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The p38 MAPK signaling pathway: A major regulator of skeletal muscle development

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Abstract

Skeletal muscle development is regulated by extracellular growth factors that transmit largely unknown signals into the cell affecting the muscle-transcription program. One intracellular signaling pathway activated during the differentiation of myogenic cell lines is p38 mitogen-activated protein kinase (MAPK). As a result of modifying the activity of p38 in myoblasts, the pathway proved essential for the expression of muscle-specific genes. P38 affects the activities of transcription factors from the MyoD and MEF2 families and participates in the remodeling of chromatin at specific muscle-regulatory regions. P38 cooperates with the myogenic transcription factors in the activation of a subset of late-transcribed genes, hence contributing to the temporal expression of genes during differentiation. Recent developmental studies with mouse and *Xenopus* embryos, substantiated and further extended the essential role of p38 in myogenesis. Evidence exists supporting the crucial role for p38 signaling in activating MEF2 transcription factors during somite development in mice. In *Xenopus*, p38 signaling was shown to be needed for the early expression of Myf5 and for the expression of several muscle structural genes. The emerging data indicate that p38 participates in several stages of the myogenic program.
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Keywords: Signaling; p38 MAPK; Skeletal muscle; Myogenesis

1. Introduction

Identification of the p38 mitogen-activated protein kinase (MAPK) signaling pathway was originally based on its activation by environmental stresses and by pro-inflammatory cytokines. Over time, it became clear that the p38 pathway functions in a large number of cellular processes unrelated to stress responses and including cell growth, cell differentiation, cell cycle arrest and apoptosis. This is not surprising since in the course of the years many extracellular stimuli including growth factors and hormones have been shown to activate the p38 pathway. The activation of p38 in response to a wide range of extracellular stimuli can be seen in part by the diverse range of MAPK kinase kinases (MAP3K) that participate in p38 activation (TAK1, ASK1, DLK, MEKK4), contributing to the complexity of this signaling pathway. The MAP3Ks phosphorylate and activate the MAPK kinases (MAP2Ks) MKK6 and MKK3 which in turn phosphorylate the p38 MAPKs. In vertebrates, there are four isoforms of p38: p38α, β, γ and δ. The isoforms can be categorized by a Thr-Gly-Tyr (TGY) dual phosphorylation motif. Once activated, p38s phosphorylate serine/threonine residues of their substrates. The list of downstream substrates of p38 keeps growing and includes other protein kinases and many transcription factors suggesting its possible role in regulating gene expression at the transcriptional level. Several of the downstream targets of p38 that are lineage-specific or playing an essential role in development have led to the identification of the central role of the p38 pathway in developmental and differentiation processes. The drosophila p38 gene has been suggested to play a role in decapentaplegic (*dpp*)-regulated wing morphogenesis (Adachi-Yamada et al., 1999). p38 was also demonstrated to affect the asymmetric development of the drosophila egg by controlling the localization of Oskar and Gurken which are essential for the posterior and dorsal specification, respectively (Suzanne et al., 1999). In mice, p38 activity was recently demonstrated to be required for the development of the 8–16 cell stage embryo (Natale et al., 2004). Essential roles for p38 signaling in several

Abbreviations: MAPK, mitogen-activated protein kinase; MAP3K, MAPK kinase kinase; MKK6, 3, MAP kinase kinase 6, 3; MEF2, myocyte enhancer factor 2; BMP, bone morphogenic protein; MRF, myogenic regulatory factor; XMyf5, *Xenopus* Myf5; XMyoD, *Xenopus* MyoD; ERK, extracellular regulated kinase; RMS, rhabdomyosarcoma; PI3-K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin

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differentiation processes including adipogenesis, neurogenesis, chondrogenesis, erythroid differentiation and myogenesis were demonstrated (reviewed in Nebreda and Porras, 2000; Zarubin and Han, 2005). The extracellular signaling molecules regulating skeletal muscle development are largely known while the intracellular signaling events are still poorly understood. Studies in recent years indicate that p38 is probably one of the major intracellular signaling pathways affecting myogenesis. The purpose of the present review is to summarize the expanding literature pertaining to the involvement of p38 signaling in skeletal myogenesis.

