



Myogenic regulatory factors in myogenesis and regeneration of skeletal muscle

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ABSTRACT

Myogenesis, or the formation of muscle tissue, is an extremely complicated process, is essential for the proper functioning of the body. Depending on the type of muscle, they play different important functions. In vertebrata skeletal (striated) muscles allow skeletal movement (locomotion) and maintaining posture. They are built of long, cylindrical cells (myofibres) with numerous nuclei. Each myofibre is composed of many alternately oriented fibrils, resulting in a conspicuous banding. These striated muscles contract quickly and fatigue rapidly. Their development and regeneration are controlled by many genetic factors and some of the most important ones are myogenic regulating factors (MRF), including MyoD (or Myod1), myogenin, Myf5 and MRF-4. These proteins regulate the activity of many genes involved in development and regeneration of muscle tissue. They act in a complementary manner and numerous studies show they are expressed to varying degrees. The synthesis of non-functional or abnormal MRFs may lead to serious consequences and even death due to abnormal development of muscle tissue. MRF-regulated inhibition of myoblasts in their growth cycle is a crucial step in muscle formation.

Keywords: myogenesis, skeletal muscles, myogenic regulatory factors

1. INTRODUCTION

Skeletal muscles are extremely important building block of the body. In case of birds, farm and game animals their muscle tissue can be look at from two different perspectives. First, it is of vital importance for these animals as it allows them to feed, breathe and move. For humans it is an important component of their diet. Regardless of the function or purpose, muscle formation is a complex process, influenced by many different factors, e.g. genetics, biochemistry and environment. Even the smallest physiological and biochemical changes may impair tissue formation and therefore lead to its abnormal structure, resulting in serious, even fatal consequences. Therefore, understanding the mechanism of muscle formation and regeneration is of utmost importance, as it may reduce some negative phenomena that occur during these processes.

Myogenic regulatory factors (MRF) seem to be of extreme importance; among them MyoD (also known as Myod1), myogenin, Myf5 and MRF4 are considered the main regulators of the processes.

2. MYOGENESIS

Myogenesis, or forming of skeletal muscles, begins at the embryonic stage and continues throughout entire life in the processes of regeneration and development of muscles. First, dermomyotomes develop into muscle cell precursors; these in turn develop into myoblasts (muscle cells) and satellite cells, the latter acting as muscle stem cells. Eventually, myoblasts undergo series of mitosis and produce successive generations of muscle cells until one of these generations is inhibited in their cycle through the action of numerous transcription factors (post-mitotic myoblasts) [1]. The next step is the formation of polynuclear cells, myotubes, by fusing mononuclear post-mitotic myoblasts. This process is possible due to the presence of signalling molecules, such as integrin and cadherin, in the cell membrane of post-mitotic myoblasts [1, 2]. These myoblasts already synthesize structural proteins of the muscle but do not show the typical muscle striations.

Constant renewal of the myoblast population is possible due to the presence of satellite cells located at the edges of the muscle fibres. These cells act as muscle stem cells and their activity increases as a result of injuries and tissue disruption and also with high or low temperature and oxygen deficiency. Damaged tissue releases cytokines and growth factor, prompting satellite cells into proliferation and differentiation into myoblasts, which eventually became fused and form new muscle fibres. The regeneration process ends with restoring satellite cell population in regenerated muscles [2].

3. MYOGENIC REGULATORY FACTORS

This complex process of muscle formation is regulated, among others, myogenic regulatory factors, e.g. MyoD (also known as Myod1), myogenin, Myf5 and MRF4 (Myf6) [3]. Research has shown that these factors are able to transform 10T1/2 fibroblasts and other non-muscle cells into myogenic cells [4]. They all are characterized by the presence of a highly conserved region that allows them to maintain the structure of the double helix

connected with a short loop; that is why they are classified into muscle-specific basic-helix-loop-helix (bHLH) factors. The presence of bHLH domain allows these factors to interact with DNA molecule in its E-box sequence [4, 5]. This motif consists of highly degenerated DNA of CANNTG consensus sequence and is present in the promoter region of many muscle-specific genes. Their transcription is activated as a result of interactions of gene sequences with the muscle-specific transcription factors – bHLH [4, 6].

