REVIEW

The myocardin family of transcriptional coactivators: versatile regulators of cell growth, migration, and myogenesis

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The association of transcriptional coactivators with sequence-specific DNA-binding proteins provides versatility and specificity to gene regulation and expands the regulatory potential of individual *cis*-regulatory DNA sequences. Members of the myocardin family of coactivators activate genes involved in cell proliferation, migration, and myogenesis by associating with serum response factor (SRF). The partnership of myocardin family members and SRF also controls genes encoding components of the actin cytoskeleton and confers responsiveness to extracellular growth signals and intracellular changes in the cytoskeleton, thereby creating a transcriptional–cytoskeletal regulatory circuit. These functions are reflected in defects in smooth muscle differentiation and function in mice with mutations in *myocardin* family members. This article reviews the functions and mechanisms of action of the *myocardin* family of coactivators and the physiological significance of transcriptional coactivation in the context of signal-dependent and cell-type-specific gene regulation.

The instructions for gene expression are “hard wired” into DNA sequence in the form of *cis*-regulatory elements that bind sequence-specific transcription factors and confer specialized patterns of gene expression (Howard and Davidson 2004). However, the binding of transcription factors to their cognate sites is often insufficient to account for the patterns of expression of their target genes. Indeed, it is not uncommon for a transcription factor to control genes with distinct or even mutually exclusive expression patterns. Many transcription factors are also relatively weak transcriptional activators and function by recruiting coactivators (or corepressors) that do not bind DNA directly but regulate transcription in a DNA sequence-specific manner by associating with DNA-bound factors (Spiegelman and Heinrich 2004). Thus, the association of DNA-binding proteins with accessory factors provides specificity and versatility to

gene regulation and expands the regulatory potential of individual *cis*-regulatory elements.

The regulation of serum response factor (SRF) and its target genes provides a classic example of the diversity of genes controlled by a single DNA-binding protein and the importance of cofactor interactions in the control of gene expression (Shore and Sharrocks 1995). Among the many target genes of SRF are genes involved in cell proliferation and muscle differentiation, which represent opposing programs of gene expression: Muscle genes are repressed by growth factor signals and generally do not become activated until myoblasts exit the cell cycle. The ability of SRF to regulate different sets of downstream effector genes depends on its association with positive and negative cofactors (Treisman 1994; Shore and Sharrocks 1995). SRF thereby serves as a platform to interpret cell identity and signaling by engaging various partners. The recent discovery and mechanistic dissection of the myocardin family of transcriptional coactivators (Wang et al. 2001, 2002), which regulate SRF activity during cell growth, migration, and myogenesis, have provided new insights into the mechanism of action of SRF, as well as the roles of coactivators in the control of gene expression in general. This article reviews the functions and mechanisms of action of the myocardin family of coactivators and considers their activities in the broader context of signal-dependent and cell-type-specific gene regulation.

SRF and MADS-box transcription factors

SRF belongs to the MADS (MCM1, Agamous, Deficiens, SRF) family of transcription factors, which share homology in a 57-amino-acid MADS-box that mediates homodimerization and DNA binding to a dyad symmetrical A + T-rich DNA consensus sequence (Shore and Sharrocks 1995). There are numerous MADS-box proteins in plants, but SRF and the four members of the myocyte enhancer factor-2 (MEF2) family (MEF2A, MEF2B, MEF2C, and MEF2D) are the only MADS-box proteins found in metazoans (Black and Olson 1998). The crystal structures of SRF and MEF2 have revealed commonalities in their modes of DNA binding (Pellegrini et al. 1995; Santelli and Richmond 2000), which are re-

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flected in the similar sequences of their binding sites: CC(A/T)₆GG (known as a CArG-box) for SRF and CTA(A/T)₄TAG for MEF2.

The conserved N-terminal region of the MADS-box forms an α -helical structure, which becomes oriented in an antiparallel manner within homodimers to form a bipartite DNA-binding domain. An N-terminal extension of the MADS-box in SRF makes critical base contacts in the minor groove that stabilize DNA binding. A domain C-terminal to the DNA-binding region is oriented away from DNA and is important for homodimerization and interaction with accessory factors. SRF, like other MADS-box transcription factors, interacts with a diverse array of transcriptional regulators to generate tissue-specific and signal-responsive patterns of gene expression (Messenguy and Dubois 2003). The SRF MADS-box induces an unusual degree of bending of the double helix, likely creating a unique shape that facilitates association with other components of the transcriptional regulatory complex (West and Sharrocks 1997).

Approximately 160 genes have been estimated to be direct transcriptional targets of SRF, and about half of these have been experimentally validated (Sun et al. 2006a). The majority of SRF target genes are involved in cell growth, migration, cytoskeletal organization, and myogenesis. The prototypical SRF target gene involved in cell growth, *c-fos*, is controlled by a single CArG-box, referred to as a serum response element (SRE), that acts in concert with surrounding *cis*-regulatory elements in the promoter (Norman et al. 1988). The first descriptions of SRF-dependent enhancers of muscle gene expression described duplicated CArG-boxes that functioned cooperatively to activate transcription (Miwa and Kedes 1987; Chow and Schwartz 1990). These initial observations led to the working hypothesis that SRF-dependent genes involved in cell growth are controlled by single CArG-boxes, while SRF-dependent muscle genes are controlled by duplicated CArG-boxes. However, recent genome-wide curation of all SRF target genes and the CArG-boxes that control their expression reveals duplicated CArG-boxes near the start of transcription of most SRF target genes (see Supplemental Material in Sun et al. 2006a). Some CArG-box-dependent muscle genes are expressed in only one muscle cell type (smooth, skeletal, or cardiac), whereas others are expressed in multiple muscle cell types. How such specificity is achieved has not been fully resolved, but likely involves positive and negative modulatory proteins in addition to SRF that act in a gene-specific manner.

The SRF target genes involved in cell growth can often be distinguished from those involved in myogenesis by the degree to which their CArG-boxes fit the perfect consensus. CArG-boxes in the promoters of several muscle genes deviate from the consensus sequence by one or more residues, resulting in a reduction in SRF-binding affinity (Chang et al. 2001), whereas the CArG-box upstream of *c-fos* fits the consensus perfectly. The nucleotide substitutions that render muscle gene CArGs non-consensus are often evolutionarily conserved, and replacement of these CArG-boxes with the perfect

consensus CArG-box from the *c-fos* promoter results in widespread (i.e., non-muscle-specific) expression (Hautmann et al. 1998; Chang et al. 2001). Thus, it appears that cell growth genes are “constitutive” SRF targets, their expression driven by a perfect consensus CArG-box that binds SRF with high affinity, while some myogenic SRF targets have only nonconsensus CArG-boxes that require coactivation, enhancement, or reinforcement of SRF binding to activate their expression in muscle cells.

SRF loss-of-function phenotypes

Experiments in cultured cells using dominant-negative SRF mutants or siRNA to down-regulate SRF expression have revealed essential roles for SRF in serum-dependent cell growth and skeletal muscle differentiation (Soulez et al. 1996; Wei et al. 1998; Kaplan-Albuquerque et al. 2005). A dominant-negative SRF mutant also blocks differentiation of coronary smooth muscle cells (SMCs) in the proepicardial organ of chick embryos (Landerholm et al. 1999) and disrupts skeletal and cardiac muscle differentiation in transgenic mice (Zhang et al. 2001).

A homozygous *Srf*-null mutation in mice results in lethality at gastrulation (Arsenian et al. 1998). SRF regulates genes involved in cell migration and adhesion, processes required for gastrulation. ES cells lacking SRF also display defects in spreading, adhesion, and migration that correlate with abnormalities in actin stress fibers and loss of expression of genes encoding stress fiber components including vinculin, talin, and a subset of actin isoforms, which are directly regulated by SRF (Schratt et al. 2002). SRF has also been shown to regulate cell survival during development via its control of the anti-apoptotic *Bcl-2* gene (Schratt et al. 2004).

The early lethality of *Srf*-null mice precludes an analysis of the potential roles of SRF in muscle development. However, conditional deletion of *Srf* from the cardiac muscle lineage results in mid-gestation lethality, accompanied by disruption of sarcomeric structure and abnormalities in muscle gene regulation (Miano et al. 2004; Parlakian et al. 2004; Niu et al. 2005). Smooth muscle deletion of *Srf* also results in a reduced number of differentiated SMCs near the dorsal aorta, and the few that are detected display cytoskeletal defects, including an absence of thick filaments and bundles of thin filaments (Miano et al. 2004). Deletion of *Srf* in skeletal muscle results in perinatal lethality from skeletal muscle hypoplasia (Li et al. 2005b).

