory mechanisms to modulate their respective level of expression (Figure 2).[23] On the other hand, it has been shown that different portions of MRF4 promoter region can be trans-activated by all other MRFs, but MRF4 has no auto-activation role to enhance its level of expression (Figure 2).[24] However, MRF4 is required to negatively regulate the level of Myogenin during terminal differentiation (Figure 2).[25]

In addition to the pivotal role of MRFs, myogenesis can be synergized by the action of another family of proteins called myocyte enhancer factor 2 (MEF-2) family. These proteins alone have almost no myogenesis potential. However, if present along with different MRFs, they can potentiate myogenesis.[26] It was shown that MEF-2 proteins can positively regulate different MRFs, specifically MyoD, Myogenin, and MRF4 (Figure 2).[27,28] Moreover, MEF-2 proteins level of expression can be positively regulated through both self-auto-activation and trans-activation by different MRFs.[29] Unexpectedly, a recent study showed

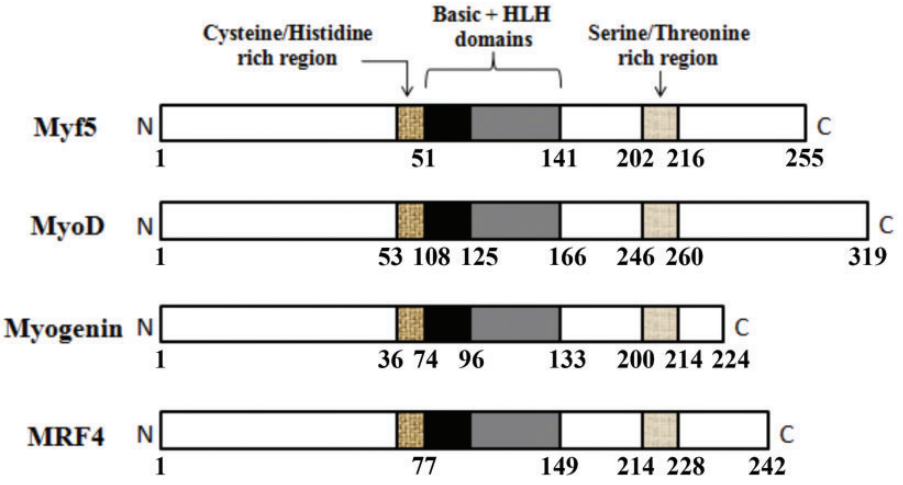

Figure 1. The primary structures for different MRFs. The three structural homologous domains of different MRFs are shown. These are; a basic domain linked to HLH region, a cysteine/histidine-rich domain that lies on the N-terminal side of the basic domain, and a serine/threonine-rich domain that is located near the C-terminal. The amino acid numbers are represented beneath each structure. (A color version of this figure is available in the online journal.)

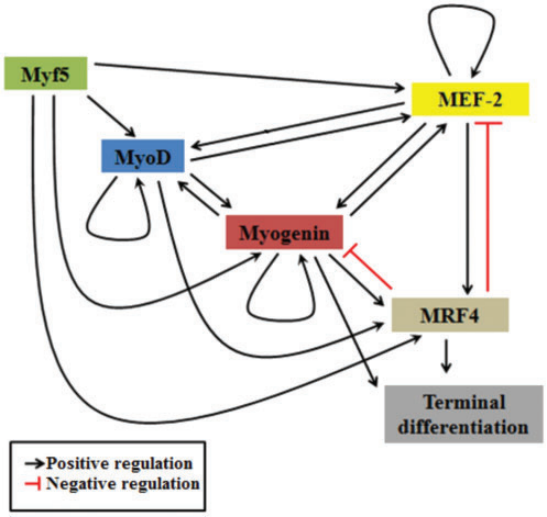

Figure 2. Signaling cascade of MRFs with their auto and cross-regulatory mechanisms. Myf5 activates all other MRFs (MyoD, Myogenin and MRF4) and MEF-2 proteins. MyoD has an auto-regulatory mechanism and cross-activation mechanism with Myogenin. Myogenin has an auto-regulatory mechanism and can induce terminal differentiation directly or/and indirectly via activation of MRF4. MRF4 directly induces terminal differentiation and can be activated by all other MRFs and MEF-2. MRF4 can inhibit both Myogenin and MEF-2 expressions. MEF-2 has an autoregulatory mechanism and can reciprocally activate both MyoD and Myogenin. (A color version of this figure is available in the online journal.)

that MRF4 can target and reciprocally repress MEF-2, leading to decreased muscle growth (Figure 2).[30]