2. p38 is necessary for muscle differentiation

2.1. Skeletal muscle development

The development of skeletal muscle in the developing somite is a multistep process in which pluripotent mesodermal cells become committed to the myogenic lineage by receiving signals from neighboring tissues. These signals, including Wnts, BMP4 (bone morphogenic protein 4) sonic hedgehog and Noggin initiate the expression of transcription factors from the MyoD family, MyoD and Myf5 in cells turning into myoblasts. Subsequently, the activities of MyoD and Myf5 are induced and lead to the withdrawal of myoblasts from the cell cycle; they also initiate the expression of other transcription factors from the MEF2 family and of myogenin. Together, myogenin and MEF2 family members cooperate in the activation of many muscle structural genes during differentiation and the formation of multinucleated myotubes.

2.2. The α and β isoforms of p38 are involved in muscle differentiation

The study of skeletal muscle differentiation has benefited during the last decades from the availability of several myogenic cell lines that allowed the biochemical dissection of the myogenic pathway. Studies using cell lines led to the identification of the transcription regulatory factors of the MyoD family (MyoD, Myf5, myogenin and MRF4) and the MEF2 family (MEF2A–D). Extracellular cues positively regulating myogenesis such as insulin-like growth factors (IGFs) were also identified using the same cell lines. Still, little is known about the intracellular signaling molecules regulating the expression and activities of the myogenic transcription factors. One approach for identifying the involvement of an intracellular pathway is to analyze the changing activities of kinases belonging to this pathway during the differentiation process. Out of the four isoforms of p38 (α, β, γ, δ), the phosphorylation and activity of α and β isoforms were gradually induced during the differentiation of myoblasts (Cuenda and Cohen, 1999; Wu et al., 2000; Zetser et al., 1999). The sustained activation of p38 during differentiation differs from the stress-induced acute activation in that the first promotes the differentiation process while the second does not affect differentiation (Puri et al., 2000; Wu et al., 2000). The prolonged activation of p38 does not occur in serum-starved and insulin-treated fibroblasts, but does occur in fibroblasts expressing MyoD (Wu et al., 2000). Thus, activation of this pathway is probably intrinsic to muscle cells expressing MyoD and serves to guarantee the complex and timely activation of the muscle program (as later discussed). The involvement of p38α and β isoforms in differentiation is supported mainly by the anti-myogenic effect of SB203580, a pharmacological inhibitor specifically inactivating these isoforms and not the others (γ and δ) (Cuenda and Cohen, 1999; Wu et al., 2000; Zetser et al., 1999). This was further supported by the immunodepletion of specific isoforms from muscle cell extracts and the finding that p38α and β isoforms comprised the entire kinase activity towards MEF2C protein (Zetser et al., 1999).

The involvement of p38γ is so far not fully explored. Interestingly, its expression profile supports a possible role in myogenesis. Transcripts of p38γ are highly and specifically expressed in skeletal muscle, and its expression is induced during differentiation of C2C12 cells (Cuenda and Cohen, 1999; Lechner et al., 1996; Li et al., 1996; Tortorella et al., 2003). An early study of Lechner and colleagues suggested that overexpression of wild type p38γ enhanced, while an inactive mutant inhibited the differentiation of C2C12 cells (Lechner et al., 1996). This data was not confirmed by others and the development of active isoforms of p38 should provide the necessary tool to access the role of individual isoforms in this process.

The p38δ isoform is not likely to function in myogenesis, as it is differentially enriched in other non-muscle tissues (reviewed in Ono and Han, 2000).