The myocardin family of SRF coactivators

The fact that SRF, which is expressed ubiquitously, is required for the expression of muscle genes suggests that muscle-specific SRF cofactors contribute to the muscle-specificity of SRF target genes. *Myocardin*, the founding member of a family of extraordinarily powerful myogenic SRF coactivators, was discovered in a bioinformatics screen for novel cardiac-restricted genes (Wang et al. 2001). The properties of myocardin highlight one of the

The myocardin family of transcriptional coactivators

difficulties of identifying transcriptional coactivators in general. Myocardin's role in activating high levels of muscle transcription as an SRF coactivator was not obvious from examination of its predicted protein structure. Subsequent experiments showed that myocardin could stimulate transcription from CARG-dependent muscle enhancers, yet did not bind DNA directly. Instead, myocardin forms a stable ternary complex on CARG-boxes by associating with SRF. In *Srf*-null cells, myocardin has no detectable transcriptional activity (Wang et al. 2002). Activities of similar coactivator proteins are also critically dependent on the cellular context in which they are assayed: In the absence of a required cofactor, they are inactive.

Consistent with its discovery as a novel cardiac-restricted gene, *myocardin* expression is largely confined to the cardiovascular system. In vertebrate embryos, the onset of myocardin expression coincides with that of *Nkx2-5*, the earliest known marker of the cardiac lineage (Wang et al. 2001). Thereafter, myocardin is expressed throughout the heart, as well as in a subset of SMCs within the cardiovascular system and internal organs.

Myocardin shares homology with myocardin-related transcription factor-A (MRTF-A, also called MAL, MKL-1, and BSAC), and MRTF-B (also called MKL-2) (Ma et al. 2001; Mercher et al. 2001; Sasazuki et al. 2002; Wang et al. 2002), which are expressed in a broad range of embryonic and adult tissues: Most cell types tested, including ES cells (Du et al. 2004), express some level of MRTF-A and MRTF-B. During embryogenesis, however, MRTF-A is significantly enriched in mesenchymal cells, muscle cells, and epithelial cells of various organs (Wang et al. 2002; S. Li, D. Wang, and E.N. Olson, unpubl.). Likewise, MRTF-B is most highly expressed in a subset of the branchial arch arteries (Oh et al. 2005) derived from the neural crest and in developing neural structures during embryogenesis.

A single member of the myocardin family exists in fruit flies (Fig. 1) and in several other arthropods, but not in fungi. The fly homolog is most closely related to MRTF-A by primary sequence homology. Thus, the ancestral *myocardin* gene was likely an ortholog of *Mrtf-A* that arose during the metazoan transition to multicellularity.

Structure-function studies

Members of the myocardin family share homology in multiple functional domains (Fig. 1). The N-terminal regions of myocardin and MRTFs contain three RPEL motifs, which mediate association of MRTFs with actin, thereby providing responsiveness to cytoskeletal signaling (Miralles et al. 2003). In NIH 3T3 cells, stimuli that promote actin polymerization release MRTFs from G-actin, allowing them to enter into the nucleus. In contrast, myocardin is localized exclusively to the nucleus (Kuwahara et al. 2005), which is likely due to sequence divergence of its RPEL domains and consequent inability to bind actin (Wang et al. 2001; Miralles et al. 2003).

Myocardin family members contain a 35-amino-acid

Figure 1. The myocardin family of proteins, their structural domains, and their interaction partners. (A) Structural domains of the vertebrate and fly myocardin-related proteins. The percent identity between each domain and the corresponding domain of myocardin is shown. (B) Functions of the various domains of myocardin in black, binding interactions in red. The RPEL domains mediate cytoplasmic localization and actin binding only in MRTF-A and MRTF-B. Nuclear localization of MRTF-A requires the basic domain. Not shown: Smad3 binds full-length myocardin.

SAP domain, named after SAF-A/B, Acinus, and PIAS, which in other proteins participates in chromosomal dynamics, nuclear breakdown, and apoptotic DNA fragmentation (Aravind and Koonin 2000). The SAP domain is predicted to adopt a helix-linker-helix structure resembling a homeodomain without a DNA recognition motif, and in a few cases, the SAP domain has been shown to serve as a weak DNA-binding module (Gohring et al. 1997; Kipp et al. 2000; Sachdev et al. 2001; Bohm et al. 2005). Deletion mutants of myocardin lacking this region retain the ability to stimulate SRF activity on some promoters, but they are defective on others (Wang et al. 2001), which suggests a possible role for this domain in mediating interactions with other promoter-specific transcription factors.

Association of myocardin and MRTFs with SRF is mediated by a short peptide sequence that includes a basic and glutamine-rich region (Wang et al. 2001, 2002). A coiled-coil motif resembling a leucine zipper mediates homo- and heterodimerization of myocardin and MRTFs and has been proposed to contribute to the cooperativity between CARG-boxes in SRF-dependent muscle genes (Wang et al. 2001, 2002; Miralles et al. 2003; Du et al. 2004). The C-terminal regions of myocardin and MRTFs are somewhat divergent in amino acid sequence, and function as transcription activation domains (TADs). Deletion of these regions generates dominant-negative mutants. The TADs can be replaced by heterologous

TADs, such as that from the viral coactivator VP16, and function normally in reporter activation *in vitro*, indicating that this domain serves a general function in transcriptional activation, but does not contribute to the specificity of these factors for SRF coactivation (Wang et al. 2001).

Myocardin recruits chromatin remodeling enzymes to SRF target genes. Association with the histone acetyltransferase p300 enhances and interaction with class II histone deacetylases represses expression of SRF target genes (Cao et al. 2005). In support of a role for myocardin in altering chromatin structure, myocardin-SRF complexes have been shown to associate with a specific variant of histone H3 on SMC gene loci *in vivo* (McDonald et al. 2006). Myocardin also appears to enhance SRF binding to nonconsensus CArG-boxes associated with smooth muscle SRF gene targets, whereas the perfect consensus CArG-boxes found upstream of the immediate early gene *c-fos* bind SRF with the same affinity in the presence and absence of myocardin (Hendrix et al. 2005). The mechanism whereby myocardin enhances SRF DNA binding has not been defined. Perhaps the interaction of the two proteins stabilizes SRF in a conformation that facilitates association with DNA, or with DNA in a particular promoter context.

Opposing roles of myocardin and Ets factors in the control of SRF

Extracellular signals regulate the transcriptional activity of SRF through two parallel signaling pathways that involve mitogen-activated protein (MAP) kinase signaling and actin dynamics. Activation of MAP kinase pathways leads to phosphorylation of the ternary complex factor (TCF) family of Ets domain proteins, which associate with SRF on a specific subset of CArG-boxes flanked by Ets-binding sites (Fig. 2; Janknecht et al. 1993).

The TCF Elk-1 interacts with SRF through a short peptide motif known as a B-box (Ling et al. 1997, 1998; Sharrocks et al. 1997). The SRF-binding regions of myocardin and MRTFs resemble the predicted secondary structure of the B-box, although they lack direct amino acid homology with this region of Elk-1. Deletion of this region of myocardin abolishes its ability to associate with SRF and activate SRF-dependent genes, and replacement of this domain with the Elk-1 B-box restores these functions (Wang et al. 2004). Myocardin and Elk-1 compete for interaction with a common docking site in the MADS-box of SRF (Miralles et al. 2003; Wang et al. 2004). Their mutually exclusive association with this site creates a binary switch in which growth signals can modulate the phenotype of SMCs. When SMCs are stimulated with PDGF, Elk-1 becomes phosphorylated by the MAP kinase signaling pathway, facilitating its association with SRF and favoring the displacement of myocardin (Wang et al. 2004). Although Elk-1 is a coactivator of SRF, its activity is substantially weaker than that of myocardin, such that the displacement of myocardin from SRF by Elk-1 results in an overall decrease in the expression of smooth muscle genes. Consistent with

Figure 2. The myocardin family of proteins controls smooth muscle gene expression, subject to regulation by TCFs. Myocardin competes with TCFs for a binding site on SRF; cytoplasmic signaling results in phosphorylation of Elk-1. Phospho-Elk-1 can then displace myocardin from SRF, resulting in downregulation of a subset of smooth muscle genes. This process is critical in the phenotypic “switch” from a contractile to a proliferative state in smooth muscle cells. MRTF-A and MRTF-B also regulate smooth muscle gene expression, as shown by defects in smooth muscle differentiation or function in knockout mice.

this model, lowering endogenous levels of Elk-1 in SMCs results in an increase in expression of a subset of smooth muscle genes, likely via derepression of SRF–myocardin complexes on the promoters of these genes (Zhou et al. 2005).