MRFs in prenatal myogenesis

Prenatal and postnatal myogenesis programs have many molecular features in common, wherein MRFs play indispensable roles.[31] Indeed, MRFs are by far the master regulatory elements of the myogenic program as they participate in a complex network of very precisely arranged regulators with varying spatio-temporal expressions.[31] Initially, groups of upstream regulators and signals have to direct undifferentiated cells to the myogenic program. Then, MRFs regulate these specified myogenic cells until their terminal differentiation.[32]

MRFs were thought to be simply divided into early factors (MyoD and Myf5) that are involved in the commitment and proliferation of the myogenic directed cells, and late factors (Myogenin and MRF4) which regulate the terminal differentiation of the committed cells.[25] However, later accumulating evidences emphasized that MRFs are functionally overlapped. For example, MyoD can also be involved in the regulation of terminal differentiation, and MRF4 can, besides, has a role in the early commitment stage.[33] Moreover, all MRFs except Myf5 can individually induce the transition of prenatal skeletal muscle precursors from proliferation to differentiation.[34]

Due to the incompletely defined head skeletal muscle origins and molecular regulation of their precursors, our review only discusses the role MRFs in skeletal muscles of the trunk and limbs.[35] In addition, investigations on prenatal skeletal muscle development in vertebrates have been mostly conducted on avian, and murine embryos.[36]

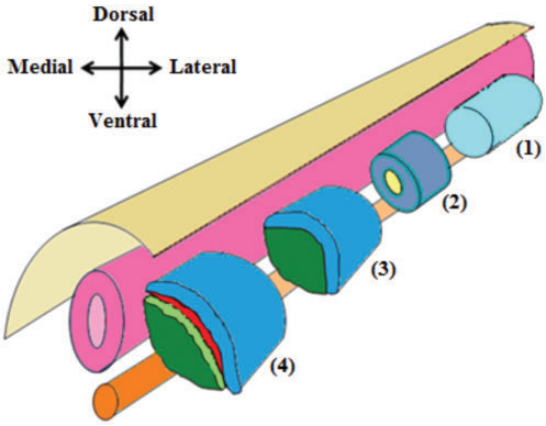

Figure 3. Differentiation of the paraxial mesoderm. Prior to somite formation, the paraxial mesoderm is composed of a single epithelial tube which flanks the neural tube and notochord (1). Later on, the paraxial mesoderm becomes segmented into somites. Each somite consists of epithelial cells with a mesenchymal core (2). Further differentiation of each somite results in the formation of two distinct layers; the dorsolateral dermomyotome, and the ventromedial sclerotome (3). After that additional two layers formed; the myotome which derived from the dermomyotome and the syndetome which derived from sclerotome (4). (A color version of this figure is available in the online journal.)

Therefore, our upcoming discussion on prenatal myogenesis will be almost confined to these two species.

General overview of prenatal myogenesis

Myogenesis program indistinctly commences early during embryonic development in vertebrates. It virtually coincides with different phases of many other developmental programs, wherein numerous multi-program regulators and signals are shared. Generally, all body skeletal muscles originate from mesoderm which eventually divides into axial (notochord), paraxial, intermediate, and lateral plates.[31] Specifically, limbs and trunk skeletal muscles originate restrictedly from paraxial mesoderm, whereas head and neck muscles are thought to be derived from the axial, paraxial, and lateral plates of mesoderm.[37,38]

The paired box transcription factor 3 (PAX3) is diffusely detected as the first ever expressed myogenic transcription factor in paraxial mesoderm.[39] PAX3 recruits and guides undifferentiated mesodermal cells toward the myogenic lineage, defining the seminal myogenic progenitor cells (MPCs).[40] Paraxial mesoderm then gets segmented into epithelial blocks each with a mesenchymal core, collectively called somites (Figure 3).[41] Myf5 henceforth appears in the somites as the first MRF to be expressed, mainly in their upper medial quadrants.[42]

Differentiation within these somites results in two distinct portions. These two portions are the ventromedial portion, which comprises sclerotome, and dorsolateral portion, that comprises dermomyotome. Sclerotome undergoes mesenchymal transformation, lose Myf5 expression, and form another distinct layer called syndetome. Sclerotome forms the precursor cells of tendons, ribs, and vertebral column. Dermomyotome preserves its epithelial characteristics, maintain Myf5 expression, and form an underlying layer called myotome. Dermomyotome acts as a source of

both precursor cells of dermis and MPCs of skeletal muscles (Figure 3).[32]

Undifferentiated mesodermal cells recruited toward the myogenic lineage are generally known as MPCs. These cells further acquire their myogenic identity by expressing different MRFs, and they are regarded henceforth as myoblasts.[31] During embryonic period, embryonic myoblasts differentiate further and establish the basis for myogenesis by elongating and forming (Mono-nucleated) primary myofibers. After that, as fetal period commences, another class of myoblasts named fetal myoblasts, begin to either fuse with one another or with the already formed primary myofibers, which collectively results in multinucleated secondary myofibers formation.[40]

