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Myocardin/MKL Family of SRF Coactivators: Key Regulators of Immediate Early and Muscle Specific Gene Expression

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Abstract Myocardin, megakaryoblastic leukemia-1 (MKL1), and MKL2 belong to a newly defined family of transcriptional coactivators. All three family members bind to serum response factor (SRF) and strongly activate transcription from promoters with SRF binding sites. SRF is required for the serum induction of immediate early genes such as *c-fos* and for the expression of many muscle specific genes. Consistent with a role in muscle specific gene expression, myocardin is specifically expressed in cardiac and smooth muscle cells while MKL1 and 2 are broadly expressed. Myocardin has particularly been shown to be required for smooth muscle development while MKL1/2 are required for the RhoA signaling pathway for induction of immediate early genes. SRF can be activated by at least two families of coactivators, p62TCF and myocardin/MKL. These factors bind to the same region of SRF such that their binding is mutually exclusive. This provides one mechanism of regulation of SRF target genes by pathways that differentially activate the coactivators. The RhoA pathway appears to activate MKL1 by altering MKL1's binding to actin and causing MKL1's translocation from the cytoplasm to the nucleus. However, this mechanism of activation of the myocardin/MKL family has not been observed in all cell types such that other regulatory mechanism(s) likely exist. In particular, rapid serum inducible phosphorylation of MKL1 was observed. The regulation of this coactivator family is key to understanding how SRF target genes are activated during muscle cell differentiation or growth factor induced cell proliferation. J. Cell. Biochem. 93: 74–82, 2004.

Key words: megakaryoblastic leukemia; myocardin; serum response factor; MKL; MAL; immediate early genes

Serum response factor (SRF) regulates the serum and growth factor induction of many cellular immediate early genes through serum response elements (SREs) [Johansen and Prywes, 1995]. The SRE is essentially equivalent to a CArG box, a regulatory element identified in many muscle specific genes that are also regulated by SRF [Miano, 2003]. One mechanism for activation of SREs is by the binding and activation of a cofactor, p62TCF, which binds to SRF and to a short sequence element on the 5′ flank of the c-fos SRE. p62TCF, encoded by the three ets-related factors Elk1, SAP1, and SAP2, is activated by MAP kinase phosphorylation of its transcriptional activation domain [Treisman, 1994]. However, binding of p62TCF can be blocked without much effect on serum induction of SRE reporter genes [Johansen and Prywes, 1995]. These results suggested that SRF can be activated by a second, TCF-independent pathway. This pathway was identified as containing RhoA since inhibition of RhoA blocked serum induction of the SRE and constitutively activated RhoA could also activate SRE reporter genes [Hill et al., 1995]. How SRF was directly regulated by this pathway remained elusive since SRF was constitutively nuclear, bound to the SRE and no modification of SRF was shown to be required for its regulation [Johansen and Prywes, 1995]. It has also been unclear how a factor involved in growth regulated gene expression could also be involved in differentiation-specific (non-proliferative) regulation of muscle genes.

The above results suggested that there must be cofactor(s) of SRF that regulate its activity in

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different cell states or tissues. While a number of SRF cofactors were identified, only p62TCF has held up as a strong SRF coactivator with most being only partially required for SRF activity [Cen et al., 2003; Majesky, 2003]. The break in this field came with the identification of myocardin from an in silico screen for cardiac specific genes [Wang et al., 2001]. Myocardin was then found to very strongly activate CArG box reporter genes and to stably bind SRF. In addition to cardiac muscle cells, myocardin was also found to be expressed in smooth muscle cells [Wang et al., 2001; Chen et al., 2002; Du et al., 2003; Wang et al., 2003; Yoshida et al., 2003].