2.3. Biological consequences of manipulating p38 activity in myoblasts

The activity of the p38 pathway was manipulated in cells either by ectopic expression of mutated MAP2K, MKK6, or by the addition of pharmacological inhibitors specific to p38. Treatment of several myogenic cell lines with the p38 inhibitor SB203580 prevented their differentiation both morphologically and biochemically. Treated myoblasts did not develop myotubes and the expression of a variety of muscle-specific genes including the cell cycle regulator $p21^{waf1}$, the myogenic regulatory factor (MRF), myogenin and structural genes like myosin heavy chain was reduced (Wu et al., 2000; Zetser et al., 1999). The expression of a constitutively active mutant of MKK6 (MKK6EE) stimulated the transcriptional activities of muscle transcription factors from the MyoD and MEF2 gene families and consequently the differentiation program. Constitutively active MKK6 could even drive the differentiation of myoblasts growing in high serum, suggesting that p38-activity could induce the withdrawal of myoblasts from the cell cycle in the presence of the mitogenic activity of serum proteins (Wu et al., 2000; Zetser et al., 1999). As mentioned above, p38 signaling induced the expression of the cyclin-dependent inhibitor $p21^{waf1}$ in myoblasts, supporting its role in regulating cell cycle withdrawal of myoblasts at the G1 stage, which is absolutely necessary for differentiation to occur. Additional strong supporting evidence to this view comes from a study that investigated the p38 pathway in rhabdomyosarcoma (RMS), a tumor originated from muscle precursors (Mauro et al., 2002; Puri et al., 2000).

RMS cells fail to differentiate in spite of the expression of MyoD, and previous studies suggested that this was due to a deficiency of a factor required for MyoD activity (Tapscott et al., 1993). The study of Puri and colleagues suggests that the missing factor is the activity of p38 (Puri et al., 2000). Sustained activity of p38 by the expression of activated MKK6 in RMS cells restored the activities of MyoD and the expression of $p21^{waf1}$ leading to growth arrest and terminal differentiation. The involvement of p38 in cell growth and its regulation of $p21^{waf1}$ were observed in other cellular systems (reviewed in Zarubin and Han, 2005).

MKK6EE can also function in shifting the expression of late-activated structural genes to earlier stages and inducing precocious differentiation (Penn et al., 2004; Wu et al., 2000).

It is worth mentioning that at least in one study, the addition of pharmacological inhibitors of p38 to primary limb bud cultures increased the expression of myogenic markers and the formation of myotubes (Weston et al., 2003). The results of this study disagree with those of the others presented above, and thus need further elucidation. One possible explanation is that the limb bud represents a different strategy of muscle specification influenced by local extrinsic factors specific to this environment only. A second possibility, suggested by the authors of the above study, is that p38 inhibitors affected post-differentiation events. According to their model, p38 may have a dual role in myogenesis; in early stages it induces differentiation, while in later stages its activity must be suppressed to allow the differentiated cells to elongate, polarize, aggregate and fuse.

Overall, changes in p38 activity affect the transcriptional pattern of muscle-specific genes, indicating that it may modulate transcription factors involved in the differentiation process.

2.4. Transcription factors affected by p38 during differentiation

The best studied transcription factors that are direct substrates of p38 belong to the MEF2 family. At least two members, MEF2A and MEF2C are directly phosphorylated by p38 at residues located in the transactivation domain of the proteins (Fig. 1) (Han et al., 1997; Zhao et al., 1999). Phosphorylation enhances MEF2-dependent gene expression, but the mechanism is still unknown. MEF2C proteins are phosphorylated in differentiating myoblasts in a p38-dependent manner, and this phosphorylation is necessary for their transcriptional activities (Wu et al., 2000; Zetser et al., 1999). Recently MEF2D was also demonstrated to be phosphorylated in a p38-dependent manner and to play a dominant role, together with MyoD, in the expression of a subset of MyoD-late induced genes (Penn et al., 2004). Inhibition of p38 activity in muscle cells reduced not only MEF2-phosphorylation but also its expression (Zetser et al., 1999). The reduced expression is probably due to the loss of MEF2 activity that induces its own transcription (Cripps et al., 2004) and/or the repression of other transcription factors such as MyoD.