Detailed roles of MRFs during prenatal myogenesis

The PAX3+/MRFs- MPCs from the hypaxial lip of dermomyotome delaminate before further myogenic differentiation. These MPCs are designated for long migratory fates such as those committed toward forelimbs, hindlimbs, cervical, and occipital mesenchyme.[43] These cells maintain their PAX3 expression and do not express MRFs while migrating.[44] Once they reach their assigned destination, they proliferate and gradually upregulate their MRFs resulting in PAX3 down-regulation. Hence, these cells are now regarded as myoblasts (Figure 4).[31] The transformation of migrating MPCs into myoblasts occurs through one of two pathways. The first canonical pathway is through PAX3 activation of Myf5, which in turn direct the expression of MyoD.[45] The second pathway is through PAX3 activation of Pitx2 expression which directly activates MyoD expression (Figure 4).[46]

Subsequently, Myf5 predominates in the epaxial part of dermomyotome, which then expresses MRF4 and MyoD. The Myf5 also appears in the hypaxial dermomyotome along with MyoD. Then, MyoD becomes the remarkably dominant MRF in the hypaxial dermomyotome as a result of Myf5 direct activation.[45] However, MyoD expression in the hypaxial dermomyotome can occur even in the absence of Myf5. This indicates that Myf5 expression is dispensable for the expression of MyoD within hypaxial dermomyotome (Figure 4).[47]

Upstream signals from different sources in the embryo (i.e. neural tube, notochord and dorsal ectoderm) are responsible for the patterning of dermomyotome and its different MRFs spatial distribution. Signals of the Wnt family including Wnt1 and Wnt3 (originating from the dorsal neural tube) and Sonic hedgehog (originating from both ventral neural tube and notochord) directly induce Myf5 expression in the epaxial portion of the dermomyotome (Figure 4).[48,49] Another signal of the Wnt family, Wnt7, from the dorsal ectoderm targets the hypaxial portion of the dermomyotome where it directly activates MyoD expression (Figure 4).[50] Bone morphogenetic protein 4 (BMP4) expressed by the lateral mesoderm counteracts Wnt7 activity and inhibits premature MyoD expression. Yet, BMP4 is in turn negatively regulated by a protein called Noggin, which is Wnt 1 and Shh dependent, leaving Wnt7 activity unopposed.[51] Other members of the Wnt signaling group such as Wnt4, Wnt5a, and Wnt6may also activate both Myf5 and MyoD expression (Figure 4).[48]

Other upstream regulators include PAX3 and PAX7 proteins. PAX3can be expressed throughout the dermomyotome, specifying all MPCs, while the expression of its paralogue, PAX7, is restricted to the central portion of the