Since myocardin is not expressed in most cell types it could not serve to regulate immediate early gene expression, which is relatively ubiquitous. About the same time as the identification of myocardin, a similar gene was identified independently by two groups at a recurrent translocation exclusively associated with childhood acute megakaryoblastic leukemia (AML) [Ma et al., 2001; Mercher et al., 2001]. MKL1, also termed MAL, MRTF-A and BSAC was found at a t(1;22) translocation fused with the RNA-binding motif protein 15 (RBM15) gene (a.k.a. OTT) [Ma et al., 2001; Mercher et al., 2001; Sasazuki et al., 2002; Wang et al., 2002]. The resulting RBM15-MKL1 fusion protein is believed to possess oncogenic properties [Ma et al., 2001; Mercher et al., 2001]. Several groups subsequently reported the independent cloning of human and mouse MKL1 cDNA and showed that it is similar to myocardin in its ability to strongly activate SRE reporter genes and to bind stably to SRF [Sasazuki et al., 2002; Wang et al., 2002; Cen et al., 2003]. A third member of the family, MKL2 or MRTF-B, that is also broadly expressed was later identified and shown to strongly activate SREs and to bind to SRF [Wang et al., 2002; Selvaraj and Prywes, 2003].

FUNCTIONAL DOMAINS

These three proteins share an overall similarity of 35%, however, they are much more strongly conserved in several conserved domains (Fig. 1). The basic and glutamine (Q)-rich domains were found to be required for myocardin binding to SRF while only the basic region was required for MKL1 binding [Wang et al., 2001; Cen et al., 2003]. A leucine zipper like domain can mediate homo- and heterodimerization of the family members. Deletion of this domain had a modest effect on MKL1/2 activation of SRE reporter genes but a larger effect on activation by myocardin [Cen et al., 2003; Selvaraj and Prywes, 2003; Wang et al., 2003]. One of the notable features of the MKL/Myocardin family is that they all contain a SAP domain, which is a conserved 35-amino acid motif that includes two amphipathic α-helices. SAP domains are found in a variety of nuclear proteins including SAF-A and -B, Acinus, and

