Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus

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Miano JM, Long X, Fujiiwara K. Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus. Am J Physiol Cell Physiol 292: C70–C81, 2007. First published August 23, 2006; doi:10.1152/ajpcell.00386.2006.—Serum response factor (SRF) is a highly conserved and widely expressed, single copy transcription factor that theoretically binds up to 1,216 permutations of a 10-base pair cis element known as the CArG box. SRF-binding sites were defined initially in growth-related genes. Gene inactivation or knockdown studies in species ranging from unicellular eukaryotes to mice have consistently shown loss of SRF to be incompatible with life. However, rather than being critical for proliferation and growth, these genetic studies point to a crucial role for SRF in cellular migration and normal actin cytoskeleton and contractile biology. In fact, recent genomic studies reveal nearly half of the >200 SRF target genes encoding proteins with functions related to actin dynamics, lamellipodial/filopodial formation, integrin-cytoskeletal coupling, myofibrillogenesis, and muscle contraction. SRF has therefore emerged as a dispensable transcription factor for cellular growth but an absolutely essential orchestrator of actin cytoskeleton and contractile homeostasis. This review summarizes the recent genomic and genetic analyses of CArG-SRF that support its role as an ancient, master regulator of the actin cytoskeleton and contractile machinery.

myocardin; smooth muscle; CArG box; knockout

Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus

SRF is a founding member of the MADS (Mcm1 and Arg80 in yeast, Agamous and Deficiens in plants, and SRF in animals) domain-containing family of transcription factors and is present in most, if not all, species from the animal, plant, and fungus kingdoms (67, 68, 99). At least one species of protist (Dictyostelium discoideum) harbors an SRF ortholog, but no SRF orthologs have yet been defined in the bacterial kingdom. SRF was so named because of its binding activity to a serum response element located in the promoter of the immediate early transcription factor, FOS (79, 108). Similar serum response elements have been found in the promoter regions of a number of other immediate early transcription factors. Signaling to SRF occurs principally through mitogen-activated protein kinase (MAPK) or RhoA pathways that converge on the nucleus to stimulate gene expression (38). SRF-induced growth-related genes encode for proteins that directly activate growth factor and cell cycle genes (51). Thus SRF is largely an indirect regulator of cell growth, and the in vitro evidence for this stems primarily from gain- and loss-of-function studies (32, 33, 86, 103). Despite the evidence supporting SRF’s role as a growth-associated transcription factor, there are some reports demonstrating an inverse relationship...
between SRF expression and cell growth. For example, SRF reverses the ras-transformed phenotype (54), and RNA interference (RNAi) of SRF phenocopies growth factor-stimulated smooth muscle cell proliferation (49). Perhaps the most direct in vitro evidence blurring the concept of SRF being essential for growth are the studies done in embryonic stem cells null for SRF. Here, although defective expression of immediate early SRF target genes is evident, cells proliferate normally (94). The latter result clearly indicates that compensatory pathways must exist in cell systems to ensure that such a critical process as growth occurs, even in the absence of SRF. As will be discussed later, cytoskeleton-contractile activity cannot be compensated for in cells where SRF expression is reduced or absent.

What in vivo evidence exists to support SRF’s role in promoting a growth phenotype? Balloon injury to the vessel wall evokes rapid increases in c-fos as well as other SRF target genes involved in growth of vascular cells (52, 71, 72). SRF can mediate regenerating epithelium in an in vivo model of gastric ulceration (14), and anti-sense SRF impedes angiogenesis in the same model (15). In yeast, mutation of the minichromosome maintenance 1 (Mcm1) gene results in defective DNA replication, and there is evidence for Mcm1 binding to bonaﬁde origins of replication in the yeast genome (18). In contrast to the above instances, much in vivo genetic evidence exists for SRF playing a critical role in processes unrelated to cell replication. For example, cardiac-restricted overexpression of either wild-type or dominant-negative SRF results in various forms of heart failure because of severe alterations in contractile gene expression (125, 126). Moreover, SRF inactivation or knockdown in D. discoideum (26), Drosophila melanogaster (91), Caenorhabditis elegans (30), and Mus musculus (1, 28, 55, 61, 70, 78, 81, 82) does not result in impaired cell proliferation. Instead, these SRF null models reveal an essential and ancient role for SRF in the organization of normal cytoskeleton and contractile systems (see below). Although the in vivo data indicate, overwhelmingly, that SRF is dispensable for cell proliferation and growth of tissues, when SRF is present it likely plays a redundant role in growth-related processes.

At the same time SRF was being characterized as a transcription factor activating growth-promoting genes, independent work from the muscle field showed SRF binding within the promoter region of several contractile genes that mark the terminally differentiated state of sarcomeric muscle (8, 59, 74). We now appreciate that SRF can activate a number of contractile genes, the expression of which spans all three differentiated muscle types. Indeed, developmental studies reveal the highest levels of SRF mRNA expression in differentiating cardiac, skeletal, and smooth muscle cell lineages (6). Smooth muscle cell-restricted SRF target genes are of particular interest, since their compromised expression is a hallmark of vascular and visceral smooth muscle cell diseases as well as metastatic cancer (80, 88). The realization that SRF controls mutually exclusive programs of gene expression (growth vs. muscle differentiation) led to an important question in the SRF world: how could a widely expressed transcription factor direct such disparate programs of gene expression? SRF, per se, effects only subtle changes in gene expression. However, nearly 50 SRF cofactors (list available upon request) have been defined that restrict SRF-mediated transcriptional activities to cell-specific gene sets.