Each of the factors in MRF group is characterized by its specific expression profile which depends on the stage of embryogenesis (table 1). Therefore it is possible to determine their most probable function and co-operation scheme [7]. MyoD and Myf5, characteristic of the early phase of muscle formation, participate in muscle cells specialization, myogenin controls differentiation, and MRF4 is associated with myotube maturation [8].

Table 1. Expression of MRF coding genes at different phases of muscle tissue development (“-“ – lack of expression; “+” – low expression ; “++” – high expression) [after 7]

	Embryonic progenitors	Satellite stem cells	Committed satellite cells	Myoblasts	Myocytes	Myotubes/myofibres
Myf5	-	++	++	+	-	-
MyoD	-	++	-	++	+	+
MYOG	-	-	-	-	++	+
MRF4	-	-	-	-	+	++

Myogenin (*myogenic factor 4*), also known as MYOG, was first isolated from rat fibroblasts and described in 1989 [9]. In the same year Sassoon et al. [10] presented the course of the expression of myogenin-coding gene during embryogenesis *in vivo* in mice. Studies on its expression during regeneration of mature muscles *in vivo* lasted a bit longer. It was found that myogenin mRNA can be regarded as a very early and specific marker that allows identifying the active skeletal muscle precursor cells in mature muscle regeneration *in vivo* [11].

MyoD was the first discovered and described transcription factor of MRF group. As early as in 1987 it was demonstrated to have the ability to transform selected cell types, e.g. fibroblasts, into cells that can fuse into myotubes [12].

Results by Grounds and his group [11] proved that both myogenin-coding gene and MyoD transcription factor gene begin their expression immediately after muscle injury. In addition, both genes show co-expression in the same cells, suggesting complementary activity of these factors [11].

Both myogenin and MyoD transcription factor share identical bHLH sequence and are largely similar in the remaining encoding sequence; nevertheless, their functions in myogenesis are significantly different [13]. While MyoD plays a central role at the beginning of the process and influences the formation of mesodermal progenitor cells, myogenin is

important in the later stages and directs the specialization of myoblasts into functional muscle fibres [14, 15].

Myogenin is an important transcription factor in the development of skeletal muscles and blocking its expression in mice results in abnormal muscle development and thus respiratory failure and death in the perinatal period [16]. Moreover, the important role of myogenin is suggested by the fact that it is the only transcription factor of MRF group which expression can be detected in all skeletal muscle cells [17].

Myf5, myogenic factor 5, is a protein that plays an important role in myogenesis and muscle differentiation. The relevant gene is expressed very early in myogenesis and its transcript is found even in dermomyotomes. Interestingly, as shown in mice, when Myf5 is either abnormal or dysfunctional, its function can be taken by both MyoD and MRF4 and it is only in the absence of all three that the development of muscles is not possible [16, 18]. Regulation of Myf5 expression is associated with Pax7 transcription factor which is characteristic for embryonic development. Pax7 transcription factor regulates muscle precursor cells proliferation as well as Myf5 gene expression, through interaction with its promoter region [19].

MRF4 (Myf6) acts as a regulating factor in myogenesis and muscle regeneration.

4. MRF PARTICIPATION IN MYOBLAST CELL CYCLE REGULATION

The permanent continuation of myoblast cell cycle is associated with the presence of mitogenic factors in the environment. These factors stimulate cells into S phase which also inhibit the differentiation of myoblast into myotubes. Therefore, permanent withdrawal of muscle cells from the cell cycle is essential for their fusion into poly-nuclear myotubes. This withdrawal requires inhibition of positive regulators of the cell cycle.

Skipping the cycle and generating of post-mitotic myoblasts are due to the co-operation of MyoD transcription factor and other mechanisms, responsible for blocking cell cycle progression [8, 20].