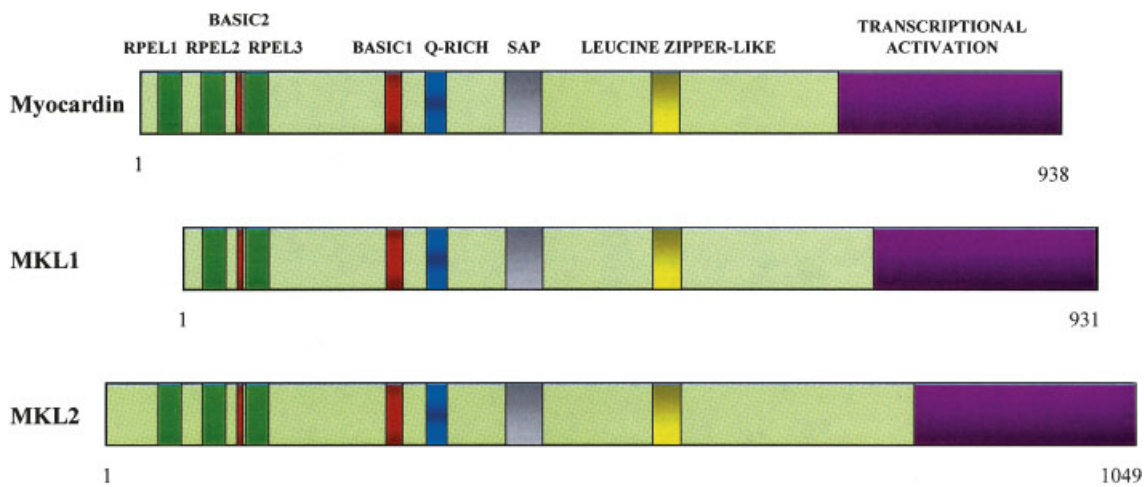

Fig. 1. Conserved domains of myocardin, megakaryoblastic leukemia-1 (MKL1) and megakaryoblastic leukemia-2 (MKL2). The positions of conserved domain discussed in the text are indicated. The GenBank accession numbers for these genes are: NM_153604, NM_020831, and AY374297. Different N-terminal regions of MKL1 have been reported, in particular one form contains three RPEL motifs [Miralles et al., 2003]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PIAS [Kipp et al., 2000]. The SAP domains of SAF-A and PIAS interact with a nuclear matrix attachment region, which has been proposed to partition DNA into independent chromatin regions [Kipp et al., 2000; Sachdev et al., 2001]. Deletion of the SAP domain of MKL1 had no effect on MKL1's transcriptional activity and MKL1–SRF complex formation [Cen et al., 2003; Miralles et al., 2003]. Point mutation of the SAP domain of myocardin also did not affect myocardin–SRF complex formation. Interestingly, the SAP domain was required for activation of some muscle-specific reporter genes but not others, suggesting that it might specifically couple myocardin to elements in some promoters [Wang et al., 2001]. Nevertheless, the myocardin/MKL SAP domain is not absolutely required for transcriptional activation and there is no evidence yet that it functions through nuclear matrix attachment.

The myocardin/MKL proteins contain strong transcriptional activation domains in their C-terminal regions when fused to GAL4's DNA binding domain further demonstrating that they are transcriptional activators. Deletion of N-terminal regions of myocardin/MKL led to much higher activation by the GAL4 fusion proteins suggesting that these regions may regulate transcriptional activation [Wang et al., 2001; Cen et al., 2003; Selvaraj and Prywes, 2003].

A particularly strongly conserved region of this family is the N-terminal MKL homology domain. This region of about 120 amino acids contains two or three RPEL motifs depending upon the isoform of the protein that has been cloned [Miralles et al., 2003]. The RPEL repeat is named after four of its conserved amino acids. The function of the RPEL repeats is relatively unknown. They are not required for transcriptional activation of SRF targets, however, the RPEL motifs in MKL1 were required for retention of the protein in the cytoplasm. As discussed later, the RPEL motifs are also critical for actin binding to MKL1 suggesting that this association sequesters MKL1 in the cytoplasm until it is activated [Miralles et al., 2003].

Lastly, nuclear localization of MKL1 can be mediated by two basic regions. The first (Basic 1) described above is also required for SRF binding in vitro. The second (Basic 2) is located between RPEL motifs 2 and 3. Removal of both is required to block nuclear accumulation of MKL1 [Miralles et al., 2003]. MKL1 may also contain nuclear export signals that have not been clearly defined. Thus it is the balance of cytoplasmic retention with nuclear import and export that control MKL1 localization.

REGULATION BY THE RhoA PATHWAY

Activation of the c-fos SRE by SRF and p62TCF is fairly well understood as it involves MAPK phosphorylation of p62TCF and activation of its transcriptional activation domain [Treisman, 1994]. The second, TCF-independent pathway is less well understood but has been shown to require the small GTPase RhoA and changes in actin filaments [Hill et al., 1995; Sotiropoulos et al., 1999]. It was unclear however, how these or other possible TCF-independent pathways would directly affect SRF. The relatively ubiquitously expressed members of the myocardin/MKL family have filled this hole (Fig. 2). Dominant negative MKL1 blocked SRE reporter gene activation by serum, activated RhoA and activated mDia, an effector of RhoA [Cen et al., 2003; Miralles et al., 2003]. Serum and RhoA activation of the reporters was also inhibited by RNA interference of endogenous MKL1 and MKL2 [Cen et al., 2003]. Complete inhibition required knockdown of both MKL1 and MKL2 suggesting that they have redundant functions. Dominant negative MKL1 also blocked serum induction of endogenous SRF target genes, especially those genes without apparent TCF binding sites, such as SRF and vinculin, whose induction is dependent upon the Rho pathway [Sotiropoulos et al., 1999; Gineitis and Treisman, 2001; Cen et al., 2003]. Chromatin immunoprecipitations showed that MKL1 and SRF bind to the SRF and vinculin promoters in vivo, further establishing MKL1 as a critical SRF coactivator [Miralles et al., 2003].