Among SRF cofactors, the myocardin (MYOCD) family of transcription factors has been of special interest in the context of vascular smooth muscle cell (SMC) differentiation (65, 84). First cloned in a bioinformatics screen for cardiac-restricted transcription factors (110), MYOCD is among the most powerful eukaryotic transactivators known. Through a close partnership with SRF, MYOCD can execute a near-complete program of SMC differentiation in vitro (19, 111, 121). Indeed, MYOCD knockout mice arrest shortly after establishment of a functional cardiovascular system because of impaired vascular SMC differentiation (63). Two other myocardin-related transcription factors (MRTF-A and MRTF-B) have been identified that are more widely expressed than MYOCD and appear to direct distinct programs of gene expression (95, 112). Interestingly, levels of MYOCD mRNA are as high in vascular SMC as they are in cardiac muscle, but the levels of MYOCD are sharply attenuated when SMC are cultured in vitro or injured in vivo (19, 41). Thus the phenomenon of an attenuated SMC differentiated phenotype, first recognized 36 years ago (31), may in part relate to altered MYOCD expression in this context, it will be of great interest to understand the transcriptional nature of MYOCD gene expression.

The binding of MYOCD family members to SRF has been delimited within a region of MYOCD proteins bearing homology to the B-box of the ELK family of SRF cofactors (113, 122). In an elegant study from the Olson laboratory, the ability of SRF to bind either ELK1 or MYOCD was shown to depend on the phenotype of SMC. When SMC are stimulated with a growth factor, the MAPK-ELK1-SRF axis is engaged and SRF-ELK1 complexes are favored over SRF-MYOCD complexes. As a result, growth-related genes are more likely to be activated than SMC contractile genes. Conversely, when serum is withdrawn and the MAPK-ELK1-SRF axis is not engaged, SRF-MYOCD complexes are favored and SMC contractile gene expression ensues (113). Similar molecular events are likely to exist with other disparate programs of SRF-dependent gene expression. The SRF-MYOCD relationship highlights one of several known mechanisms wherein ubiquitously expressed SRF can direct specific programs of gene expression in a cofactor-mediated manner (Fig. 1). Another critical determinant of SRF-dependent gene expression is SRF’s binding to a diverse assortment of DNA cis element sequences, a subject we take up next.

FUNCTIONAL SRF BINDING SITES: THE CarGome

As indicated earlier, SRF binds serum response elements in the promoter of various immediate early transcription factors. The core sequence of the serum response element is a CC ATT rich GG sequence or CarG box (74), which is common to all SRF target genes. Serum response elements, by definition, contain an adjacent cis element (called an ETS site) that binds members of the ELK family of SRF cofactors (11). A misconception regarding serum response elements and CarG boxes continues to exist in the SRF world and is in need of clarification. Specifically, serum response elements and CarG boxes are often referred to as one in the same; however, they should not be considered synonymous since CarG boxes are not always associated with adjacent ETS sites. Therefore, in this
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Fig. 1. Serum response factor (SRF) controls disparate programs of gene expression through cofactor associations. Schematic illustrates some of the SRF-dependent gene programs that are specified by the recruitment of cell-restricted cofactors. A growing number of actin cytoskeleton genes are direct targets of SRF although the cofactors involved in such gene activation have yet to be fully disclosed. See text for more details.

review, we will only refer to SRF-binding sites as CArG boxes or CArG elements.

Binding site selection and scores of promoter studies have helped elucidate the base sequence rules for SRF binding (60, 68, 119). Accordingly, we may classify CArG elements in two broad categories, consensus CArG and CArG-like. Consensus CArG boxes are 10-base pair sequences of DNA conforming to the general rule, CCWWWWWWGG (where W may be either an A or T nucleotide). There are 64 possible consensus CArG elements to which SRF may bind (either A or T across 6 nucleotides = 2^6 or 64). SRF binds with high affinity to consensus CArG boxes. CArG-like elements, to which SRF binds with weaker affinity, are essentially a consensus CArG boxes with base pair deviation from the consensus CCW6GG (e.g., ACW6GG, CCW6TG, CCGW6GG, etc.). If we consider each central W substituted with either C or G (e.g., CCWSW4GG, CCWWSW3GG, etc), then there are 6 x 2^6 = 384 deviations from the consensus CArG. Furthermore, there are 12 potential single nucleotide changes across the terminal CC (either C may be substituted with A, G, or T) or GG (either G may be substituted with A, C, or T) of an otherwise consensus CArG yielding 12 x 2^6 = 768 additional deviations from consensus CArG. Thus there are 384 + 768 = 1,152 theoretical CArG-like elements. Combined with consensus CArGs, SRF can theoretically bind 1,152 + 64 = 1,216 permutations of CArG. These theoretical data have been of enormous utility in developing a bioinformatic approach to identify genome-wide, functional CArG elements, which is our next topic of discussion.