Studies on synchronized myoblasts cultures and *in vivo* demonstrated that MyoD and Myf5 are characterized by different patterns of their expression, associated with myoblast cell cycle stages. MyoD protein reaches their highest level early in G1 phase of the cycle and then lowers its level to the minimum in S phase, while Myf5 protein cannot be detected in differentiating myoblasts, which are characterized by high levels of MyoD and myogenin. Myf5, though, is expressed in myoblast permanently inhibited in G0 phase (i.e. leaving the cell cycle) and not undergoing further divisions. Expression of both MyoD and myogenin cannot be detected in post-mitotic myoblasts [21]. Moreover, MyoD transcription factor in growing muscle cells is phosphorylated by cdk1 and cdk2 (cyclin-dependent kinases); their action weakens when myoblasts start their differentiation into myotubes [22].

During the transition of muscle cells from G1 to synthesis phases a rapid decline in MyoD levels is observed. Knowing the consequences of cdk-dependent phosphorylation in ubiquitin-dependent MyoD degradation, it may be concluded that MyoD phosphorylation by cdk2 is involved in ubiquitin-dependent MyoD degradation during the transition between these phases [8]. Cyclin D1 is also important in myogenesis inhibition through MyoD and is indispensable to induce cyclin E, which forms cdk2-cyclin complexes. These complexes are

essential for the phosphorylation of this myogenic factor and its eventual ubiquitination and degradation [8].

In accordance with its role, MyoD concentration increases at an early stage of myoblast differentiation, as opposed to Myf5 concentration, which drops significantly after initiation of differentiation and then is maintained at a constant, low level until the late stage. The different nature of the changes in the level of both factors confirms the ability of MyoD to inhibit Myf5 expression. It also reflects the ability of Myf5 to induce proliferation of myoblasts, in contrast to MyoD, which stops proliferation and inhibits cell cycle [23, 24].

Existing research confirm the presence of Myf5 in both cytoplasm and nucleus of undifferentiated cells. Following the start of differentiation, when Myf5 level drops significantly, it is still present in the cytoplasm, yet it is concentrated near to the nucleus, and is gradually transported into it. This is most likely due to the transcriptional function of this protein, which inhibits transcription of muscle-related genes, encoding cell cycle progression and start of differentiation. These factors are present in different muscle precursor cells and, given their expression, these cells can be classified into two populations: MyoD-positive that fuses into myotubes, and Myf5-positive that represents resting, non-differentiating satellite cells [8].

Myogenin expression varies differently from Myf5 expression; the latter increases significantly just after the start of differentiation and then decreases in its subsequent stages. A similar character of expression changes between MyoD and myogenin suggests interaction of both factors, based on positive feedback [8, 25]. Additionally, myogenin expression regulates the synthesis of proteins in contractile apparatus of muscle cells [26].

MRF4 expression increases significantly in the middle and late stages of differentiation, when myoblasts fusion has already begun, and this confirms its activity in myotubes maturation [8].

Existing research allow to determine many of MRF functions, their co-operation and mutual interactions. The most commonly accepted hypothesis picks MRF4 gene as a determinant gene. MyoD, Myf5 and MRF4 most likely determine the identity of multi-potential muscle cell progenitors, whereas MyoD, myogenin and MRF4 support the process of their differentiation [8, 27].

5. CONCLUSIONS

Myogenic regulatory factors are one of the most important modulators of muscle tissue formation and regeneration. By regulation of muscle-specific gene expression they are actively involved at all stages of muscle development. Existing research led to a partial understanding of MRF action, genes they regulate, as well as their influence on myoblast cell cycle.

Further studies are of utmost importance as they may lead to the development of therapeutic strategies in the treatment of abnormalities in muscle formation and/or regeneration. Moreover, more accurate knowledge of these factors and deeper understanding of their action and co-operation may allow the introduction of targeted gene therapy. With this therapy it will be possible to replace inactive or abnormal proteins with the functional ones.

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