Activated RhoA causes the formation of actin stress fibers suggesting that this function might also regulate SRF and MKL1. In favor of this model inhibitors of actin filament assembly, such as latrunculin B, block SRE activation [Sotiropoulos et al., 1999]. In addition, downstream effectors of RhoA that affect stress fiber formation, the protein kinase ROCK and mDia, are also required for SRE activation in some cell types and activated mDia activates both actin filament formation and SRE reporter genes [Tominaga et al., 2000; Geneste et al., 2002]. An argument against actin filament formation

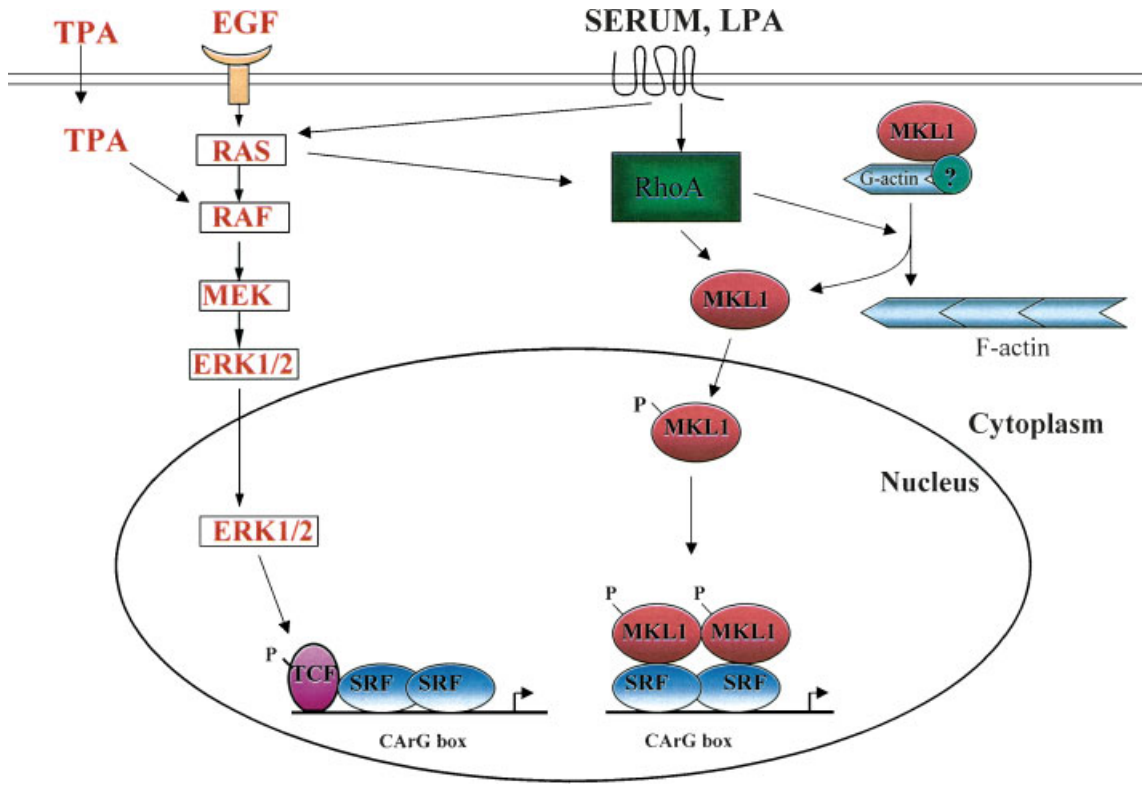

Fig. 2. Model for growth factor and serum-induced serum response factor (SRF) activation pathways. SRF bound to CArG boxes (SREs) can be activated by either p62TCF or MKL1/2, however, their binding to SRF is mutually exclusive. p62TCF is activated by ERK1/2 and other MAPK phosphorylation. RhoA is required for TCF-independent activation by serum and lysophosphatidic acid (LPA) which activate G protein coupled receptors. RhoA activates stress fibers. This results in the depletion of G-actin from the cell into F-actin fibers, release of MKL1 from a G-actin complex and translocation of MKL1 to the nucleus. MKL1 may also be activated by other RhoA-induced pathways and inducible phosphorylation of MKL1 has been observed suggesting that RhoA induction of an MKL1-kinase may be a critical regulatory step. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