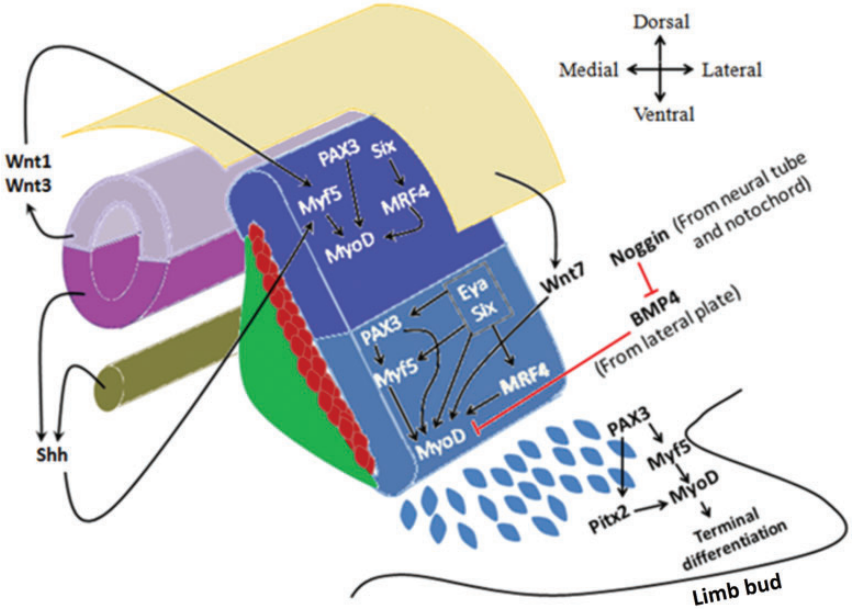

Figure 4. Different epaxial and hypaxial myogenic factors in dermomyotome and the upstream signals. Wnt1, Wnt3, and Shh signals activate Myf5 in the epaxial dermomyotome which in turn activates MyoD expression. In addition, Six proteins and PAX3 can activate MyoD expression independent of Myf5. However, in the hypaxial dermomyotome, Myf5 expression is dependent on PAX3, Six, and Eya proteins. Never the less, all the aforementioned factors can directly activate MyoD expression, while BMP4 inhibits its expression in the hypaxial dermomyotome. In the limb bud, delaminated (PAX3+/MRFs−) cells do not express MyoD until they reach their destination. MyoD expression in these cells can be induced either canonically through PAX3 and Myf5, or non-canonically by PAX3 and Pitx2. (A color version of this figure is available in the online journal.)

dermomyotome.[31] However, Myf5 expression in the hypaxial dermomyotome appears to be PAX3 dependent, while it is not within the epaxial dermomyotome (Figure 4).[45,52] In general, during the early embryonic period, both PAX3 and Myf5 lie upstream of MyoD in the myogenic cascade. Later on, as PAX7 upregulates, Myf5 and MyoD together lie downstream of PAX3 and PAX7.[52] Nevertheless, PAX7 exerts much more important roles during postnatal myogenesis of growth and regeneration.[53]

Other upstream signaling proteins include the sine oculus family members Six1 and Six4, and eye absent family members Eya1 and Eya2.[54] These signaling proteins can act directly on MRFs or indirectly through regulating PAX3 expression.[55] In epaxial dermomyotome, PAX3 expression appears to be independent of Six or Eya proteins. However, in hypaxial dermomyotome, both Six and Eya are immensely required for PAX3 to be expressed.[56] On the other hand, in epaxial dermomyotome, only MRF4 has been found to be under direct activation of Six proteins.[54] Nevertheless, Myf5, MRF4, and MyoD can be directly activated by both Six and Eya proteins in the hypaxial dermomyotome (Figure 4).[56]

It has been revealed that the expressed Myf5 in epaxial dermomyotomal MPCs is required for the development of back muscles (epaxials). MyoD in the MPCs of the hypaxial dermomyotome is essential for development of limbs, tongue, and diaphragm muscles (hypaxials). However, intercostal and abdominal wall muscles have dual origins (epaxial–hypaxial).[57] Furthermore, it has been disclosed that Myf5 and MyoD are not only MPCs fate determinants, but are also important requisites for their proliferation.[58]

Subsequently, groups of the MPCs, initially from the epaxial followed by hypaxial, caudal, and rostral lips of the dermomyotome, migrate underneath the dermomyotome forming the primary (early) myotome. Another waves of the dermomyotomal MPCs successively invade the primary myotome aiding its growth and differentiation leading to formation of the secondary (late) myotome.[59] Myotome is regarded as the first differentiated skeletal muscles to occur in the body.[60] The MPCs in the myotome will downregulate PAX3 expression and upregulate MRFs expression to become myoblasts. These cells eventually elongate, fuse with each other, and terminally differentiate forming myotubes and myofibers.[61]

The growing myotome remains in place serving as a source of myogenic cells for some axial muscles.[62] The myogenic regulatory factor Myf5 has been found to be a substantial requisite for early myotome development, while it is not during further consolidation and differentiation of the early myotome.[63] However, even in the absence of myotome, as a result of Myf5 and MRF4 inactivation, there will be no effects on the muscle development, and the myogenesis process can proceed normally.[64]

It has been revealed in mice, that loss of both MyoD and Myf5 during the embryonic period results in complete absence of myoblasts and consequently skeletal muscles postnatally. However, single mutation of either one has inconsiderable effects, raising the possibility of compensation of one another supposedly via their partial redundant action. Early MPCs lacking Myf5 can only be compensated by MyoD or to a lesser extent MRF4 to pursue their myogenic pathway, otherwise they adopt other non-myogenic fates.[65] Unexpectedly, it has been shown that MRF4 can rescue myogenesis in the early stages even in complete absence of both Myf5 and MyoD.[66] On the other hand, myogenin absence leads to loss of skeletal muscles, despite the presence of normal committed myoblasts and death shortly after birth.[67] This indicates that there is no possible compensation for myogenin absence, and it has an indispensable role in terminal differentiation of myoblasts.[68] In addition, previous studies reported the presence of a differential transcriptional activation among the four MRFs. For example, it has been shown that MyoD, Myf5, and Myogenin are capable of activating several muscle specific genes including Myosin light chain, muscle creatine kinase, and acetylcholine receptor α subunit chain, while MRF4 is inefficient in activating the transcription of the aforementioned genes.[69,70]