Although MyoD can be directly phosphorylated in vitro by p38 at serine 5, this event does not affect its transcriptional activity (Wu et al., 2000). Still, the p38 pathway induced MyoD-dependent transcription indirectly. The physical interaction between MyoD and MEF2 family members on muscle-specific promoters may explain the indirect effect of p38 on MyoD (Molkentin et al., 1995). A second possible mechanism of indirect regulation of MyoD is through its heterodimerization with E47 (Lluis et al., 2005). Data of a recent study indicate that phosphorylation of E47 at serine 140 by p38 induces MyoD/E47 association and activation of muscle-specific transcription (Fig. 1). Since MyoD/E47 heterodimer is the functional unit in muscle transcription, the regulation of its formation by p38 is a major step in muscle-specific transcription. Interestingly, not all MRFs are positively regulated by p38. MRF4, which is involved in late stages of myogenesis is phosphorylated by p38 at two serine residues in the amino-terminal transactivation domain, resulting in its reduced transcriptional activity (Fig. 1) (Suelves et al., 2004). It was suggested that downreg-

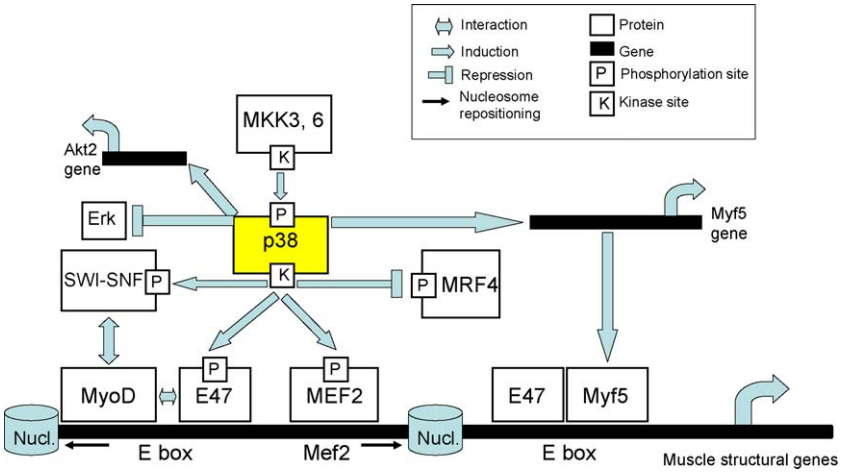

Fig. 1. A model for the activity of p38 in myogenesis. P38 is activated in muscle cells by its MAP2K, MKK3, 6 and phosphorylates a variety of substrates contributing to the myogenic process. It phosphorylates MEF2 proteins, inducing their transcriptional activity. It phosphorylates E47, promoting its heterodimerization with MyoD. It phosphorylates MRF4, inhibiting its transcriptional activity. It also induces the expression of Myf5, probably via the phosphorylation of an unknown mediator(s) affecting the transcription of Myf5. It affects chromatin remodeling at muscle-specific loci, by phosphorylating and recruiting the SWI–SNF complexes to muscle promoter regions. It can also affect other signaling pathways; it represses the activity of ERK indirectly and it induces transcription of the Akt2 gene.

ulation of MRF4 activity caused selective silencing of specific muscle genes in the terminal stage of muscle differentiation. The paradox of p38 inhibiting muscle transcription via MRF4 can be solved as MRF4 is predominantly expressed in fully differentiated skeletal muscle and is believed to maintain muscle-specific gene transcription in the adult tissue. Since p38 activity is undetectable in differentiated adult muscle cells, MRF4 should be fully functional in maintaining E-box-mediated gene transcription needed for the function and viability of these cells.