regulating SRF is first that inhibiting actin filaments is a large morphological and structural change for the cell such that there may be many indirect effects. Second, mutants in RhoA were made to distinguish effector pathways. Two groups identified mutants that either activated stress fibers, but not SREs, or activated SREs, but not stress fibers [Sahai et al., 1998; Zohar et al., 1998]. We have also identified novel RhoA mutants that cause stress fiber formation but poorly activate SREs (unpublished results). These results strongly suggest that other pathways must exist downstream of RhoA for activation of SRF independent of stress fibers. Indeed, a recent report has identified CNK1 as a RhoA effector that is at least partially required for RhoA activation of SRF, but not stress fibers [Jaffe et al., 2004]. CNK1 was previously identified as a modifier of ras and ksr signaling in *Drosophila* [Therrien et al., 1998]. It is thought to act as a scaffold protein for signaling components, but it is unknown how it might affect SRF. While there is clearly a connection between the cytoskeleton and SRF activation, the above results suggest that there are alternative or interconnected pathways for serum and/or RhoA activation of the SRE (Fig. 2).

The overexpression of actin in cells causes the inhibition of serum induced SRF activation. This led to the model that free G-actin might be the functional molecule for SRF regulation by inhibiting a pathway component [Sotiropoulos et al., 1999]. This model was bolstered by the analysis of actin mutants [Posern et al., 2002]. Mutants that cannot form filaments were still able to inhibit SRE activation. In contrast, actin mutants that constitutively formed into F-actin fibers, without affecting G-actin levels, caused constitutive activation of SREs. This latter result suggests that F-actin can in fact signal to SRF, as opposed to free G-actin, however, it is also possible that the ratio of F- to G-actin is sensed by the cell or

that the mutations in actin create an activating form of actin monomer.

CELLULAR LOCALIZATION OF MKL1

The RhoA-actin pathway was directly connected to MKL1 regulation by the coimmunoprecipitation of a complex of MKL1 and actin [Miralles et al., 2003]. At the same time it was found that serum caused translocation of MKL1 from the cytoplasm to the nucleus in NIH3T3 cells. Serum-induced MKL1 translocation was dependent upon the RhoA-actin pathway [Miralles et al., 2003]. These results suggest that actin binding retains MKL1 in the cytoplasm and that depletion of G-actin into stress fibers allows MKL1 to translocate to the nucleus and activate SREs (Fig. 2). Supporting this model, actin binding was mapped to the RPEL motifs at the N-terminus of MKL1 and deletion of these motifs led to nuclear localization of the protein. In vitro binding of actin and MKL1 could not be detected such that a yet to be identified cofactor is likely required. It is interesting to speculate that one of the cells many actin binding proteins might serve as an adaptor of MKL1 to actin and provide a connection to other signaling factors bound to the cytoskeleton.

We also found that MKL1 is predominantly cytoplasmic in serum starved NIH3T3 and HeLa cells, but we have not been able to detect the movement of endogenous MKL1 to the nucleus by fractionation of cell extracts [Selvaraj and Prywes, 2003]. We and others have detected serum-induced movement of transfected MKL1 from the cytoplasm to the nucleus in NIH3T3 cells (unpublished results) [Du et al., 2004], but we did not observe this movement in HeLa cells. Transfected MKL1 was also found to be constitutively nuclear in smooth muscle cells [Du et al., 2004]. These differences may reflect the precise cell lines used or the levels of expression of MKL1. Myocardin was also found to be nuclear localized suggesting that this family member is not regulated by changes in localization [Wang et al., 2001]. We found that moderate overexpression of MKL1 in stably transfected cells resulted in the protein being constitutively expressed in the nucleus. This did not result in the constitutive activation of SRE reporter genes suggesting that additional activation steps must be required (unpublished results).