The acquisition of the genetic code of life in a growing number of organisms heralds an unprecedented opportunity to scan different species of genomes for similarities in sequence composition (i.e., comparative genomics). This approach has clearly assisted investigators in defining coding exons that average ~150 base pairs. Defining regulatory sequences (e.g., CArG boxes), averaging between 8 and 12 base pairs, is much more challenging, particularly if the binding rules of the transcription factor are poorly resolved. With much of the rules governing SRF binding to CArG understood, we set out to perform a genome-wide scan for functional CArG elements in an attempt to further define the mammalian CArGome (106). Our approach involved two important filters to reduce “genomic noise.” First, we confined our search to CArG boxes that were 100% conserved in sequence and space between human and mouse. It is thought that nucleotide sequence constraint signifies important functions such as transcriptional regulation (22). Second, we only scanned an 8-kb window of sequence around protein-coding genes with annotated transcription start sites (4 kb upstream and 4 kb downstream of the annotated start site). The rationale for this filter was that virtually all functional CArG elements are within the boundaries of this window (68). Applying these filters to the mouse and human genomes resulted in the recovery of nearly 200 putative CArG-containing target genes, 10 of which had previously been validated (106). The distribution of these CArG sequences across the 8-kb window approximated a bell curve with an equal likelihood of finding a considered CArG box in the proximal promoter or downstream intronic/exonic sequence. Interestingly, nearly 10% of the novel CArG sequences were found within coding exons, which generally do not contain regulatory sequences for transcription (45). After manually validating the computer’s prediction of conserved CArG elements, a total of 89 targets were cloned for experimental validation using luciferase reporter, gel shift, chromatin immunoprecipitation (ChIP), and RNAi knockdown of SRF. Most (60) of the targets evaluated were found to be functional in two or more of the assays employed. Remarkably, the Gene Ontology categorization of each CArG element’s associated gene revealed nearly half to be associated with the actin cytoskeleton or contractile systems (106). Several independent groups using different methodologies have also reported a number of SRF target genes with known functional roles in cytoskeleton and contractile systems (3, 83, 96, 124). A comprehensive list of all functionally validated SRF target genes is available upon request.

SRF-REGULATED ACTIN CYTOSKELETON GENES

An impressive list of SRF target genes with clear physiological functions in the actin cytoskeleton and contractile systems exists with the number of actin cytoskeleton genes now surpassing those of contractile genes (Table 1). It is interesting to point out that, although the transcriptional regulation of most cytoskeleton genes in Table 1 has only recently been revealed, much information exists regarding the role of the cytoskeleton in gene transcription. In this context, SRF target genes are known to be governed by dynamic changes in the actin cytoskeleton. For example, the small GTPase, RhoA, which induces the formation of stress fibers, can activate SRF-mediated gene expression (43). Subsequent studies showed that the increase in SRF activity via RhoA was through the depletion of globular (G) actin during filamentous (F) actin polymerization (102). At least two RhoA-dependent pathways mediate a reduction in G-actin levels. One pathway involves members of the formin family of cytoskeleton proteins (Dia-

phenous) and the other is through the ROCK-LIMK-cofilin axis. Both RhoA-dependent pathways act to stabilize F-actin (35, 102). It is evident that one consequence of decreasing G-actin levels is the liberation of MRTF-A (normally bound to G-actin in the cytosol), its translocation to the nucleus, and subsequent association with SRF to direct context-dependent

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Table 1. Cytoskeleton-contractile SRF target genes

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HUGO gene nomenclature symbols (human) are indicated for each category of serum response factor (SRF) target genes. The designations are not meant to be absolute, and we acknowledge that some targets could be categorized in more than one column (e.g., CNN1 is known to be involved in both cytoskeleton and contractile processes). *Actin filament-associated genes; **filament reorganization genes; ***integrin coupling-associated genes; ****smooth muscle cell-restricted genes; $$$skeletal muscle cell-restricted genes; $$$$cardiac muscle cell-restricted genes; $$$$$calcium homeostasis-related genes. This table was constructed from data obtained in Refs. 3, 68, 83, 96, 106, and 124. See text for further details.

gene expression (58, 75). SRF itself is subject to cytoplasmic-nuclear shuttle in some cellular contexts (48, 64). In addition to RhoA-induced signaling to SRF, there are LIM proteins (CRP1 and CRP2) that can associate with SRF in the nucleus to direct SRF-dependent gene expression (16). Moreover, β-actin is found in the nucleus and has been implicated in the assembly of a preinitiation complex for gene transcription (44). These studies represent a sampling of data supporting an increasingly important role for cytoskeleton-associated proteins in the control of gene transcription. It is noteworthy that a growing number of actin cytoskeleton genes are themselves direct targets of SRF, implying the existence of a feedback loop for SRF-dependent control of actin cytoskeleton-related processes (84, 106). The subject of actin cytoskeleton biology is a daunting one to address, so we confine our discussion in this review to the role of the actin cytoskeleton in one biological process where SRF is clearly of importance—cell migration.