Roles of the myocardin family in Rho and cytoskeletal signaling

Signaling by the Rho family of small GTPases stimulates SRF activity via actin polymerization, independently of MAP kinase activation and TCFs (Sotiropoulos et al. 1999). Members of the Rho family, including RhoA, Cdc42, and Rac1, use multiple pathways to increase the F-actin:G-actin ratio in different cell types (Geneste et al. 2002). In fibroblasts, multiple downstream effectors of activated RhoA promote F-actin accumulation: mDia1 by promoting assembly of F-actin (Copeland and Treisman 2002), and ROCK by stabilizing F-actin (Sotiropoulos et al. 1999). Calcium influx through voltage gated calcium channels during SMC contraction stimulates myocardin expression via a Rho/ROCK-dependent pathway (Wamhoff et al. 2004), and myocardin siRNA sup-

presses expression of contractile protein gene targets of SRF and myocardin, demonstrating that myocardin expression is regulated by, and part of the response to, changes in actin dynamics associated with smooth muscle physiology.

The actin-sensitivity of SRF has been proposed to be due to sequestration of MRTF-A in the cytoplasm by direct binding to G-actin via the N-terminal RPEL motifs (Miralles et al. 2003; Posern et al. 2004). Actin polymerization in response to Rho signaling results in activation of SRF as a consequence of the translocation of MRTF-A/MAL to the nucleus (Fig. 3; Miralles et al. 2003; Posern et al. 2004). However, significant areas of uncertainty remain regarding the actin-sensitivity of MRTF nuclear localization. For example, mutants of RhoA have been isolated that stimulate SRF activity but do not promote stress fiber formation (Sahai et al. 1998; Cen et al. 2004), indicating that, among the many pathways regulated by RhoA, there may be an alternate effector of SRF transcriptional activity. It remains to be determined whether these SRF-activating mutants of RhoA affect nucleocytoplasmic partitioning of the MRTFs. The mechanisms that regulate nuclear import of MRTF-A also remain to be defined. How is MRTF-A

dislodged from actin monomers (Miralles et al. 2003; Posern et al. 2004)? Does actin polymerization mask the domain of actin that binds MRTF-A, or is there an actin-polymerizing factor that binds G-actin with a higher avidity than does MRTF-A?

Striated Muscle Activator of Rho Signaling (STARS), an evolutionarily conserved muscle-specific protein, binds actin through a unique protein domain and stimulates SRF activity (Arai et al. 2002). STARS promotes nuclear localization of MRTF-A and MRTF-B, suggesting that it may compete with their RPEL motifs for association with actin and thereby provide a mechanism for signaling from the contractile apparatus to the nucleus (Kuwahara et al. 2005). STARS may also drive MRTFs into the nucleus indirectly, by stabilizing F-actin and decreasing the cytoplasmic F:G actin ratio. STARS is up-regulated during normal heart development and pathological cardiac hypertrophy (G.C.T. Pipes, K. Kuwahara, and E.N. Olson, in prep.), settings in which subsets of the muscle *actin* genes (all of which are CArG-dependent) are activated and G-actin monomers are incorporated into an expanding sarcomere. Some mechanism must exist to shield SRF activity from feedback inhibition by binding of actin monomers to MRTFs during periods of sarcomeric growth. STARS or some other muscle actin-binding protein is likely to serve this function.

The regulation of *actin* gene expression by SRF and the sensitivity of MRTFs to the state of actin polymerization creates a regulatory loop whereby changes in cell shape that influence cytoskeletal structure can promote actin synthesis (Fig. 3). This transcriptional–cytoskeletal regulatory circuit appears to be quite ancient, as studies in multiple lower metazoans show that inactivation of SRF causes cytoskeletal defects (Guillemin et al. 1996; Escalante et al. 2004; Sun et al. 2006a). SRF, the multiple CArG-dependent *actin* genes, their protein products, and the MRTFs thus constitute a novel mechanism that maintains cell shape, extrusive activity, and/or contractile potential in homeostatic balance with SRF transcriptional output. Nucleation of stress fibers (via Rho) or expression of F-actin-binding proteins (such as STARS during sarcomeric expansion) tips this balance toward stimulation of SRF transcriptional activity. Inhibition of SRF activity by expression of a naturally occurring splice variant of SRF (Davis et al. 2002; Chang et al. 2003) or actin depolymerization tips the balance toward loss of contractility and sarcomere regression.

Figure 3. Actin signaling to MRTFs. Actin dynamics and SRF transcriptional activity comprise a regulatory circuit that responds to and controls cytoskeletal and sarcomeric dynamics. MRTFs bind actin monomers directly and are sequestered in the cytoplasm. Extracellular signals stimulate actin polymerization via Rho family members, shifting the cytoplasmic F-actin:G-actin ratio. In response to actin polymerization, an unknown mechanism results in MRTF translocation to the nucleus, where it coactivates high levels of transcription of CArG-dependent genes, including many *actin* genes and other regulators of actin-based cellular behaviors, which then feed back to negatively regulate SRF activity. Expression of proteins that alter actin dynamics in different cell types (such as the actin-bundling protein STARS in muscle) can indirectly activate SRF transcription via actin dynamics.

Roles of myocardin and MRTFs in myogenesis

Numerous lines of evidence underscore the importance of myocardin in heart development. For example, expression of a dominant-negative myocardin mutant or disruption of myocardin expression with morpholino knockdown methods in *Xenopus* embryos interferes with cardiac development and inhibits cardiac gene expression (Wang et al. 2001; Small et al. 2005). Conversely, ectopic expression of myocardin in *Xenopus* embryos results in activation of cardiac gene expression throughout the embryo; cardiac gene expression in re-

sponse to myocardin is particularly robust in spinal cord neurons for reasons that are unclear. Similarly, injection of myocardin mRNA into *Xenopus* animal cap explants results in expression of numerous, but not all, myocardin genes (Small et al. 2005). Forced expression of myocardin in fibroblasts or stem cells is sufficient to activate the expression of smooth muscle genes, with only a subset of cardiac genes being expressed (Wang et al. 2003; Pipes et al. 2005; van Tuyn et al. 2005). This promyogenic activity requires the SRF-binding region and TAD of myocardin (Wang et al. 2003).

MRTF-A and MRTF-B can also activate smooth muscle gene expression when overexpressed in transfected fibroblasts (Wang et al. 2002; Du et al. 2004; Selvaraj and Prywes 2004). Why, then, don't MRTF-A and MRTF-B activate smooth muscle genes in the many non-muscle cell types in which they are expressed? Non-muscle tissues may express negative regulators of MRTF activity or lack essential cofactors or signals required for establishment of an SRF-MRTF complex on smooth muscle promoters. Perhaps there is a threshold level of expression of MRTFs that is required for smooth muscle gene activation, but not achieved by the endogenous levels of expression of MRTFs. It is also possible that the state of chromatin renders muscle target genes of SRF-MRTF inaccessible in nonmuscle cells.

MKL-1/MRTF-A has been implicated in skeletal muscle differentiation in vitro. Si-RNA for MRTF-A blocks fusion of skeletal myoblasts into multinucleated myotubes and prevents activation of SRF-dependent muscle genes (Selvaraj and Prywes 2003). Similarly, transgenic expression of dominant-negative MRTF-A in skeletal muscle in vivo results in abnormally thin muscle fibers with significant fibrosis, consistent with MRTF activity being required for muscle growth. This form of myopathy resembles that in mice lacking *Srf* in skeletal muscle (Li et al. 2005b). Expression levels of muscle genes (both CArG-dependent and -independent) are vastly decreased in these mice.

Myocardin is constitutively nuclear and specifically expressed in cardiac and smooth muscle myocytes, while the MRTFs shuttle between the nucleus and the cytoplasm in fibroblasts. In striated muscle, however, the activity of STARS protein is predicted to drive MRTFs into the nucleus and effectively make them constitutively nuclear (although this has not yet been shown in vivo). Thus, in contrast to other cell types, muscle cells have multiple mechanisms driving high levels of SRF transcriptional activity via myocardin family members, particularly during sarcomeric expansion, myocyte growth, and hypertrophy.