Nuclear factor kappa B (NFκB) is a transcription factor mainly active in cells of the immune system, but may function in many other lineages. The role of NFκB in muscle differentiation is controversial. Some studies suggest that its transcriptional activity is necessary for the differentiation process (Baeza-Raja and Munoz-Canoves, 2004; Conejo et al., 2001, 2002) while others claim that it is a negative regulator of myogenesis, and that chronic activation of NFκB is associated with muscle wasting (Reviewed by Guttridge, 2004). Studies supporting a promotion of differentiation function of NFκB suggest that activation of its transcriptional activity is mediated by p38 (Baeza-Raja and Munoz-Canoves, 2004; Conejo et al., 2001). Active NFκB induced the expression of interleukin 6 (IL6) which by itself is able to increase myogenic differentiation. Hence, a promyogenic signaling pathway involving p38-NFκB-IL6 was suggested (Baeza-Raja and Munoz-Canoves, 2004).

2.5. p38 functions on a subset of muscle-specific promoters to regulate their timely expression

Based on the effect of p38 signaling on master regulatory transcription factors, it was assumed in the early studies that the whole myogenic program was affected. However, recent studies indicate that p38 may play a more complex role in the timely expression of a subset of muscle-specific genes. The first indication of p38 activity on a subset of genes regulated by MyoD was provided by a study that examined the pattern of genes regulated during MyoD-induced myogenesis (Bergstrom et al., 2002). MyoD was found to orchestrate multiple subprograms of gene expression. The induction of MyoD in the presence of SB 203580 prevented the expression of a small subset of MyoD-target genes predominantly composed of late muscle structural genes but not of the majority of genes expressed during myogenesis. Interestingly, the binding of MyoD to the promoters of SB 203580-inhibited genes was not affected. Also, histone acetylation at the promoter region was relatively unaffected, suggesting that p38 was acting on parallel or downstream transcriptional events. Further analysis of the mechanism of p38-mediated muscle transcription demonstrated that inhibition of p38 did not affect the recruitment of acetyltransferases p300 and PCAF to p38-affected promoters but prevented the engagement of BRG1 and BRM, subunits of the chromatin remodeling SWI–SNF complex, to the same promoters (Simone et al., 2004). Consequently, chromatin remodeling allowing the access of transcription factors to these promoters was prevented. P38 was demonstrated to phosphorylate the SWI–SNF subunit BAF60 in vitro, and to recruit SWI–SNF complexes to the affected promoters, probably through their interactions with MyoD (Fig. 1) (de la Serna et al., 2001, 2005). Therefore, this study establishes a link between p38 and chromatin remodeling at muscle-specific promoters. Nevertheless, the selective activity of p38 at a subset of promoters is not explained by this study. A recent study by Penn and colleagues analyzed the effect of precociously elevated p38 activity, by ectopically expressing activated MKK6 on the expression profile of muscle-specific genes (Penn et al., 2004). Microarray analysis indicated that the p38-affected genes were normally expressed in the late stages of differentiation. Precocious activation of p38 resulted in earlier expression of a number of late genes like Desmin and Myosin Light Chain. Ectopic expression of MEF2D, a substrate of p38, in addition to that of MKK6 resulted in still earlier expression of the late genes. Chromatin immunoprecipitation assays (ChIPs) demonstrated that p38 activation shifted the binding of MEF2 isoforms and the MyoD protein to an earlier period of differentiation at the relevant promoters. This data indicates that p38 activity regulated the formation of a MyoD–MEF2 complex at a set of late promoters. The expression of the MEF2D isoform was rate limiting, and its binding to the subset of late promoters correlated well with their expression. Indeed, only the combinatorial binding of MyoD and MEF2D and the activity of p38 could cause the early recruitment of polymerase II and the formation of an active transcription complex at these promoters. These findings have led to the suggestion of a feed-forward model for explaining the temporal expression of genes during the differentiation process. According to the model, a single transcription factor, MyoD initiates the whole myogenic program. It induces the expression of "early genes" like myogenin and MEF2 isoforms, including MEF2D and the activity of the p38 pathway via an unknown mechanism. The phosphorylated p38 becomes an active kinase and phosphorylates MEF2D, permitting it to bind to promoters of late-transcribed genes and activate their transcription together with MyoD. Transcription of "late genes" is not activated by MyoD until MEF2D is expressed and p38 is active. Therefore, an initial single event of MyoD activation can induce more events necessary for the subsequent activation of later genes in a cascade-type mechanism. The feed-forward mechanism imposes temporal order of MyoD-mediated gene expression. This model is discussed in greater detail in a recently published review (Tapscott, 2005).