Cell migration is central to myriad developmental (e.g., gastrulation) and pathological (e.g., metastasis) processes. We can consider cell migration occurring in a series of at least four steps (62, 100). First, a portion of the cell membrane must buckle outwardly in the direction of migration to form a lamellipodium in a process known as cell protrusion. Second, focal adhesions must be made at the leading edge of the migrating cell (i.e., at the lamellipodium) to create the necessary matrix-cell-cytoskeleton connections for migration to proceed. Third, the interior actin cytoskeleton must engage myosin motors to elicit active contraction. Fourth, as contraction occurs, adhesions at the “rear” or trailing edge of the cell must dissipate to allow cell propulsion in the direction of the lamellipodium. Each of these steps involves a complex interplay between the cell’s extracellular matrix and the interior actin cytoskeleton, mediated by cell adhesion proteins such as integrins (see below). The actin cytoskeleton can therefore be thought of as a critical link between the extracellular milieu surrounding a cell and the interior cell signaling pathways that ultimately converge in the nucleus to effectuate gene expression control. For purposes of brevity, we shall focus on three aspects of actin cytoskeleton-associated cell migration to highlight the intimate role of SRF in the control of this important biological process.

Actin Filament Dynamics

The actin cytoskeleton, long thought to be a static scaffold for the maintenance of cell shape, polarity, and mechanical support, undergoes dynamic remodeling involving scores of proteins that regulate the cytoskeleton (21, 85). One of the central features of actin filament dynamics and the ability of a cell to migrate is a process known as actin treadmilling, which, as alluded to earlier, plays a key role in RhoA-mediated SRF activation (102). Fundamentally, actin treadmilling involves the polymerization of ATP-G-actin into an existing actin filament at the plus or barbed end and depolymerization of ADP-actin at the minus or pointed end. Several SRF target genes encode for proteins that play a key role in actin treadmilling (Table 1). For example, profilin 1 (PFN1) is known to bind and sequester G-actin but can readily facilitate the polymerization of G-actin into F-actin by virtue of its ability to...
accelerate nucleotide exchange of ADP-actin for the more polymerization-competent ATP-actin (117). Recently, PFN1-mediated polymerization of actin filaments was shown to be accelerated in the presence of various members of the formin family (e.g., Diaphenous; see Ref. 56). Although no formin genes have yet to be described as direct targets of SRF, there are two formin-binding proteins (FNBP) that are hypothesized to be SRF dependent (Table 1). PFN1 knockout mice arrest early during embryogenesis, presumably because of a defect in cytokinesis (117). A hypomorphic allele of the PFN1 ortholog in Drosophila (chickadee) elicits clear defects in border cell migration during development (109). A second, neural-restricted profilin gene (PFN2) is presently a hypothetical SRF target (Table 1). Four of the most widely cited actin depolymerization or severing proteins [destrin (DSTN), coflin (CFL) 1, CFL2, and gelsolin (GSN)] are now known to be direct transcriptional targets of SRF (Table 1). GSN, which has several functions in actin cytoskeleton remodeling, severs actin filaments in a calcium-dependent manner (105). Fibroblasts null for GSN exhibit elevated stress fibers and a defect in cell migration (105). DSTN and CFL depolymerize F-actin by increasing the dissociation constant of ADP-actin at the pointed end of actin filaments, thereby increasing pools of ADP-actin for sequestration by PFN1 (4). DSTN- and CFL-mediated depolymerization is balanced, in part, through competitive binding of F-actin by tropomyosin (TPM), which acts to stabilize F-actin filaments. Several isoforms of TPM are known or hypothetical SRF targets (Table 1). Another stabilizer of F-actin (and SRF target) is the SMC-restricted calponin (CNN1) gene, which is found in association with stress fibers along the long axis of SMC in culture (37, 69). Importantly, DSTN-CFL activity is negatively regulated by LIMK-mediated phosphorylation of these proteins at an NH2-terminal serine residue (120). Such phosphorylation can occur through RhoA signaling, which ultimately activates SRF (see above). The fate of phosphorylated DSTN-CFL is unclear, but it is intriguing to consider that such proteins could be destined for degradation. In this setting, the elevation of SRF activity could provide for a mechanism of restoring DSTN-CFL levels. The phenotype of DSTN-CFL null mice is unknown; however, worms deficient in CFL exhibit a severe impairment in myofibrillogenesis (4). Given the critical role of actin filament dynamics in a variety of cell functions, particularly cell migration, and the fact that several key genes in actin filament regulation are direct transcriptional targets of SRF, it is entirely predictive that cells in which SRF is deleted or reduced will show concomitant defects in actin dynamics and cell migration (see below).