Loss-of-function phenotypes of myocardin family genes

The phenotypes of mice lacking members of the myocardin family have uncovered unique roles of each gene and have also raised questions about functional redundancy. A homozygous null mutation of *myocardin* in mice results in embryonic lethality at embryonic day 10.5 (E10.5) accompanied by a loss of smooth muscle

gene expression, but no apparent decrease in cardiac gene expression (Li et al. 2003). The lack of smooth muscle gene expression in null embryos is, surprisingly, not due to a cell-autonomous defect in SMC differentiation in the embryo proper, as the creation of chimeric mice derived from wild-type and *myocardin*^{-/-} ES cells revealed that *myocardin*-null SMCs can differentiate and be incorporated into vascular tissues with normal morphology (Pipes et al. 2005). Recent studies suggest that the lethality of *myocardin*-null embryos reflects an essential role of myocardin in a population of extraembryonic SMCs (S. Li, D.Z. Wang, and E.N. Olson, unpubl.). Proof of this hypothesis awaits tissue-specific deletion of myocardin from embryonic and extraembryonic SMCs.

The potential role of myocardin in heart development has been clouded by the lack of a cardiac phenotype in *myocardin*-null embryos. How can the lack of a cardiac phenotype of *myocardin*-null mice be explained in light of the ability of myocardin siRNA or dominant-negative mutants to disrupt heart development in *Xenopus* embryos? It seems likely that MRTFs or other cardiac transcription factors can compensate for the loss of myocardin function in the early heart. The generation of compound mutant mice will be required to address this possibility. With respect to myocardin's apparent ability to induce cardiac gene expression in *Xenopus* embryos (Small et al. 2005) but not in cultured mammalian cells (Wang et al. 2003), the environment of the embryo may be permissive for the actions of myocardin due to the presence of cofactors or signaling molecules that are absent from cultured cells. Alternatively, or in addition, negative regulators may interfere with the cardiogenic activity of myocardin in vitro.

Mice lacking MRTF-A are viable and display an intriguing defect specific to lactating females in which myoepithelial cells, which provide the contractility required for secretion of milk from the mammary gland, fail to differentiate and undergo apoptosis (Li et al. 2006; Sun et al. 2006b). As a result, offspring of MRTF-A-null mothers fail to thrive. MRTF-B is also expressed in myoepithelial tissue, but despite its high degree of similarity to MRTF-A, is unable to replace the function of MRTF-A, suggesting there are significant differences in how these factors are regulated, or in their interactions with other transcription factors. Alternatively, it may be that MRTF-A and MRTF-B are wholly redundant, and deleting MRTF-A lowers the amount of transcriptional activation of SRF target genes below a required threshold.

Mice lacking MRTF-B display defects in differentiation of SMCs derived from the neural crest, although two different targeted alleles of the gene result in phenotypes of differing severity. A lacZ enhancer trap allele results in vascular defects and perinatal lethality with incomplete penetrance (Li et al. 2005a), whereas a deletion mutation introduced into the gene by homologous recombination results in lethality at E13.5 with complete penetrance (Oh et al. 2005). The α -smooth muscle actin gene, which is SRF-dependent, is expressed normally in the heart and aorta of MRTF-B-null mice, but is down-regulated in the neural-crest-derived SMCs, which dis-

play the mutant phenotype. Thus, in a subset of smooth muscle tissue (i.e., neural crest-derived SMCs), the developmental roles of MRTF-B appear to have diverged from the roles of myocardin and MRTF-A. An interesting question for future investigation is why MRTF-A cannot substitute for MRTF-B in neural crest cells, and why MRTF-B cannot substitute for MRTF-A in differentiating myoepithelial cells.

The SRF–myocardin partnership in fruit flies

blistered is the fruit fly homolog of *Srf*, and is required for development of the tracheal system, a branching tubular network that functions in gaseous exchange, analogous to the combined functions of the vascular and respiratory systems in vertebrates. In *blistered* mutant flies, tracheal branching is perturbed due to an inability of cells at the tracheal termini to extend cytoplasmic processes toward target tissues (Affolter et al. 1994; Guillemin et al. 1996). There is a single myocardin family member in *Drosophila*, referred to as MAL-D or DMRTF, which, like its mammalian counterparts, functions as a strong SRF coactivator (Han et al. 2004; Somogyi and Rorth 2004). Determination of the function of DMRTF during embryogenesis has been complicated by a strong maternal contribution to the embryo. Nevertheless, loss of DMRTF function results in abnormalities in tracheal branching similar to, but less severe than, those in *blistered* mutant embryos. The defects in tracheal development in DMRTF RNAi-injected embryos are similar to those of embryos lacking Rac1 and Rac2 (Chihara et al. 2003), suggesting that DMRTF, like its mammalian counterparts, responds to Rho family members.

blistered also plays an important role in *Drosophila* wing development by promoting the formation of intervein tissue and suppressing vein formation. Expression of a dominant-negative DMRTF mutant in wing imaginal discs reduces wing interveins, whereas overexpression of DMRTF promotes the formation of excess intervein tissue, defects reminiscent of *blistered* loss- and gain-of-function phenotypes, respectively. Expression of dominant-negative DMRTF also disrupts dorsal migration of mesodermal cells, a process required for regional specification of cardiac, somatic, and visceral muscles (Han et al. 2004).

Migration of border cells, a specialized group of follicle cells, into germline tissue during oogenesis requires directed movement and extension of filopodial processes, which is accompanied by nuclear accumulation of DMRTF (Somogyi and Rorth 2004). In DMRTF mutant flies, border cells are unable to migrate effectively due to a defective actin cytoskeleton.

The target genes of SRF–DMRTF that control cell migration and tracheal branching in *Drosophila* remain to be defined. However, the striking similarities between the defects resulting from loss of function of SRF and myocardin family members in vertebrates and *Drosophila* suggest that these proteins represent an ancient and evolutionarily conserved system for coupling changes

in cell shape and extracellular signaling with cell migration during development.

Modulation of myocardin activity during pathological growth and remodeling of muscle cells

The functions of myocardin and the MRTFs are not confined to development and have been implicated in the control of muscle gene expression during disease. Overexpression of myocardin in vitro increases cardiac myocyte size and activates the expression of *atrial natriuretic factor* (ANF) (Badorff et al. 2005; Xing et al. 2006), a sensitive marker of cardiac myocyte hypertrophy controlled by SRF. Myocardin transcriptional activity is also negatively regulated via phosphorylation of myocardin by glycogen synthase kinase-3 β , a known suppressor of hypertrophic signaling (Badorff et al. 2005). Furthermore, *myocardin* transcript levels are up-regulated in failing heart (Torrado et al. 2003), consistent with the up-regulation of CArG-dependent muscle genes during pathological remodeling of the heart. Given that heart failure and cardiac hypertrophy are accompanied by increases in CArG-dependent muscle gene expression (with some exceptions), the increase in myocardin expression may represent a maladaptive response to cardiac injury, suitable for future therapeutic intervention.

Pathological remodeling of the vessel wall during atherosclerosis and restenosis involves a switch in SMC phenotype, from a differentiated, contractile to a proliferative, “synthetic” state (Owens et al. 2004). Signals that perturb myocardin activity will, in turn, block transcription of SMC contractile genes, and may promote phenotypic switching (Fig. 4). The forkhead transcription factor Foxo4 is up-regulated in vascular SMCs following injury and interacts with myocardin with consequent inhibition of transcriptional activity. The association of Foxo4 and myocardin appears to play a key role in modulation of smooth muscle growth and differentiation in this process, as Foxo4 and myocardin can both be isolated from the chromatin of smooth muscle genes under proliferating conditions (Liu et al. 2005). The Kruppel transcription factor KLF4 is also up-regulated during phenotypic dedifferentiation of SMCs and blocks activation of SMC contractile genes by decreasing myocardin expression (Liu et al. 2005) and interfering with SRF binding to CArG-boxes in intact chromatin, as well as recruiting repressive chromatin remodeling activities to SMC promoters (McDonald et al. 2006). The in vivo relevance of KLF4-mediated repression of myocardin expression is difficult to reconcile with recent reports of vast increases in myocardin immunoreactivity in synthetic SMCs found following vascular injury (Doi et al. 2005), although other reports describe a decrease in *myocardin* transcript levels under similar conditions (Hendrix et al. 2005).