3. Relationship of the p38 pathway with other signaling pathways in myogenesis

In addition to p38, several other signaling pathways are involved in myogenesis, and an interesting question is the relationship of these pathways to the p38 pathway. A major pathway essential for differentiation as well as survival of myotubes is the phosphoinositide 3-kinase (PI3-K) which affects downstream targets such as Akt and mTOR/p70S6 kinase. Akt is phosphorylated by similar kinetics to p38, raising the question whether these pathways crosstalk with one another or constitute two separate pathways. There is controversy over this matter in the literature. Some studies show that PI3-K and p38 are activated by two separated pathways during myogenic differentiation; both are required for muscle differen-

tiation. Inhibition of PI3-K with the pharmacological inhibitor LY294002 did not affect the phosphorylation and activity of p38 (Li et al., 2000; Sarker and Lee, 2004; Tamir and Bengal, 2000). Though these studies suggest that the two pathways are parallel, they may either affect different downstream targets or may converge on shared targets that require input from both signaling pathways. One common target of the two pathways is MEF2 that can be phosphorylated and activated by these two distinct pathways (Tamir and Bengal, 2000). Other studies support crosstalk between the pathways. One study that used pharmacological inhibitors of p38 and of mTOR suggested that inhibition of any of these kinases suppresses the activity of the other. Therefore it was suggested that a positive feedback loop exists between these two pathways that drives differentiation to completion (Conejo et al., 2002; Cuenda and Cohen, 1999). A mechanism of the crosstalk between p38 and Akt was suggested in studies that demonstrated that Akt acts downstream of p38 in myogenic differentiation (Cabane et al., 2004; Gonzalez et al., 2004). P38 affected the expression of Akt2 (and not of Akt1) and increased the phosphorylation at serine 473 of Akt2 in a PI3-kinase-dependent manner (Gonzalez et al., 2004). Akt2 promoter activity and protein levels were induced by the activation of p38 signaling, thus providing the mechanism for this crosstalk (Fig. 1). Although in agreement about the function of Akt downstream of p38, these two studies disagree about the reciprocal relationships; one argues that Akt does not affect p38 (Cabane et al., 2004) while the other claims that PI3-kinase regulates p38 activity upstream of MKK6 (Gonzalez et al., 2004). The excessive usage of pharmacological inhibitors at concentrations that could inhibit the activity of kinases in a non-specific manner could be a possible explanation for the conflicting results (Lali et al., 2000). Further studies should clarify the confusing results emerging from the current literature about the relationships between these two myogenic-promoting signaling pathways.