**Formation of a Leading Edge**

The protrusive force at the leading edge of a migrating cell represents the epicenter of cytoskeleton activity requisite for cell migration to proceed. Although an explosive actin polymerization is ongoing at the leading edge, there are several additional actin-binding proteins that reorganize actin filaments to facilitate the formation of the lamellipodium. At this point, it may be instructive to define lamellipodia vs. related membrane alterations called filopodia, both of which are involved in cell migration. Lamellipodia are broad outward bucklings of the cell membrane, whereas filopodia are “spikes” at the cell periphery. Whether a cell membrane is deformed to create a lamellipodium or a filopodium relates largely to the presence or absence of key actin-binding proteins (66). Chief among the proteins involved in these two important membrane alterations are actin-associated nucleating, branching, cross-linking, and capping factors. Each of these classes of actin-binding proteins has at least one known SRF target gene (Table 1). The actin-related protein 3 (ACTR3) gene encodes for one component of a seven-member multiprotein composite known as Arp2/3 complex. The Arp2/3 complex binds the side of an existing actin filament and nucleates actin polymerization such that the uncapped barbed end is able to elongate rapidly, generating protrusive force for the lamellipodium (42). The Arp2/3 complex requires signal-induced activation by the WASp/Scar/WAVE family of proteins, which bring the Arp2/3 complex in close apposition to the existing F-actin (42). Interestingly, the Arp2/3 complex moves away from the membrane under conditions favoring filopodial formation, suggesting this membrane alteration does not require Arp2/3 complexes (66). At present, ACTR3 has not been genetically inactivated, so its direct role in cell motility is not known. The process of nucleating an actin filament is coupled closely to filament branching, which has the effect of creating a lattice of actin filaments (so called dendritic nucleation; see Ref. 42). It is the collective forces associated with branching actin filament polymerization (absent myosin motors) that overcome the forces of the cell membrane, thereby facilitating the formation of lamellipodia in the direction the cell will eventually migrate toward (42, 62). Coronin 1a (CORO1A) and two filamins (FLNA and FLNC) are SRF-dependent target genes (Table 1) that are also located at the leading edge of a cell to assist in actin filament cross-linking (29, 42, 92). Several mutations in FLNA have been defined in humans, and mice deficient in FLNA show evidence of defects in cell migration, particularly in the central nervous system (29). It is important to note that the process of actin filament nucleation/branching/cross-linking is carefully regulated by capping proteins that bind barbed ends of F-actin, thereby stabilizing these filaments. RNAi knockdown of capping protein (CAPG) blocks lamellipodia and accentuates the formation of filopodia that have far less F-actin branching but instead exhibit elongating actin filaments (66). It is unclear whether cells deficient in CAPG have any defects in cell migration (66). One capping protein that has been shown to be SRF dependent is CAPZA3 (Table 1). This capping protein is particularly enriched in male germ cells where it is thought to play a critical role in their motility (114). This brief summary of a very complex process of leading edge formation points out several F-actin reorganization steps that are subject to direct control through SRF-mediated target gene activation.

Up to this point, our discussion has focused mainly on cytoplasmic cytoskeleton SRF target genes involved in actin filament modifications necessary for cellular migration. Such changes in the actin cytoskeleton largely involve signals emanating from the cell’s exterior (e.g., chemotactic factors). Certain signaling events for cell migration, therefore, begin at the membrane-matrix interface before the formation of lamellipodia and filopodia (100). Two classes of membrane receptors comprising several SRF target genes serve to bridge the exterior milieu of a cell to its internal actin cytoskeleton: integrins and syndecans. Integrins are noncovalently bound heterodimers of α- and β-subunits that have diverse functions.
in cell biology, including cell migration (46). Integrin β-1 (ITGB1), a known SRF target gene, dimerizes with the majority of α-integrin subunits, including three that are SRF dependent (Table 1). Hence, SRF indirectly regulates many distinct integrin-dependent pathways through ITGB1 gene regulation. Interestingly, ITGB1 is involved in a RhoA-dependent pathway of SRF stimulation beginning at focal adhesions with the activation of focal adhesion kinase (116). Focal adhesions are present at the leading and trailing edge of migrating cells and function to create an attachment point for the cell to interface its actin cytoskeleton with extracellular ligands bound to clustered integrin receptors. Gene inactivation of ITGB1 results in peri-implantation lethality, but several conditional ITGB1 knockouts suggest important functions related to adhesion and migration (9). Two syndecans (SDC2 and SDC4) are also known SRF targets and function at focal adhesions to stabilize integrin receptor-ligand interactions, thereby assisting in the transmittance of mechanical information to the cell interior (118). SDC4 interacts with a PDZ domain-containing protein called synectin, which functions to increase cell spreading and reduce cell migration (118). Moreover, fibroblasts from SDC4 null mice show impaired migration in a scrape wound assay (118). Characterization of SDC2 null mice has yet to be reported.