PHOSPHORYLATION

One potential mechanism for MKL1 regulation is by its phosphorylation. Serum induces a shift in MKL1 mobility in SDS–PAGE that was sensitive to phosphatase treatment and we have observed induced ^{32}P-labelling of MKL1 [Miralles et al., 2003] (unpublished results). This induced phosphorylation is rapid, correlating well with target gene induction. Inhibition of RhoA or MEK1 (part of the ERK1/2 MAPK pathway) partially blocked phosphorylation suggesting that these signaling pathways are involved. Deletion mapping suggests that the site(s) of phosphorylation are in the C-terminal region. While this region is not required for nuclear localization it is still possible that phosphorylation at a specific site could affect localization as well as activity. The identification of the phosphorylation sites is necessary to determine whether this modification is required to regulate MKL1 activity. If so, the identification of MKL1 protein kinases and phosphatases will add another step to the SRF signaling pathway.

SMOOTH AND CARDIAC MUSCLE DIFFERENTIATION

The role of myocardin in cardiac muscle differentiation was first demonstrated in *Xenopus* embryos by injection of a dominant negative form of myocardin that blocked heart formation and the expression of cardiac muscle genes [Wang et al., 2001]. Myocardin is expressed in smooth muscle cells as well as cardiac tissue and myocardin knock-out mice died by embryonic day 10.5 with a lack of vascular smooth muscle cells, although heart development appeared normal [Li et al., 2003]. Since MKL1 and MKL2 expression was detected in the heart [Wang et al., 2002; Selvaraj and Prywes, 2003; Du et al., 2004], the redundancy of these factors may explain the normal heart development while myocardin is specifically required in the vascular smooth muscle cells. While MKL1 and MKL2 are expressed in smooth muscle cells [Wang et al., 2002; Du et al., 2004], it is unclear whether they are expressed in these specific vascular smooth muscle cells or whether there is a myocardin-specific activity essential for the development of these cells. MKL2 knockout mice were previously generated in a gene trap experiment. These mice died at birth demonstrating the requirement for this coactivator but

the exact cause of death is not known [Skarnes et al., 1992]. Results with MKL1 knockout or double knockout mice have not yet been reported.

Overexpression of either myocardin or MKL1 can activate smooth muscle specific genes in several non-muscle cell lines [Chen et al., 2002; Cen et al., 2003; Wang et al., 2003; Du et al., 2004]. These results suggest that MKL1 may also regulate smooth muscle genes in some cell types.

SKELETAL MUSCLE DIFFERENTIATION

Since MKL1 and 2 were expressed at elevated levels in skeletal muscle tissue, we sought to elucidate the role of these factors in skeletal muscle differentiation using the C2C12 skeletal myoblast cell line. Dominant negative MKL2 protein blocked the differentiation of skeletal myoblasts to myotubes and also blocked the expression of endogenous markers of skeletal muscle differentiation such as skeletal α-actin and skeletal α-myosin heavy chain [Selvaraj and Prywes, 2003]. MKL1 and MKL2 are both expressed in these cells, but not myocardin, implicating MKL1 and/or MKL2 in skeletal muscle differentiation. Consistent with the fact that SRF is required to maintain the rate of cell proliferation of skeletal myoblasts [Gauthier-Rouviere et al., 1996], dominant negative MKL2 also reduced the growth rate of these cells [Selvaraj and Prywes, 2003]. These results show that the MKL family members are required for optimal cell growth and for myogenic differentiation of the C2C12 skeletal myoblasts.

It is odd that SRF and the myocardin/MKL family are required for both growth-induced and differentiation specific gene expression (Fig. 3). The activation of these antagonistic gene expression programs could be explained by differential activation of the myocardin/MKL proteins or due to the association of other factors at the target gene promoters. While MKL1 is activated by cellular localization and/or phosphorylation, as discussed above, it is unclear whether it is regulated during muscle cell differentiation. Myocardin and MKL1 were found in the nucleus of COS and smooth muscle cells, respectively [Wang et al., 2001; Du et al., 2004]. We found that MKL1 was predominantly cytoplasmic in C2C12 skeletal myoblasts but there was no significant shift in distribution to the nucleus upon differentiation [Selvaraj and Prywes, 2003]. Inhibition of actin polymerization in smooth muscle cells caused MKL1 to translocate to the cytoplasm suggesting that signaling pathways that had been previously activated may control MKL1 localization [Du et al., 2004]. It will be interesting to determine whether myocardin and/or MKL1/2 are activated during muscle differentiation and, if so, at what stage this occurs.