Another interesting relationship between the p38 and the ERK MAPK pathways that could explain the function of p38 in cell cycle withdrawal was suggested. Inhibition of ERK activity enhanced the activity of p38 and inhibition of p38 enhanced ERK activity (Fig. 1) (Khurana and Dey, 2002). This interaction between p38 and ERK MAPK activities could explain the results of a previous study describing the role of p38 in the induction of growth arrest and myogenic differentiation in RMS cells (Puri et al., 2000). Treatment of RMS cells with an inhibitor of the ERK pathway, U0126, induced the expression of myosin heavy chain that was prevented if cells were also treated with the inhibitor of p38, SB203580 (Mauro et al., 2002). Our recent studies indicate that inhibition of ERK activity in RMS cells markedly induced the activity of p38, while constitutive activation of p38 reduced ERK activity (Y.T and E.B, unpublished results). Together, these observations suggest that the effects of p38 on RMS cells can be separated into two: indirect and direct. The indirect effect is mediated by the reduction in ERK activity allowing the withdrawal from the cell cycle. The direct effect is mediated by the activity of p38 participating in the expression of both cell cycle and muscle-specific genes. Thus, the overt effect of p38 in restoring normal differentiation to RMS tumor cells is a consequence of the complex crosstalk with other signaling pathways such as the ERK MAPK.

4. Animal model systems for studying the involvement of p38 in myogenesis

The studies of p38 function in myogenic cell lines are limited to specific stages of myogenesis, and are isolated from the real developmental context. Targeted inactivation of the mouse p38α gene cause a placental defect that could be repaired, and then embryos developed relatively normally (Adams et al., 2000; Tamura et al., 2000). The lack of any severe developmental phenotype in the mouse might be attributed to the redundant function of other isoforms of p38, such as p38β. Thus, double and/or triple knockout mice strains might be needed in order to analyze the function of p38. A simple way to avoid this problem was recently presented in a study that used to inhibit p38 with the pharmacological inhibitor, SB 203580 in somite explants and whole mouse embryos (de Angelis et al., 2005). Using a MEF2 transgenic "sensor" mouse containing a MEF2-driven reporter gene as an indicator for MEF2 activity, they found that inhibition of p38 signaling blocked MEF2 activity in the somites. Loss of MEF2 activity was probably the reason for the concurrent loss of myosin light chain 3F promoter activity representing myotomal myogenesis. Although the differentiation process was affected, the commitment to the myogenic lineage was not affected since the expression of the Myf5 locus was not changed by p38 inhibition. Overall, this study confirmed the function of p38 known from tissue culture studies as a positive regulator of MEF2 and muscle differentiation.

A second recently published study analyzing the effect of p38 on early development of the frog *Xenopus laevis* identified distinct myogenic phenotypes (Keren et al., 2005). In this study, different approaches were used to inhibit p38α in the developing *Xenopus* embryo, including injection of antisense morpholino to p38, or kinase inactive MKK6, and treatment of mesodermal explants with SB 203580. Interference with the p38 pathway specifically prevented the expression of XMyf5, but not of XMyoD. Consequently, several defects in muscle development were observed. At gastrula–neurula stages, convergent extension movements were prevented and segmentation of the paraxial mesoderm was delayed, probably due to the failure of cells to withdraw from the cell cycle. Expression of a subset of muscle structural genes was reduced. At later stages, there was a general deterioration of somites borders as a result of myotube degeneration and massive apoptosis in most parts of the trunk somites. Interestingly, morpholino-mediated knockdown of XMyf5 and rescue experiments indicated that the developmental defects caused by p38-knockdown were mediated by the loss of XMyf5 expression. Therefore this study identified multiple defects in myogenesis probably caused by the inhibited expression of XMyf5 at crucial stages of muscle development. It also delineates a specific intracellular pathway involving p38 and XMyf5 proteins (Fig. 1). However the study raises some questions because of the different results when compared with other experimental systems. Obviously, a developing embryo is a very different experimental system than tissue culture cells.