The clustering of integrins and syndecans at focal adhesions link the matrix world to the actin cytoskeleton through an array of SRF-regulated actin-binding proteins, including vinculin (VCL), dystrophin (DMD), dystrobrevin-α (DTNA), and talin (TLN) (Table 1). VCL is a classic actin-binding protein that serves to stabilize focal adhesions and regulate cell motility (20). Homozygous VCL knockout mice are embryonic lethal because of severe dysmorphogenesis of the heart and brain (presumably, in part, because of faulty migration), and heterozygous mice are susceptible to stress-induced cardiomyopathy stemming from cytocontractile defects in the myocyte (123). DMD and DTNA are closely associated proteins at the membrane cytoskeleton that have important roles in muscle membrane structural integrity and hence normal muscle physiology. Mutations in these genes, particularly DMD, are not well tolerated and result in muscle atrophy and death (90). TLN activates the COOH-terminal tails of β-integrin receptors and stabilizes their interaction with underlying F-actin (12, 46). TLN gene inactivation results in a gastrulation defect resulting from abnormal cell migration and not altered programs related to growth or apoptosis (76). It is expected that cells without TLN will also show defective integrin signaling, a subject that has yet to be addressed.

Several membrane-associated cytoskeleton SRF target genes play demonstrable or hypothetical roles in the regulation of cytoskeleton processes. For example, AKAP12 is a tumor suppressor gene that functions, in part, to establish a normal cytoarchitecture conducive for a cell’s stationary state. When AKAP12 expression is compromised, notable defects in the cytoarchitecture are observed, and the cells display heightening migratory behavior (34). Interestingly, the AKAP12 locus comprises three distinct transcription units with only the AKAP12 α gene under direct control of SRF (104). Another recently described SRF target gene is the mammalian homolog of Enabled (ENAH), which appears to promote actin filament extension along filopodia, enabling cell migration (89). Melusin (ITGB1BP2), a muscle-restricted gene that interacts with the cytoplasmic tail of ITGB1, is hypothesized to be a membrane sensor of mechanical stress in the heart (10). These few examples further demonstrate the critical role SRF plays in mediating both mechanosensory and migratory responses through membrane-associated cytoskeleton target gene activation.

**Cellular Contraction**

The role of SRF in controlling muscle contractile gene expression and differentiation has been reviewed previously (47, 57, 68). New SRF target genes involved in sarcomeric muscle assembly and differentiation include TGFBI11 and BIN1 (98, 115). In addition, many of the genes listed in Table 1 (e.g., DMD, ACTN1) associate with specialized structures in striated muscle (Z- and intercalated disks) to stabilize sarcomeres. In SMC, the contractile apparatus is physically and functionally linked to the actin cytoskeleton, since agents that disrupt the actin cytoskeleton (e.g., cytochalasins) interfere with SMC contraction; sarcomeric muscle is refractory to such treatment apparently because their contractile filaments are stabilized through actin-capping proteins (36). The close relationship between SMC actin cytoskeleton and contractile apparatus is thought to be necessary for the distribution of contractile force across the cell. When SMC lose their native phenotype in various diseases (68, 80), the actin cytoskeleton adopts a more promigratory phenotype as contractile elements dissipate, probably because of a decrease in SRF- and MYOCMd-mediated gene expression.

As for nonmuscle cells, the net effect of changes at focal adhesions and lamellipodia-filopodia after exterior stimuli for migration is the generation of actin-myosin cross-bridging within the cytosol. The sliding filaments create tracional forces that drag the rear part of the cell in the direction of the leading edge (62). Clearly, SRF target genes involved in actin dynamics (see above) are engaged as are SRF target genes that function to stabilize F-actin (CNN1 and TPM1), thereby extending the half-life of mature actin filaments. The principal actin genes involved with nonmuscle contraction are the cytoplasmic β- and γ-actin genes (ACTB and ACTG1), both of which are SRF dependent (Table 1). Until recently, the underlying mechanism guiding spatial distribution of the proteins encoded by ACTB and ACTG1 was unknown. We now understand that the β-actin protein undergoes arginylation, which allows for β-actin redistribution to the leading edge of a cell and the formation of a lamellipodium necessary for cell motility (50). A hypomorphic allele of ACTB has been reported in mice that results in embryonic lethality, although insight into the underlying mechanism for this phenotype is lacking (97). Interestingly, none of the nonmuscle myosin genes appear to be SRF dependent, which contrasts with many muscle myosin genes known to require SRF for optimal expression (Table 1). However, for actin-myosin force generation to proceed in nonmuscle cells and muscle cells, specific myosin light chains must be phosphorylated in a calcium-dependent manner. Several SRF target genes are involved both in the handling of calcium (ATP2A2, CASQ1, PLN, and SLC8A1) and the phosphorylation of myosin light chains (MYLKv6 and MYLKv7). Recently, the CaR element in MYLKv7 (also known as telokin) was deleted in mice, and the phenotype revealed abolished telokin expression and impaired relaxation of SMC.
The latter result provides the first (and only) genetic proof of an “endogenous” CArG element’s essential role in controlling gene expression and contractile activity. Although each of the above SRF target genes relates largely to muscle contractility, a role in nonmuscle contraction cannot be ruled out entirely. In summary, scores of SRF target genes play direct or indirect roles in several aspects of actin cytoskeleton biology relating to migration (Fig. 2). It is particularly striking that the bare minimum number of actin-binding proteins sufficient for actin dynamics (i.e., Arp2/3 complex, ACTB, DSTN/CFL, and CAPZ; see Ref. 85) are all SRF dependent. Clearly, knocking down or inactivating SRF completely would be expected to have an overt phenotype related to defects in actin-cytoskeleton and contractile activity. The data in support of this concept are considered next.