MRFs in postnatal myogenesis

As in prenatal skeletal muscle development, MRFs play a pivotal role in myogenesis during postnatal life.[71] Postnatal growth, repair, and regeneration have nearly the same molecular mechanisms, wherein several growth factors activate quiescent SCs so they adopt myogenesis program that is precisely regulated by the MRFs orchestra.[72] While prenatal development of skeletal muscles is achieved by the maturation of temporally different groups of myoblasts (embryonic and fetal), the postnatal growth and regeneration are indispensably dependent on SCs.[40] Although, SCs are described to be heterogeneous with regard to their origin and functional status.[73]

During the early postnatal life, SCs are plentiful in skeletal muscle tissue where they account for nearly 30% of all nuclei identified underneath the basal lamina. This relative abundance of SCs gradually declines to reach 5% or less within adult skeletal muscles.[74] SCs are small cells that are uniquely interposed between the myofiber plasma and basement membranes, while myonuclei are located within the myofiber just underneath the plasma membrane.[7,75] SCs have distinct structural characteristics including their scant cytoplasm and relatively small nuclear size in comparison with that of myonuclei.[75] In addition, these cells are characterized by their specific expression of PAX7 protein which is always upstream of MRFs. It is known that PAX7 has an anti-apoptotic role in SCs. In case of PAX7 absence, SCs are lost postnatally and skeletal muscles undergo atrophy and cannot regenerate even in the presence of PAX3. This indicates that PAX3 has no compensatory potential for PAX7 absence in SCs.[76,77]

General overview of postnatal myogenesis

Postnatal and adult skeletal muscle tissue, like any other living tissue, is in a dynamic state with continuous turnover due to daily minor traumas and small lesions.[78] However, in the case of prominent or recurrent traumas, regenerative myogenesis is accompanied by fibrosis and adipogenesis. This occurs due to the activation of skeletal muscle resident cell population called fibro/adipogenic progenitors (FAPs) rather than SCs.[79] However, signals coming from activated

FAPs have been shown to be required during differentiation of SC-derived myogenic cells. Thus, a balanced action of both SC and FAP populations is important for normal myogenesis.[80]

In general, the process of postnatal myogenesis due to an injury of skeletal muscle can be divided into three main stages; degeneration, regeneration, and remodeling.[81] During degeneration, necrosis accompanied by inflammatory process occurs in the injured tissue. This is followed by the overlapped stages of regeneration and remodeling mediated via the process of SC activation, proliferation, and differentiation which is orchestrated by the expression of different MRFs within SCs.[82]

Detailed roles of MRFs during postnatal myogenesis

MRFs during SC quiescence. SCs are usually quiescent cells, which means that they are arrested in G0 phase.[7] These quiescent SCs are almost negative for MRFs. This is achieved by their ubiquitous high expression of PAX7 and, to a lesser extent, PAX3.[76] However, it has been, surprisingly, found that disparate levels of Myf5 protein are detectable within most of quiescent SCs (Figure 5).[83] It was shown that about 90% of SCs have an active Myf5 transcriptional locus, which indicates that these quiescent SCs are already directed to the myogenic pathway.[84] Significant levels of Myf5 translation are inhibited within quiescent SCs by miR31 which sequester myf5 mRNA in mRNP particles.[85] The remaining (~10%) of SCs are described as stem cells rather than myogenic progenitors, due to their lack of Myf5 expression (Figure 5).[77]

Interestingly, it is the Myf5-ve SC pool which undergoes depletion in mdx mice leading to diminished SC self-renovation capacity. This occurs because Myf5-SCs tend to have a relatively higher self-renovation ability compared with Myf5+ ones.[86] On the other hand, almost all SCs have been shown to be derived from prenatal MyoD+ precursors.[87] However, neither the MyoD mRNA transcript nor MyoD protein are detectable within postnatal quiescent SCs.[88] MyoD locus is maintained non-transcriptable within the peripheral SC nuclear heterochromatin by a dimethyltransferase protein called Suv4–20h1 to promote SC quiescence.[89]

MRFs during SC activation. Different stimuli can induce myogenic SC activation such as exercise,[90] electrical stimulation,[91] and some pharmaceutical preparations, notably anabolic androgenic steroids.[2,3,5,6] In general, upon activation, most of SCs gradually downregulate their expression of PAX7[92] and, if present, PAX3.[76] Meanwhile, they begin to upregulate their MRFs as the myogenic program ensues.[93]