First, studies of tissue culture could investigate the effect of the p38 pathway on the differentiation stage only, whereas animal studies investigated several developmental stages including the stage of commitment of mesoderm cells to the myogenic lineage. In the *Xenopus* study, the elimination of XMyf5 expression in p38-knockdown embryos hindered the study of downstream events such as the differentiation stage investigated in cell culture studies. Although p38 regulated XMyf5 expression it is still possible that it also modified the transcriptional activities of other MRFs and MEF2 proteins. The differences between the two animal studies could be attributed to the different embryological systems as well as the different methodologies used. The mouse work used the pharmacological inhibitor SB203580 as the sole means to inhibit p38 in explants and whole embryos, whereas the *Xenopus* work used several approaches to inhibit p38 activity. Major disadvantages of pharmacological inhibitors are their toxicity and limited penetration, especially when working with three dimensional explants and whole embryos. Thus, one cannot deny that limited tissue penetration explains only partial inhibition and some of the effects that could be detected by other methods to inhibit p38 could be missed. A second major difference between mouse and *Xenopus* is the biology of muscle formation. The initiation of Myf5 expression occurs at very different developmental stages in mouse and *Xenopus*. In *Xenopus* the expression of XMyf5 occurs in early gastrula stages in the presomitic mesoderm whereas in mouse Myf5 expression occurs much later in the myotome compartment of the somite. Thus, differences in temporal Myf5 expression could reflect changes in Myf5 transcriptional regulation between the two systems. Future studies should uncover such differences, if they exist.

5. Perspectives

In recent years the main focus has been given to the identification of signaling molecules inducing myogenesis of somitic mesodermal cells. Secreted molecules such as Wnts, Sonic hedgehog, BMP4 and noggin promote somite myogenesis (reviewed in Cossu et al., 2000). Still the linkage of extracellular signals to the transcriptional control of myogenesis is largely missing. Thus, the study of intracellular signaling pathways affecting myogenesis is a major step in understanding the full program of cell specification in the developing somite. An interesting direction for future studies is the identification of the specific extracellular molecules inducing the p38 pathway. Typically p38 is activated in response to various physical and chemical stresses and by various cytokines. According to some studies, a typical stress-mediated activation of p38 does not induce muscle differentiation (Puri et al., 2000; Wu et al., 2000). Therefore, one should investigate receptor-mediated signals commonly functioning during development that may affect p38. For example, Wnts, known to activate intracellular canonical and non-canonical pathways could affect the p38 pathway. A recent study suggested that the receptor for advanced glycation end products (RAGE) of the immunoglobulin superfamily is involved in the stimulation of myogenic differentiation in a p38-dependent manner (Sorci et al., 2004). An efficient approach for searching ligands and receptors of the p38 might be by using existing data of early induced MyoD-responsive genes (Bergstrom et al., 2002). According to the feed-forward model (Penn et al., 2004) MyoD is expected to induce the expression of a mediator(s) of p38 activity, if it indeed enables the activation of this pathway during myogenesis.

Future studies should aim at explaining the transcriptional specificity of p38 in the activation of a subset of muscle genes. Both tissue culture (Bergstrom et al., 2002; Penn et al., 2004) and animal (Keren et al., 2005) studies identify a subgroup of "late-expressed" muscle genes that are affected by p38. Is p38 selectively brought to certain promoters to remodel chromatin at these promoters only? Can the selective regulation of muscle determination genes (Myf5) and not of others (MyoD) explain the control of specific muscle-programs by p38? How does p38 regulate the expression of Myf5? Does p38 regulate the withdrawal of muscle precursors from the cell cycle and how?

Though much is yet to be studied, a more complete picture of p38 involvement in myogenesis has been emerging. This pathway has evolved to serve the complex and temporal expression pattern of muscle-specific genes in myogenesis. According to current data, p38 seems to be involved in several aspects of the muscle transcriptional machinery (Fig. 1). It can affect the expression of determination genes like Myf5 and the activities of transcription factors from the MEF2 and MyoD families. It can either regulate positively MEF2 family members and E47–MyoD heterodimers, or negatively the MRF4 by direct phosphorylation. P38 is recruited to muscle loci possibly by interacting with some of its target myogenic transcription factors and locally remodel promoter regions through the interaction with the SWI–SNF complex. The open chromatin structure at these promoters allows binding of more myogenic transcription factors, their activation by p38 and the active transcription by polymerase II.

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