GENETIC EVIDENCE SUPPORTING SRF AS A MASTER REGULATOR OF THE ACTIN CYTOSKELETON AND CONTRACTILE APPARATUS

Multiple species of SRF have either been genetically inactivated or knocked down with RNAi or cell-restricted, Cre-mediated excision techniques. In this section of the review, we provide an up-to-date summary of the experimental data supporting SRF’s role as a master regulator of the actin cytoskeleton and contractile apparatus. A central theme that runs parallel to the preceding section is the concept that SRF controls many aspects of actin cytoskeleton biology related to cell migration (Fig. 2). We therefore emphasize migratory phenotypes in the context of SRF disruption.

In yeast, several mutant alleles of the SRF ortholog Mcm1 exist, displaying a range of phenotypes from loss in viability to defective cell polarization and mating (23). Although no published data exist regarding the state of the cytoskeleton in Mcm1 mutants or the presence of CArG elements in the promoter regions of genes involved in cytoskeleton homeostasis, it is plausible that there are defects in expression of some cytoskeleton-associated genes given the critical role of the cytoskeleton in yeast mating (17). The power of yeast genetics and genomics should be exploited to interrogate its genome for functional CArG elements within cytoskeleton promoters and to examine the basic cytoarchitecture of Mcm1 mutants.

The protist, D. discoideum, carries one SRF ortholog (Srfa), which was originally inactivated by insertional mutagenesis (25). Mutants show normal spore differentiation markers but display defective spore morphology and loss of viability under stress-induced conditions (25). Ultrastructural analysis of mutant spores reveals faulty development of intracellular actin rods, which are required for proper spore maturation (27). At least one known cytoskeleton gene (cofilin B) is reduced in Srfa mutants. Cofilin B likely functions in the proper deployment of actin rods within spores (24, 27). In a separate study with a different mutant Srfa allele, multicellular slugs showed reduced migration compared with normal slugs, suggesting widespread perturbations in cytoskeletal processes relating to locomotion (26). Another invertebrate, C. elegans, also displays a defect in locomotion upon RNAi knockdown of SRF (30). It will be of major interest to characterize functional CArG elements in the promoter of cofilin B as well as additional SRF-dependent cytoskeleton genes in D. discoideum and C. elegans.

D. melanogaster SRF (DSRF) plays a vital role in the terminal branching of tracheal cells (that form oxygen-carrying lumens) of the respiratory system (39) and the correct specification of intervein cells (carrying hemolymph) in the wing (77). The respiratory phenotype arises because of the inability of terminal cells of the tracheal system to migrate and extend long cytoplasmic extensions in the correct spatial manner, and the wing phenotype is the result of a flaw in terminal differentiation. Overexpressing DSRF causes exuberant tracheal cell extensions in wild-type flies and can partially rescue the tracheal phenotype in mutants (39). These results suggest that DSRF controls cytoskeletal events surrounding adhesion, migration, and cytoplasmic outgrowth of tracheal cells. Mutants of the fly ortholog of MRTF-A (SRF cofactor) show similar deficiencies in the migration of tracheal cells as well as altered cardiac and ovarian border cell migration (40, 101). Consistent with mouse studies (see below), DSRF/DMRTF-A mutant phenotypes do not manifest perturbations in cell proliferation (39, 91, 101). Instead, altered cytoarchitecture appears to underlie the migratory phenotypes in these mutant strains of fly. In this context, the Drosophila cytoplasmic actin 5C gene harbors two CArG boxes in as many promoters that may be important targets of DSRF/DMRTF-A for correct migratory responses (7). It is unknown whether these CArG elements bind SRF and regulate directly the promoter activities of Drosophila actin 5C.

Most of the data generated on phenotypes arising from loss in SRF expression have been obtained in mice. The first genetic knockout of SRF was shown to arrest mouse embryos at gastrulation (e7.0 days), with impaired folding of the ectoderm and endoderm and the complete absence of a mesoderm (2). In addition, no primitive streak could be found in mutant embryos. As embryogenesis was normal through e6.5 days, a proliferation defect was considered unlikely even though expression of several SRF-dependent growth genes was reduced (2). It appears then that SRF is essential for mesoderm formation, possibly as a mediator of complex cell migratory events at this stage of development. Embryonic stem (ES) cells have provided further insight into this possibility. SRF null ES cells show clear evidence of attenuated cortical actin networks and cell protrusions, both of which are essential for the cell’s ability to mount a migratory response (94). Subsequent work showed that SRF null ES cells exhibit impaired expression of ITGB1, TLN, VCL, and ZYX (all SRF targets; Table 1) and faulty formation of focal adhesions (93). Moreover, levels of F-actin were severely reduced probably because of the profound loss in SRF target genes involved with actin polymerization (see above). Consequently, cells in which SRF expression is compromised would be expected to fail to migrate, a concept that has been demonstrated in a boyden chamber assay using ES cells null for SRF (93). Conversely, overexpression of SRF has been reported to increase the migration of cultured...
SMC (14). Collectively, there is mounting in vitro evidence supporting a role for SRF in the migration of various cell types. As discussed next, there is growing support for a link between SRF and cell migration in vivo as well.