The process of SC activation starts principally as the level of Myf5 protein gradually increases in most of the activated SCs leaving small number of them as Myf5-. This non inclusive Myf5 expression gives rise to the "PAX7+/Myf5+ SC majority" which can divide only symmetrically. However, the "PAX7+/Myf5- SC minority" can adopt either symmetrical or asymmetrical divisions (Figure 5). Progenies of "PAX7+/Myf5+ cells" are arranged in close proximity to the plasma membrane of myofibers to pursue their myogenic fate via adopting further proliferation and differentiation. On the other hand, PAX7+/Myf5- cells become adjacent to the basal lamina where they are capable to return to the quiescent state to maintain SC pool.[77]

In addition to Myf5 and PAX7 expression, SCs start to express MyoD protein in early stages of their activation.[94] PAX7 is substantially required to bring about the activation of SCs by its direct binding to the enhancer and promoter regions of Myf5 and MyoD, respectively.[95] Activated progeny of SCs that is committed for myogenesis is thought

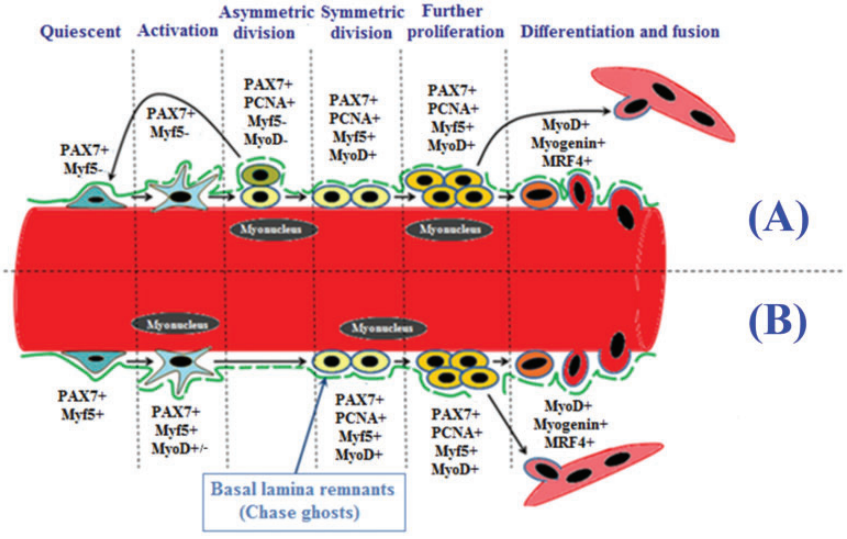

Figure 5. Expression of myogenic regulatory factors during different stages of satellite cells. Satellite cells are divided into two groups according to their self-renewal ability; myogenic stem (~10%, PAX7+/Myf5−) and myogenic precursor (~90%, PAX7+/Myf5+) cells. (A) Myogenic stem cells can undergo asymmetric divisions where some daughter cells return back to quiescence. (B) Myogenic precursor cells undergo symmetrical divisions where all daughter cells are committed to the myogenic fate. Proliferating satellite cells can be identified by the expression of PCNA. Upregulation of MyoD along with the expression of Myogenin and MRF4 induces these cells into the differentiating stage. Terminally differentiated cells may either fuse into pre-existing myofibers, or into newly formed one. Chase ghost act as a scaffold for myogenic stem and precursor cells during activation, proliferation, and differentiation. (A color version of this figure is available in the online journal.)

to be guided by basal lamina remnants (chase ghosts) to migrate and proliferate in the injured myofibers (Figure 5).[96]

The stability of both Myf5 and MyoD factors is regulated at the translational level by miRNAs and RNA binding proteins. During SC quiescence, miRNA-31 sequester the Myf5 mRNA in isolated granules, which will dissociate upon SC activation.[85] Moreover, fragile X mental retardation protein, in cooperation with miRNA pathway, inhibits Myf5 translation by direct binding to Myf5 transcripts and impacting its deadenylation.[97] The mRNA binding protein tristetraprolin (TTP) was found to promote the decay of MyoD mRNA through binding to MyoD mRNA 3′ region. However, upon SC activation, the p38 mitogen-activated protein kinases α and β were found to promote TTP inactivation and therefore stabilize MyoD mRNA.[98]

MRFs during SC proliferation. The most prominent myogenic factors expressed in proliferating SCs are Myf5 and MyoD.[94] These two factors are also expressed in activated SCs. Yet, the best distinguishing marker for proliferating SCs is the expression of proliferating cell nuclear antigen (PCNA). After proliferation, the majority of cells start to downregulate their PAX7 and maintain MyoD to proceed for differentiation. The remaining cells maintain PAX7 expression and suppress MyoD to return back to quiescence (Figure 5).[94]