Potential roles in human disease

In acute megakaryocytic leukemia, the human *MRTF-A* gene (also referred to as *MAL/MKL1*) is translocated and

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Figure 4. Myogenic and anti-myogenic signaling focusing on myocardin expression and activity. Myocardin activates the expression of multiple smooth muscle contractile genes through SRF. Foxo4 binds to and inhibits myocardin transcriptional activity. Akt signaling alleviates this repression. Growth factor signaling (such as PDGF signaling) results in activation of multiple anti-myogenic pathways. In response to vascular injury and in response to PDGF, KLF4 blocks expression of the *myocardin* gene and also decreases SRF–myocardin binding to CARG elements upstream of smooth muscle contractile genes. HRT2 expression blocks myocardin transcriptional activity through an unknown mechanism.

fused with the gene encoding One-twenty-two (OTT/RBM15), a putative RNA-binding protein (Mercher et al. 2001, 2002; Dastugue et al. 2002; Hrusak and Porwit-MacDonald 2002; Ballerini et al. 2003; Duchayne et al. 2003; Gilliland et al. 2004; Hsiao et al. 2005). The resulting RBM15–MRTF-A fusion protein contains almost the entire OTT protein sequence fused near the N terminus of MRTF-A, after the first predicted RPEL domain. All of the domains of MRTF-A required for activation of SRF target genes are thus present in this fusion protein. The mutant protein causes uncontrolled cell proliferation through an unknown mechanism. The oncogenic fusion protein displays enhanced transcriptional activity toward the growth-factor-inducible *c-fos* and *Egr1* promoters, but its ability to activate smooth muscle genes is unaltered (Cen et al. 2003). How the RBM15–MRTF-A fusion promotes tumorigenesis remains to be determined, but given the role of SRF/MRTFs in regulating cytoskeletal assembly during cell migration, it will be of interest to determine whether this protein partnership plays a role in invasive cell migration during cancer metastasis.

Other partners of the myocardin family

All but one of the factors that modulate myocardin transcriptional activity described to date require SRF and an

intact CARG-box in order to function. The exception is the TGF- β effector Smad3, which interacts with myocardin and stimulates its activity in a CARG-independent manner (Qiu et al. 2005). Mutation of the CARG-boxes, in the SM22 promoter, the prototypical smooth muscle target gene of SRF, reduced but did not ablate synergistic activation of the promoter by myocardin and Smad3. In contrast, Smad1 interacts with myocardin, resulting in transcriptional synergy in a CARG-dependent manner (Callis et al. 2005). While these findings suggest that myocardin can coactivate transcription through a DNA-binding partner other than SRF, it should be kept in mind that Smad3 also binds SRF, raising questions as to whether this form of coactivation is truly SRF-independent (Qiu et al. 2005). Furthermore, given the stability of the complex formed by SRF and myocardin on CARG-containing DNA, it is unclear how this alternative interaction with myocardin could form in the presence of SRF *in vivo*.

The transcriptional activity of myocardin is subject to repression by a variety of corepressor proteins. The hairy-related transcription factor (HRT)-2, which is expressed in developing vasculature (Nakagawa et al. 1999), associates with myocardin and represses its activity through a mechanism yet to be defined (Proweller et al. 2005). Similarly, GATA factors are capable of repressing myocardin's coactivation of SRF by competing for a common docking site on SRF, although this effect is only observed on a subset of smooth muscle enhancer elements. On the CARG-dependent enhancer of the *smooth muscle myosin heavy chain* gene, GATA-6 can synergize with myocardin and SRF to coactivate high levels of transcription, highlighting the context-dependent activity of myocardin (Yin and Herring 2005). Similarly, GATA-4 binds directly to myocardin and can synergize with myocardin to activate transcription from the cardiac-specific *Nkx2-5* enhancer in a CARG-box-dependent manner, yet it prevents activation of the cardiac *ANF* promoter by myocardin (Oh et al. 2004).

Implications and questions for the future

Gene activation requires sequence-specific DNA-binding activity coupled to stimulation of the transcriptional machinery, functions that reside most commonly in a single protein. However, SRF is a relatively weak transcriptional activator and acquires potent transcriptional activity by recruiting myocardin and MRTFs, which provide their powerful TADs to the SRF DNA-binding domain, thereby creating a functional transcriptional complex. Why might this bipartite mode of transcriptional control have evolved for SRF, as well as for its close relative MEF2 (McKinsey et al. 2002)? Gene regulation by the recruitment of a TAD to a sequence-specific DNA-binding protein expands the regulatory potential of the DNA-bound transcription factor by allowing for the exchange of multiple positive and negative cofactors. This form of transcriptional regulation is suited for the rapid activation or repression of sets of genes that are unlinked and cannot be controlled by a shared enhancer,

such as muscle genes. This mechanism is also especially amenable to mediating rapid changes in gene expression in response to extracellular signals, a central function of SRF and MEF2.

The evolution of SRF and myocardin proteins as separate components of a transcriptional activator, rather than being contained in a single polypeptide, is also reflective of, and likely responsible for, the plasticity of the smooth muscle phenotype. In this regard, it is instructive to compare and contrast the activities of myocardin, as a regulator of smooth muscle differentiation, with MyoD, a master regulator of skeletal muscle development. Both proteins are capable of activating downstream muscle genes associated with the smooth and skeletal muscle phenotypes, respectively. However, there are key differences in the biology of smooth and skeletal muscle cells that reflect, at least in part, the properties of myocardin-SRF and MyoD.

In contrast to SMCs, which can switch their phenotypes between proliferative and differentiated states in response to extracellular signals, when skeletal muscle cells differentiate, they fuse to form terminally differentiated myofibers that are permanently post-mitotic. The reversible association of myocardin with SRF allows for plasticity of the differentiated state inherent in the smooth muscle phenotype, for example, by the competition between myocardin and Elk-1 for docking on SRF. By comparison, MyoD possesses an intrinsic TAD and acts as a strong transcriptional activator on its own, and therefore lacks this regulatory potential inherent in SRF and myocardin. MyoD and other members of the MyoD family also autoregulate their own expression, which has been proposed to stabilize the skeletal muscle phenotype (Lassar et al. 1989). This type of feed-forward mechanism of transcriptional activation ensures that skeletal muscle cells differentiate irreversibly. Because of their ability to stably maintain their own expression, members of the MyoD family are only expressed in skeletal muscle; expression elsewhere would be expected to transform nonmuscle cells to skeletal muscle. Forced expression of myocardin in fibroblasts does not activate the endogenous *myocardin* gene (S. Li and E.N. Olson, unpubl.). The lack of a myocardin autoactivation loop allows for reversibility of the differentiation program in SMCs. SRF, MRTF-A, and MRTF-B are also widely expressed and play significant roles in modulating adult cellular physiology (e.g., turning on and off the differentiation of myoepithelial cells during the lactation cycle), in addition to their roles in cell fate determination during development.

The discovery of the myocardin family has opened numerous avenues of exploration into the mechanisms of cell growth, differentiation, and signaling, and many interesting questions remain. Myocardin and MRTFs are large proteins (~1200 amino acids) and use a relatively short domain of ~30 amino acids to associate with SRF. Does myocardin have additional partners that associate with other domains? Because of their potency, members of the myocardin family must be stringently regulated. What are the mechanisms for such regulation? Myocar-

din expression marks the onset of the cardiac lineage. What are the upstream regulators that activate its expression? Given the association of SRF and myocardin family members with many disease states, their intimate connection to the mitogenic response and role in regulating the expression of immediate early genes, could modulation of a myocardin family member provide a suitable target for therapeutic intervention?

The downstream target genes of myocardin and MRTFs also remain to be defined. Which of the estimated 160 target genes of SRF are regulated by myocardin family members will undoubtedly depend on cell identity and signaling. In this regard, identification of genes dysregulated by a dominant-negative form of MRTF-A in a cell line stimulated by serum revealed a subset of SRF targets that are also regulated by MRTF (Selvaraj and Prywes 2004). Similar approaches will undoubtedly provide other clues as to which cellular processes in addition to SMC phenotypic switching, the mitogenic response, SMC differentiation, and muscle gene transcription are regulated by members of the myocardin family of coactivators.

The myocardin family of transcriptional coactivators joins a growing array of transcriptional coactivators that provide a layer of regulation to their DNA-bound partners (Spiegelman and Heinrich 2004). Because such coactivators are not readily recognizable from sequence inspection, discovery of other such proteins will require functional assays to reveal their transcriptional activity. The majority of such factors will likely function in reversible, signal-responsive changes in gene expression that can be better served by a dissociable transcriptional complex than by a classical transcriptional activator that contains a DNA-binding motif covalently bound to a transcriptional activation domain.