The process of skeletal muscle determination is dependent on a family of muscle-regulatory factors (MRFs) that belong to the basic helix-

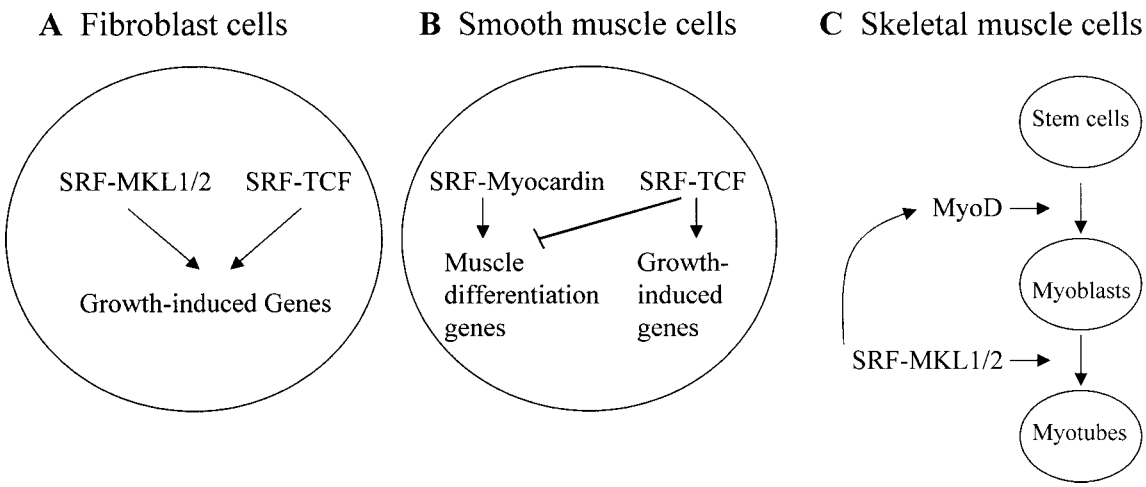

Fig. 3. Role of SRF coactivators in proliferation and differentiation. Both MKL1/2 and TCF are involved in growth induced gene expression in fibroblasts (**A**), but TCF activation inhibits target gene activation in smooth muscle cells by blocking activation by myocardin (**B**). In skeletal muscle cells MKL1/2 is required for the differentiation step from myoblasts to myotubes (**C**). SRF is also required for MyoD expression which occurs earlier in a differentiation pathway and we speculate that MKL1 and 2 are also involved in promoting MyoD expression.

loop-helix protein family and include MyoD and Myf5 (Fig. 3C) [Rudnicki and Jaenisch, 1995]. MyoD is expressed constitutively in both proliferating myoblasts and myotubes but not in non-muscle cells. MyoD expression in myoblasts and myotubes is dependent on the RhoA-SRF pathway and recently a CArG box has been identified in the distal promoter region of MyoD [Gauthier-Rouviere et al., 1996; Carnac et al., 1998; L'Honore et al., 2003]. Since the RhoA-SRF pathway has been implicated in the regulation of MyoD expression, it is possible that the MKL family members also have a role in early myogenesis by regulating MyoD expression (Fig. 3C).

COMPETITION BETWEEN MYOCARDIN/MKL AND TCF

One possible mechanism for SRF and myocardin/MKL activating muscle specific differentiation genes versus growth inducible gene targets is by the presence or activation of a TCF binding site flanking the SRE. Some SRF target genes such as *c-fos* contain TCF sites while others, such as vinculin, do not have an identifiable TCF site. The redundancy of the TCF and myocardin/MKL pathways and the presence or absence of a TCF site can explain why dominant negative MKL1 only slightly affects serum induction of c-fos, but strongly inhibits vinculin induction [Cen et al., 2003]. Mutation of the TCF site in SRE reporter genes also results in much higher activation by myocardin and MKL1 suggesting that TCF binding might block activation by these coactivators [Wang et al., 2004] (unpublished results). This would fit well with previous results that RhoA activation of SREs is increased by TCF site mutation of SRE reporters [Wang et al., 1998].