Activation of Ras-ERK pathway is important to maintain SCs in proliferative state by inducing the retinoblastoma protein expression which leads to the suppression of myogenin. Thus, to induce SCs differentiation through MRFs canonical expression, Ras-ERK pathway must be suppressed (Figure 6).[99] Many proteins have been identified to block the Ras-ERK pathway including Sprouty,[100] Impedes Mitogenic signal Propagation,[101] Raf-1 Kinase Inhibitor Protein,[102] and DA-Raf1.[99] In addition, activated and intra-nuclear translocated Notch-1 protein can promote SC proliferation through keeping MyoD expression at low levels (Figure 6).[103] It is important to note that upregulation of MyoD expression is required for SCs to exit the cell cycle and enter the differentiation state.[104] Previous studies reported that MyoD expression induces differentiation by increasing the expression of cyclin-dependant kinase inhibitor protein p21.[104]

Role of growth factors in SC activation and proliferation

Several growth factors are expected to play an important role in SC activation and proliferation. For example, fibroblast growth factor 2 (FGF-2) recruits more SCs to the cell cycle, providing more (PCNA+/MyoD+ SCs) to the myogenesis program (Figure 6). This occurs without affecting the transit time from proliferation (PCNA+/MyoD+ SCs) to differentiation (MyoD+/Myogenin+ SCs).[105] Insulin-like growth factors (IGF-I and IGF-II) have been shown to induce proliferation (PCNA+/MyoD+ SCs). However, IGF-I appear to be much more potent in differentiation (MyoD+/Myogenin+ SCs).[106] Hepatocyte growth factor (HGF) has been shown to induce SC activation and proliferation, while it inhibits heterodimerization process of MRF and E proteins. This results in inhibition of the progression toward differentiation (Figure 6).[107] However, myostatin which is a member of the transforming growth factor β (TGF-β) superfamily, prevents activation of SCs and maintain their quiescence.[108] Yet, follistatin antagonize the inhibitory action of many TGF-β family members, including myostatin which results in SC activation and progression into myogenesis (Figure 6).[109]

MRFs during SC differentiation. Transition of SCs from proliferation to differentiation is regulated principally at a level above the MRFs. This is achieved by progressive switch in the expression from Notch proteins in proliferating SCs to Wnt proteins in differentiated SCs. Wnt proteins

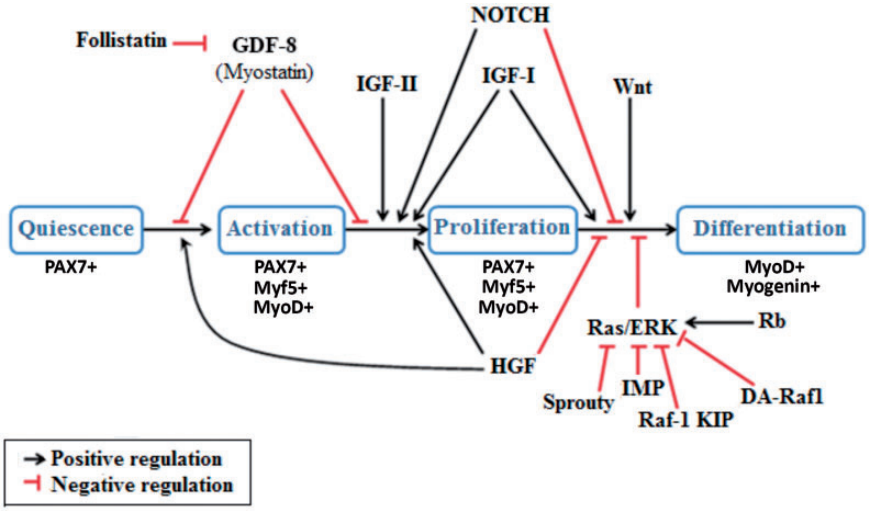

Figure 6. Illustrating diagram for several growth factors and signaling molecules that regulate different stages of satellite cells. Transition from quiescence into activation and proliferation is stimulated by HGF and inhibited by myostatin (GDF-8). IGF-II induces proliferation. IGF-I can induce both proliferation and differentiation. HGF and NOTCH sustain proliferation and inhibit transition to differentiation. Transition to differentiation is also inhibited by Rb protein through activating Ras/ERK pathway. Transition to differentiation can be induced either directly by the Wnt family of proteins, or indirectly via inhibition of the Ras/ERK pathway by Sprouty, IMP, Raf-1 KIP, and DA-Raf1. (A color version of this figure is available in the online journal.)

promote stabilization of β-catenin which activates transcription of several muscle specific genes required for differentiation (Figure 6).[110]

At the level of MRFs, transition from proliferation to differentiation is marked by the progressive downregulation of PAX7 expression with greater expression of MyoD. In addition, Myogenin expression upregulates and directs differentiating SCs to subsequently become terminally differentiated into myonuclei.[7,75] It has been shown that the lack of MyoD delays proliferation to differentiation transition time.[111]