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References

- Affolter, M., Montagne, J., Walldorf, U., Groppe, J., Kloter, U., LaRosa, M., and Gehring, W.J. 1994. The *Drosophila* SRF homolog is expressed in a subset of tracheal cells and maps within a genomic region required for tracheal development. *Development* **120**: 743–753.
- Arai, A., Spencer, J.A., and Olson, E.N. 2002. STARS, a striated muscle activator of Rho signaling and serum response factor-dependent transcription. *J. Biol. Chem.* **277**: 24453–24459.
- Aravind, L. and Koonin, E.V. 2000. SAP—A putative DNA-binding motif involved in chromosomal organization. *Trends Biochem. Sci.* **25**: 112–114.
- Arsenian, S., Weinhold, B., Oelgeschlager, M., Ruther, U., and Nordheim, A. 1998. Serum response factor is essential for

- mesoderm formation during mouse embryogenesis. *EMBO J.* **17**: 6289–6299.
- Badorff, C., Seeger, F.H., Zeiher, A.M., and Dimmeler, S. 2005. Glycogen synthase kinase 3 β inhibits myocardin-dependent transcription and hypertrophy induction through site-specific phosphorylation. *Circ. Res.* **97**: 645–654.
- Ballerini, P., Blaise, A., Mercher, T., Pellegrino, B., Perot, C., van den Akker, J., Gatbois, E., Adam, M., Douay, L., Berger, R., et al. 2003. A novel real-time RT-PCR assay for quantification of OTT-MAL fusion transcript reliable for diagnosis of t(1;22) and minimal residual disease (MRD) detection. *Leukemia* **17**: 1193–1196.
- Black, B.L. and Olson, E.N. 1998. Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. *Annu. Rev. Cell Dev. Biol.* **14**: 167–196.
- Bohm, F., Kappes, F., Scholten, I., Richter, N., Matsuo, H., Knippers, R., and Waldmann, T. 2005. The SAF-box domain of chromatin protein DEK. *Nucleic Acids Res.* **33**: 1101–1110.
- Callis, T.E., Cao, D., and Wang, D.Z. 2005. Bone morphogenetic protein signaling modulates myocardin transactivation of cardiac genes. *Circ. Res.* **97**: 992–1000.
- Cao, D., Wang, Z., Zhang, C.L., Oh, J., Xing, W., Li, S., Richardson, J.A., Wang, D.Z., and Olson, E.N. 2005. Modulation of smooth muscle gene expression by association of histone acetyltransferases and deacetylases with myocardin. *Mol. Cell. Biol.* **25**: 364–376.
- Cen, B., Selvaraj, A., Burgess, R.C., Hitzler, J.K., Ma, Z., Morris, S.W., and Prywes, R. 2003. Megakaryoblastic leukemia 1, a potent transcriptional coactivator for serum response factor (SRF), is required for serum induction of SRF target genes. *Mol. Cell. Biol.* **23**: 6597–6608.
- Cen, B., Selvaraj, A., and Prywes, R. 2004. Myocardin/MKL family of SRF coactivators: Key regulators of immediate early and muscle specific gene expression. *J. Cell. Biochem.* **93**: 74–82.
- Chang, P.S., Li, L., McAnally, J., and Olson, E.N. 2001. Muscle specificity encoded by specific serum response factor-binding sites. *J. Biol. Chem.* **276**: 17206–17212.
- Chang, J., Wei, L., Otani, T., Youker, K.A., Entman, M.L., and Schwartz, R.J. 2003. Inhibitory cardiac transcription factor, SRF-N, is generated by caspase 3 cleavage in human heart failure and attenuated by ventricular unloading. *Circulation* **108**: 407–413.
- Chihara, T., Kato, K., Taniguchi, M., Ng, J., and Hayashi, S. 2003. Rac promotes epithelial cell rearrangement during tracheal tubulogenesis in *Drosophila*. *Development* **130**: 1419–1428.
- Chow, K.L. and Schwartz, R.J. 1990. A combination of closely associated positive and negative *cis*-acting promoter elements regulates transcription of the skeletal α -actin gene. *Mol. Cell. Biol.* **10**: 528–538.
- Copeland, J.W. and Treisman, R. 2002. The diaphanous-related formin mDial controls serum response factor activity through its effects on actin polymerization. *Mol. Biol. Cell* **13**: 4088–4099.
- Dastugue, N., Lafage-Pochitaloff, M., Pages, M.P., Radford, I., Bastard, C., Talmant, P., Mozziconacci, M.J., Leonard, C., Bilhou-Nabera, C., Cabrol, C., et al. 2002. Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): A study of the Groupe Francais de Cytogenetique Hematologique (GFCH). *Blood* **100**: 618–626.
- Davis, F.J., Gupta, M., Pogwizd, S.M., Bacha, E., Jeevanandam, V., and Gupta, M.P. 2002. Increased expression of alternatively spliced dominant-negative isoform of SRF in human failing hearts. *Am. J. Physiol. Heart Circ. Physiol.* **282**: H1521–H1533.
- Doi, H., Iso, T., Yamazaki, M., Akiyama, H., Kanai, H., Sato, H., Kawai-Kowase, K., Tanaka, T., Maeno, T., Okamoto, E., et al. 2005. HERP1 inhibits myocardin-induced vascular smooth muscle cell differentiation by interfering with SRF binding to CArG box. *Arterioscler. Thromb. Vasc. Biol.* **25**: 2328–2334.
- Du, K.L., Chen, M., Li, J., Lepore, J.J., Mericko, P., and Parmacek, M.S. 2004. Megakaryoblastic leukemia factor-1 transduces cytoskeletal signals and induces smooth muscle cell differentiation from undifferentiated embryonic stem cells. *J. Biol. Chem.* **279**: 17578–17586.
- Duchayne, E., Fenneteau, O., Pages, M.P., Sainty, D., Arnoulet, C., Dastugue, N., Garand, R., and Flandrin, G. 2003. Acute megakaryoblastic leukaemia: A national clinical and biological study of 53 adult and childhood cases by the Groupe Francais d'Hematologie Cellulaire (GFHC). *Leuk. Lymphoma* **44**: 49–58.
- Escalante, R., Yamada, Y., Cotter, D., Sastre, L., and Sameshima, M. 2004. The MADS-box transcription factor SrfA is required for actin cytoskeleton organization and spore coat stability during *Dictyostelium* sporulation. *Mech. Dev.* **121**: 51–56.
- Geneste, O., Copeland, J.W., and Treisman, R. 2002. LIM kinase and Diaphanous cooperate to regulate serum response factor and actin dynamics. *J. Cell Biol.* **157**: 831–838.
- Gilliland, D.G., Jordan, C.T., and Felix, C.A. 2004. The molecular basis of leukemia. *Hematology (Am. Soc. Hematol. Educ. Program)* **2004**: 80–97.
- Gohring, F., Schwab, B.L., Nicotera, P., Leist, M., and Fackelmayr, F.O. 1997. The novel SAR-binding domain of scaffold attachment factor A (SAF-A) is a target in apoptotic nuclear breakdown. *EMBO J.* **16**: 7361–7371.
- Guillemin, K., Groppe, J., Ducker, K., Treisman, R., Hafen, E., Affolter, M., and Krasnow, M.A. 1996. The pruned gene encodes the *Drosophila* serum response factor and regulates cytoplasmic outgrowth during terminal branching of the tracheal system. *Development* **122**: 1353–1362.
- Han, Z., Li, X., Wu, J., and Olson, E.N. 2004. A myocardin-related transcription factor regulates activity of serum response factor in *Drosophila*. *Proc. Natl. Acad. Sci.* **101**: 12567–12572.
- Hautmann, M.B., Madsen, C.S., Mack, C.P., and Owens, G.K. 1998. Substitution of the degenerate smooth muscle (SM) α -actin CC(A/T-rich)₆GG elements with c-fos serum response elements results in increased basal expression but relaxed SM cell specificity and reduced angiotensin II inducibility. *J. Biol. Chem.* **273**: 8398–8406.
- Hendrix, J.A., Wamhoff, B.R., McDonald, O.G., Sinha, S., Yoshida, T., and Owens, G.K. 2005. 5' CArG degeneracy in smooth muscle α -actin is required for injury-induced gene suppression in vivo. *J. Clin. Invest.* **115**: 418–427.
- Howard, M.L. and Davidson, E.H. 2004. *cis*-Regulatory control circuits in development. *Dev. Biol.* **271**: 109–118.
- Hrusak, O. and Porwit-MacDonald, A. 2002. Antigen expression patterns reflecting genotype of acute leukemias. *Leukemia* **16**: 1233–1258.
- Hsiao, H.H., Yang, M.Y., Liu, Y.C., Hsiao, H.P., Tseng, S.B., Chao, M.C., Liu, T.C., and Lin, S.F. 2005. RBM15-MKL1 (OTT-MAL) fusion transcript in an adult acute myeloid leukemia patient. *Am. J. Hematol.* **79**: 43–45.
- Janknecht, R., Ernst, W.H., Houthaeve, T., and Nordheim, A. 1993. C-terminal phosphorylation of the serum-response factor. *Eur. J. Biochem.* **216**: 469–475.
- Kaplan-Albuquerque, N., Van Putten, V., Weiser-Evans, M.C., and Nemenoff, R.A. 2005. Depletion of serum response factor by RNA interference mimics the mitogenic effects of