The binding of TCF and myocardin/MKL1 to SRF is in fact mutually exclusive [Wang et al., 2004]. The B box of Elk-1 (one member of the TCF family) competes for MKL1 binding to SRF, suggesting that they bind to the same region on SRF [Miralles et al., 2003]. Since TCF is activated by MAP kinase phosphorylation, this would allow growth signals to prefer TCF while differentiation signals could prefer myocardin or MKL1. In smooth muscle cells the smooth muscle cell specific *SM22* gene is regulated by a CArG box with a flanking TCF site. PDGF induction induces smooth muscle cell growth and blocks differentiation. PDGF also activated TCF and blocked myocardin binding to the SM22 promoter [Wang et al., 2004]. In addition, serum induction inhibited myocardin activation of the SM22 promoter in a TCF site dependent manner. These results suggest that growth signals that induce MAP kinase phosphorylation of TCF block the myocardin pathway and that myocardin is involved in the differentiation program of smooth muscle cells. Phosphorylated TCF does not appear to activate the smooth muscle specific genes strongly such that its more important effect is to block strong activation by myocardin [Wang et al., 2004]. This provides a switch for choosing between cell growth (active TCF, myocardin blocked) or differentiation (inactive TCF, activation by myocardin) (Fig. 3B).

Target genes activated by myocardin/MKL may be influenced by TCF sites or other flanking sites. In microarray experiments of serum inducible gene in NIH3T3 cells we found that dominant negative MKL1 blocked the induction of 28 out of 150 serum-inducible genes (A.S. and R.P., submitted). This demonstrates that the MKL family is required for induction of a subset of immediate early genes. It will be important to determine whether redundant pathways, such as TCF, are required for the other serum-inducible genes and what sequences determine the MKL-dependency since many more of the immediate early genes are regulated by SRF.

OTHER FUNCTIONS

MKL1 was also isolated (and termed BSAC) in a screen for genes whose overexpression inhibits tumor necrosis factor (TNF) induced cell death [Sasazuki et al., 2002]. This anti-apoptotic activity fits wells with MKL1's activation of growth-associated immediate early genes and the original identification of MKL1 at a translocation associated with AML.

MKL1 was fused with the RBM15 protein in multiple cases of AML with t(1;22)(p13;q13) translocations [Ma et al., 2001; Mercher et al., 2001]. The role of MKL1 in activating SRF and immediate early genes, along with its anti-apoptotic activity described above, suggests that it may be the active part of the RBM15-MKL1 protein causing AML. Consistent with this notion we found that RBM15-MKL1 activated SRE reporter genes much more strongly than wt MKL1 [Cen et al., 2003]. RBM15 contains three RNA-recognition motifs (RRM) at its

N-terminus and a C-terminal domain similar to *Drosophila* spen, but the function of RBM15 remains unknown. It will be interesting to determine whether its conserved domains are required for enhanced SRF activation by the RBM15-MKL1 fusion protein as well as for leukemogenesis. These domains may alter MKL1's cellular localization or otherwise activate it. It is also intriguing to understand why this translocation causes this particular leukemia and not others, possibly because critical target genes are selected in these cells.

CONCLUSIONS

The myocardin/MKL family of proteins have been established as strong coactivators of SRF and as critical components for immediate early and muscle specific gene expression. Since SRF activity does not appear to be regulated by its direct modification, these coactivators provide a novel point for regulation of SRF target genes. MKL1 is regulated by the RhoA pathway, likely by changes in its cellular localization and/or phosphorylation. It remains to be determined how this family is regulated in specific muscle cell types. While actin was found to complex with MKL1 in immunoprecipitates, no direct interaction was detected in vitro suggesting that additional MKL1 regulatory factors need to be identified [Miralles et al., 2003]. The role of MKL1 in leukemogenesis has also opened a connection of activation of the immediate early gene pathway directly to cancer. The discovery of the myocardin/MKL family has provided a new step in pathways for immediate early and muscle specific gene expression and should allow the identification of additional factors that govern these key regulatory decisions.

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