If SCs maintain PAX7 expression and suppress MyoD level, then the progression toward differentiation is impeded. However, once SCs express Myogenin, it can directly suppress PAX7 expression and differentiation ensues, which suggesting a reciprocal inhibitory mechanism between MRFs and PAX7.[112] PAX7 is substantially required to bring about the activity of MRFs cascade.[104] The levels of MRFs expression are adjusted by counter regulatory proteins like Id2 and Id3 which also require PAX7 to induce their expression. This provides adequate control of the myogenesis machinery that is achieved in the case of Id proteins via prevention of the unwanted early differentiation of proliferating SCs.[113]

MRFs are almost similarly expressed within both the prenatal MPCs-derived myoblasts and postnatal activated SCs. Interestingly, however, MRF4 is not expressed in SCs unless they become terminally differentiated. Moreover, the expression of MRF4 becomes more evident and detectable within myonuclei during the process of regeneration.[114] On the other hand, Myf5 and MyoD expressing SCs contain a constellation of miRNAs, including miR206 and miR1, which are important to downregulate the expression of PAX proteins within proliferating and differentiating SCs.[32] In addition, a recent study by Kim *et al.*[115] demonstrated that miR1 and miR206 can induce MRFs expression, notably MyoD and Myogenin.

Summary

In conclusion, MRFs are considered key players in prenatal skeletal muscle formation and postnatal skeletal muscle growth and regeneration. Since their discovery 30 years ago, substantial research has been conducted to understand their exact signaling pathways and mechanisms of action. The vital role of MRFs in regulating myogenesis renders them as an excellent target for manipulation in potential stem cells therapeutic protocols for muscular degenerative disorders. For example, Tedesco *et al.*[116] demonstrated that transduction of the MyoD gene by a lentiviral vector resulted in myogenic differentiation of pluripotent mesoangioblast-like stem cells derived from healthy individuals and from patients affected by limb-girdle muscular dystrophy type 2D (LGMD2D). Transplantation of these MyoD transducted cells into LGMD2D mice resulted in functional improvement of the dystrophic phenotype.[116] Moreover, forced expression of MyoD in the human adipose-derived stem cells promoted them into the myogenic fate. These cells were able to fuse with Duchenne muscular dystrophy myoblasts and to improve dystrophin expression *in vitro*.[117]

Despite many research findings, several concepts pertaining to these factors are still incompletely understood. However, certain facts have been established regarding these factors and their role in myogenesis. All MRFs (Myf5, MyoD, Myogenin and MRF4) are members of class II bHLH superfamily of regulatory factors which are solely expressed in skeletal muscle tissue. In order to exert their role, MRFs can either heterodimerize with the class I bHLH E-proteins or, to a lesser degree, homodimerize with another MRF before binding to DNA. Prenatally, MRFs expression in the MPCs of the dermomyotome differs according to the cells location and eventual migratory route. Long fate migratory MPCs derived from the hypaxial lip of dermomyotome express PAX3 and do not express any MRF until they reach their final destination. These cells are considered the precursors of limbs, tongue, and diaphragm skeletal muscles. Other dermomyotomal cells delaminate and form a distinct layer called myotome starting from the epaxial (Myf5 dominant) and then hypaxial (MyoD dominant) regions. Myotome serves as the source of precursor cells of some prevertebral muscles. Many upstream factors and signals regulate Myf5 and MyoD expression in epaxial and hypaxial portions of the dermomyotome, including PAX3, PAX7, Shh, and Wnt family of proteins.

Postnatally, skeletal muscle growth and regeneration are mediated by activation, proliferation, and differentiation of SCs where they ultimately fuse to myofibers and their nuclei become new myonuclei. Many upstream growth factors and signals regulate the progression of SC from quiescence to activation, proliferation, and differentiation, including HGF, TGFβ, IGFs, Notch, and Ras-Erk pathway ligands. All quiescent SCs express PAX7; however, most of them (90%) also express Myf5 in addition to PAX7. The expression of Myf5 in these cells indicates that they are designated for symmetrical division giving rise to committed myogenic cells. Once activated, these SCs are characterized by their expression of MyoD. Activated SCs continue to express MyoD during subsequent stages even after their differentiation into myonuclei, which explains the high percentage (>90%) of MyoD+ Myonuclei. During proliferation, SCs can be further distinguished by their expression of PCNA. The terminally differentiated SCs can be identified by their expression of Myogenin, MRF4 as they cease PAX7 expression to become Myonuclei.

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DECLARATION OF CONFLICTING INTERESTS

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