- platelet derived growth factor-BB in vascular smooth muscle cells. *Circ. Res.* **97**: 427–433.
- Kipp, M., Gohring, F., Ostendorp, T., van Drunen, C.M., van Driel, R., Przybylski, M., and Fackelmayer, F.O. 2000. SAF-Box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. *Mol. Cell. Biol.* **20**: 7480–7489.
- Kuwahara, K., Barrientos, T., Pipes, G.C., Li, S., and Olson, E.N. 2005. Muscle-specific signaling mechanism that links actin dynamics to serum response factor. *Mol. Cell. Biol.* **25**: 3173–3181.
- Landerholm, T.E., Dong, X.R., Lu, J., Belaguli, N.S., Schwartz, R.J., and Majesky, M.W. 1999. A role for serum response factor in coronary smooth muscle differentiation from proepicardial cells. *Development* **126**: 2053–2062.
- Lassar, A.B., Buskin, J.N., Lockshon, D., Davis, R.L., Apone, S., Hauschka, S.D., and Weintraub, H. 1989. MyoD is a sequence-specific DNA binding protein requiring a region of myc homology to bind to the muscle creatine kinase enhancer. *Cell* **58**: 823–831.
- Li, S., Wang, D.Z., Wang, Z., Richardson, J.A., and Olson, E.N. 2003. The serum response factor coactivator myocardin is required for vascular smooth muscle development. *Proc. Natl. Acad. Sci.* **100**: 9366–9370.
- Li, J., Zhu, X., Chen, M., Cheng, L., Zhou, D., Lu, M.M., Du, K., Epstein, J.A., and Parmacek, M.S. 2005a. Myocardin-related transcription factor B is required in cardiac neural crest for smooth muscle differentiation and cardiovascular development. *Proc. Natl. Acad. Sci.* **102**: 8916–8921.
- Li, S., Czubyrt, M.P., McAnally, J., Bassel-Duby, R., Richardson, J.A., Wiebel, F.F., Nordheim, A., and Olson, E.N. 2005b. Requirement for serum response factor for skeletal muscle growth and maturation revealed by tissue-specific gene deletion in mice. *Proc. Natl. Acad. Sci.* **102**: 1082–1087.
- Li, S., Chang, S., Qi, X., Richardson, J.A., and Olson, E.N. 2006. Requirement of a myocardin-related transcription factor for development of mammary myoepithelial cells. *Mol. Cell. Biol.* (in press).
- Ling, Y., Lakey, J.H., Roberts, C.E., and Sharrocks, A.D. 1997. Molecular characterization of the B-box protein–protein interaction motif of the ETS-domain transcription factor Elk-1. *EMBO J.* **16**: 2431–2440.
- Ling, Y., West, A.G., Roberts, E.C., Lakey, J.H., and Sharrocks, A.D. 1998. Interaction of transcription factors with serum response factor. Identification of the Elk-1 binding surface. *J. Biol. Chem.* **273**: 10506–10514.
- Liu, Z.P., Wang, Z., Yanagisawa, H., and Olson, E.N. 2005. Phenotypic modulation of smooth muscle cells through interaction of Foxo4 and myocardin. *Dev. Cell* **9**: 261–270.
- Ma, Z., Morris, S.W., Valentine, V., Li, M., Herbrick, J.A., Cui, X., Bouman, D., Li, Y., Mehta, P.K., Nizetic, D., et al. 2001. Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. *Nat. Genet.* **28**: 220–221.
- McDonald, O.G., Wamhoff, B.R., Hoofnagle, M.H., and Owens, G.K. 2006. Control of SRF binding to CArG box chromatin regulates smooth muscle gene expression in vivo. *J. Clin. Invest.* **116**: 36–48.
- McKinsey, T.A., Zhang, C.L., and Olson, E.N. 2002. MEF2: A calcium-dependent regulator of cell division, differentiation and death. *Trends Biochem. Sci.* **27**: 40–47.
- Mercher, T., Coniat, M.B., Monni, R., Mauchauffe, M., Khac, F.N., Gressin, L., Mugneret, F., Leblanc, T., Dastugue, N., Berger, R., et al. 2001. Involvement of a human gene related to the *Drosophila* spen gene in the recurrent t(1;22) translocation of acute megakaryocytic leukemia. *Proc. Natl. Acad. Sci.* **98**: 5776–5779.
- Mercher, T., Busson-Le Coniat, M., Khac, F.N., Ballerini, P., Mauchauffe, M., Bui, H., Pellegrino, B., Radford, I., Valensi, F., Mugneret, F., et al. 2002. Recurrence of OTT-MAL fusion in t(1;22) of infant AML-M7. *Genes Chromosomes Cancer* **33**: 22–28.
- Messenguy, F. and Dubois, E. 2003. Role of MADS box proteins and their cofactors in combinatorial control of gene expression and cell development. *Gene* **316**: 1–21.
- Miano, J.M., Ramanan, N., Georger, M.A., de Mesy Bentley, K.L., Emerson, R.L., Balza Jr., R.O., Xiao, Q., Weiler, H., Ginty, D.D., and Misra, R.P. 2004. Restricted inactivation of serum response factor to the cardiovascular system. *Proc. Natl. Acad. Sci.* **101**: 17132–17137.
- Miralles, F., Posern, G., Zaromytidou, A.I., and Treisman, R. 2003. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**: 329–342.
- Miwa, T. and Kedes, L. 1987. Duplicated CArG box domains have positive and mutually dependent regulatory roles in expression of the human α -cardiac actin gene. *Mol. Cell. Biol.* **7**: 2803–2813.
- Nakagawa, O., Nakagawa, M., Richardson, J.A., Olson, E.N., and Srivastava, D. 1999. HRT1, HRT2, and HRT3: A new subclass of bHLH transcription factors marking specific cardiac, somitic, and pharyngeal arch segments. *Dev. Biol.* **216**: 72–84.
- Niu, Z., Yu, W., Zhang, S.X., Barron, M., Belaguli, N.S., Schneider, M.D., Parmacek, M., Nordheim, A., and Schwartz, R.J. 2005. Conditional mutagenesis of the murine serum response factor gene blocks cardiogenesis and the transcription of downstream gene targets. *J. Biol. Chem.* **280**: 32531–32538.
- Norman, C., Runswick, M., Pollock, R., and Treisman, R. 1988. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. *Cell* **55**: 989–1003.
- Oh, J., Wang, Z., Wang, D.Z., Lien, C.L., Xing, W., and Olson, E.N. 2004. Target gene-specific modulation of myocardin activity by GATA transcription factors. *Mol. Cell. Biol.* **24**: 8519–8528.
- Oh, J., Richardson, J.A., and Olson, E.N. 2005. Requirement of myocardin-related transcription factor-B for remodeling of branchial arch arteries and smooth muscle differentiation. *Proc. Natl. Acad. Sci.* **102**: 15122–15127.
- Owens, G.K., Kumar, M.S., and Wamhoff, B.R. 2004. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol. Rev.* **84**: 767–801.
- Parlakian, A., Tuil, D., Hamard, G., Tavernier, G., Hentzen, D., Concordet, J.P., Paulin, D., Li, Z., and Daegelen, D. 2004. Targeted inactivation of serum response factor in the developing heart results in myocardial defects and embryonic lethality. *Mol. Cell. Biol.* **24**: 5281–5289.
- Pellegrini, L., Tan, S., and Richmond, T.J. 1995. Structure of serum response factor core bound to DNA. *Nature* **376**: 490–498.
- Pipes, G.C., Sinha, S., Qi, X., Zhu, C.H., Gallardo, T.D., Shelton, J., Creemers, E.E., Sutherland, L., Richardson, J.A., Garry, D.J., et al. 2005. Stem cells and their derivatives can bypass the requirement of myocardin for smooth muscle gene expression. *Dev. Biol.* **288**: 502–513.
- Posern, G., Miralles, F., Guettler, S., and Treisman, R. 2004. Mutant actins that stabilise F-actin use distinct mechanisms to activate the SRF coactivator MAL. *EMBO J.* **23**: 3973–3983.
- Proweller, A., Pear, W.S., and Parmacek, M.S. 2005. Notch signaling represses myocardin-induced smooth muscle cell dif-

- ferentiation. *J. Biol. Chem.* **280**: 8994–9004.
- Qiu, P., Ritchie, R.P., Fu, Z., Cao, D., Cumming, J., Miano, J.M., Wang, D.Z., Li, H.J., and Li, L. 2005. Myocardin enhances Smad3-mediated transforming growth factor- β 1 signaling in a CArG box-independent manner: Smad-binding element is an important *cis* element for SM22 α transcription in vivo. *Circ. Res.* **97**: 983–991.
- Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F., and Grosschedl, R. 2001. PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. *Genes & Dev.* **15**: 3088–3103.
- Sahai, E., Alberts, A.S., and Treisman, R. 1998. RhoA effector mutants reveal distinct effector pathways for cytoskeletal reorganization, SRF activation and transformation. *EMBO J.* **17**: 1350–1361.
- Santelli, E. and Richmond, T.J. 2000. Crystal structure of MEF2A core bound to DNA at 1.5 Å resolution. *J. Mol. Biol.* **297**: 437–449.
- Sasazuki, T., Sawada, T., Sakon, S., Kitamura, T., Kishi, T., Okazaki, T., Katano, M., Tanaka, M., Watanabe, M., Yagita, H., et al. 2002. Identification of a novel transcriptional activator, BSAC, by a functional cloning to inhibit tumor necrosis factor-induced cell death. *J. Biol. Chem.* **277**: 28853–28860.
- Schratt, G., Philippar, U., Berger, J., Schwarz, H., Heidenreich, O., and Nordheim, A. 2002. Serum response factor is crucial for actin cytoskeletal organization and focal adhesion assembly in embryonic stem cells. *J. Cell Biol.* **156**: 737–750.
- Schratt, G., Philippar, U., Hockemeyer, D., Schwarz, H., Alberti, S., and Nordheim, A. 2004. SRF regulates Bcl-2 expression and promotes cell survival during murine embryonic development. *EMBO J.* **23**: 1834–1844.
- Selvaraj, A. and Prywes, R. 2003. Megakaryoblastic leukemia-1/2, a transcriptional co-activator of serum response factor, is required for skeletal myogenic differentiation. *J. Biol. Chem.* **278**: 41977–41987.
- . 2004. Expression profiling of serum inducible genes identifies a subset of SRF target genes that are MKL dependent. *BMC Mol. Biol.* **5**: 13.
- Sharrocks, A.D., Brown, A.L., Ling, Y., and Yates, P.R. 1997. The ETS-domain transcription factor family. *Int. J. Biochem. Cell Biol.* **29**: 1371–1387.
- Shore, P. and Sharrocks, A.D. 1995. The MADS-box family of transcription factors. *Eur. J. Biochem.* **229**: 1–13.
- Small, E.M., Warkman, A.S., Wang, D.Z., Sutherland, L.B., Olson, E.N., and Krieg, P.A. 2005. Myocardin is sufficient and necessary for cardiac gene expression in *Xenopus*. *Development* **132**: 987–997.
- Somogyi, K. and Rorth, P. 2004. Evidence for tension-based regulation of *Drosophila* MAL and SRF during invasive cell migration. *Dev. Cell* **7**: 85–93.
- Sotiropoulos, A., Gineitis, D., Copeland, J., and Treisman, R. 1999. Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. *Cell* **98**: 159–169.
- Soulez, M., Rouviere, C.G., Chafey, P., Hentzen, D., Vandromme, M., Lautredou, N., Lamb, N., Kahn, A., and Tuil, D. 1996. Growth and differentiation of C2 myogenic cells are dependent on serum response factor. *Mol. Cell. Biol.* **16**: 6065–6074.
- Spiegelman, B.M. and Heinrich, R. 2004. Biological control through regulated transcriptional coactivators. *Cell* **119**: 157–167.
- Sun, Q., Chen, G., Streb, J.W., Long, X., Yang, Y., Stoeckert Jr., C.J., and Miano, J.M. 2006a. Defining the mammalian CArGome. *Genome Res.* **16**: 197–207.
- Sun, Y., Boyd, K., Xu, W., Ma, J., Jackson, C., Fu, A., Shillingford, J.M., Henninghausen, L., Kitzler, J.K., Ma, Z., et al. 2006b. Acute myeloid leukemia-associated *Mkl1* is a key regulator of normal cardiogenesis and mammary gland function. *Mol. Cell. Biol.* (in press).
- Torrado, M., Lopez, E., Centeno, A., Medrano, C., Castro-Beiras, A., and Mikhailov, A.T. 2003. Myocardin mRNA is augmented in the failing myocardium: Expression profiling in the porcine model and human dilated cardiomyopathy. *J. Mol. Med.* **81**: 566–577.
- Treisman, R. 1994. Ternary complex factors: Growth factor regulated transcriptional activators. *Curr. Opin. Genet. Dev.* **4**: 96–101.
- van Tuyn, J., Knaan-Shanzer, S., van de Watering, M.J., de Graaf, M., van der Laarse, A., Schali, M.J., van der Wall, E.E., de Vries, A.A., and Atsma, D.E. 2005. Activation of cardiac and smooth muscle-specific genes in primary human cells after forced expression of human myocardin. *Cardiovasc. Res.* **67**: 245–255.
- Wamhoff, B.R., Bowles, D.K., McDonald, O.G., Sinha, S., Somlyo, A.P., Somlyo, A.V., and Owens, G.K. 2004. L-type voltage-gated Ca²⁺ channels modulate expression of smooth muscle differentiation marker genes via a rho kinase/myocardin/SRF-dependent mechanism. *Circ. Res.* **95**: 406–414.
- Wang, D., Chang, P.S., Wang, Z., Sutherland, L., Richardson, J.A., Small, E., Krieg, P.A., and Olson, E.N. 2001. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. *Cell* **105**: 851–862.
- Wang, D.Z., Li, S., Hockemeyer, D., Sutherland, L., Wang, Z., Schratt, G., Richardson, J.A., Nordheim, A., and Olson, E.N. 2002. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc. Natl. Acad. Sci.* **99**: 14855–14860.
- Wang, Z., Wang, D.Z., Pipes, G.C., and Olson, E.N. 2003. Myocardin is a master regulator of smooth muscle gene expression. *Proc. Natl. Acad. Sci.* **100**: 7129–7134.
- Wang, Z., Wang, D.Z., Hockemeyer, D., McAnally, J., Nordheim, A., and Olson, E.N. 2004. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* **428**: 185–189.
- Wei, L., Zhou, W., Croissant, J.D., Johansen, F.E., Prywes, R., Balasubramanyam, A., and Schwartz, R.J. 1998. RhoA signaling via serum response factor plays an obligatory role in myogenic differentiation. *J. Biol. Chem.* **273**: 30287–30294.
- West, A.G. and Sharrocks, A.D. 1997. The role of DNA-bending in MADS-box transcription factor function. *Biochem. Soc. Trans.* **25**: S639.
- Xing, W., Zhang, T.C., Cao, D., Wang, Z., Antos, C.L., Li, S., Wang, Y., Olson, E.N., and Wang, D.Z. 2006. Myocardin induces cardiomyocyte hypertrophy. *Circ. Res.* **98**: 1089–1097.
- Yin, F. and Herring, B.P. 2005. GATA-6 can act as a positive or negative regulator of smooth muscle-specific gene expression. *J. Biol. Chem.* **280**: 4745–4752.
- Zhang, X., Chai, J., Azhar, G., Sheridan, P., Borrás, A.M., Furr, M.C., Khrapko, K., Lawitts, J., Misra, R.P., and Wei, J.Y. 2001. Early postnatal cardiac changes and premature death in transgenic mice overexpressing a mutant form of serum response factor. *J. Biol. Chem.* **276**: 40033–40040.
- Zhou, J., Hu, G., and Herring, B.P. 2005. Smooth muscle-specific genes are differentially sensitive to inhibition by Elk-1. *Mol. Cell. Biol.* **25**: 9874–9885.

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