Over the last two years, several papers have been published in which SRF inactivation was restricted to specific cell types using Cre-LoxP technology. A common finding across virtually all of these studies is the severe disruption of the actin cytoskeleton and/or the contractile apparatus, resulting in defective cell migration and/or force generation. The first report of a conditional knockout (cKO) of SRF used a cardiac-restricted promoter (beta myosin heavy chain) controlling Cre expression to circumvent the early gastrulation phenotype associated with the standard knockout (82). Although the targeting strategy resulted in a truncated SRF protein product of unknown function, mutant mice arrested in midgestation with thinning of the myocardial compact zone, attenuated trabeculations throughout the ventricular chamber, and cardiac dilation (82). Such perturbations in the heart suggest that embryonic development arrested because of heart failure. Significantly, no alterations in myocardial cell proliferation or apoptosis were evident at e10.5 days of development. Higher apoptotic indexes were noted 1 day later, but this was likely a consequence of hypoxia. The attenuated expression of several cardiac transcription factors led the authors to conclude that SRF was critical in the determination and differentiation of myocardial cells (82). A second cKO of SRF used a targeting strategy in which LoxP sequences flanked the proximal promoter and first exon, resulting in complete loss of any SRF product upon Cre-mediated excision with a cardiac/vascular SMC-restricted promoter (SM22-α; see Ref. 70). Here, ultrastructure analysis suggests that loss in SRF expression throughout the cardiovascular system results in defective myofibrillogenesis (in the heart) and cytoskeletal assembly (in the dorsal aorta). Furthermore, an apparent decrease in SMC migration to the dorsal aorta was noted, consistent with the absence of lamellipodial extensions in mutant SMC. No evidence of impaired myocardial or SMC proliferation was observed in mutants. However, several genes involved in cytoskeleton and contractile physiology were severely reduced in expression (70). Similar data were obtained in a separate report where SRF was deleted in the heart using a different cardiac-restricted Cre driver (α-myosin heavy chain; see Ref. 78). In addition, cultured cardiomyocytes carrying deleted SRF alleles show drastically reduced levels of numerous genes involved with sarcomeric assembly and contractile function with virtually no discernable contractile apparatus (3). Moreover, deletion of SRF in the adult heart using tamoxifen-induced, Cre-mediated excision results in cardiac failure and death resulting from dilated cardiomyopathy. Consistent with the embryonic phenotypes, adult hearts absent SRF show highly disorganized sarcomeres (81). Finally, a skeletal muscle cKO of SRF (using a myogenin Cre driver) results in perinatal lethality because of dysmyofibrillogenesis in skeletal muscle (61). The fact that mice were born with skeletal muscle indicates that loss of SRF during embryogenesis was not linked to skeletal muscle hypoplasia, further supporting the notion of SRF being dispensable for proliferation. Thus all three muscle types in which SRF is deleted show, consistently, the critical need for this transcription factor in directly regulating expression of target genes necessary for proper myofilament assembly and the generation of contractile force. Figure 3 shows how RNAi knockdown of SRF alters the actin cytoskeleton in human coronary artery SMC, an effect that would be expected to prohibit SMC migration. The migratory defect seen in vascular SMC in vivo (70) is consonant with defective migratory responses in more ancient species of animal where SRF is deleted (see above). As detailed next, a series of cKO in the central nervous system provide further support for SRF’s salient role in mammalian cell migration.

Perhaps no other organ requires as exquisitely timed and controlled cellular migration as the brain. At least some of this complex neuronal migration is under direct control of SRF and its effects on the actin cytoskeleton. Alberti and colleagues (1) made the first cKO of SRF in the forebrain using a Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II-Cre driver and showed...
defects in neuronal migration with consequent demise at 3 wk of life. They went on to demonstrate marked attenuation of F-actin content in cultured neurons and a parallel decrease in migration. Furthermore, SRF null neurons exhibit markedly reduced GSN mRNA and elevated levels of phosphorylated CFL, both of which have the effect of reducing depolymerization of actin filaments and an attenuating perturbation in actin dynamics (1). These results indicate that SRF is essential for the establishment of a normal cytoarchitecture conducive to direct forebrain neuronal cell migration. Curiously, a competing report using a similar Cre driver did not find defective cytoarchitecture in forebrain neurons, although cell migration was not the focus of this study (87). In a follow-up report, the laboratory of Knöll et al. (55) showed SRF to be necessary for neurite outgrowth and proper hippocampal circuitry. In a bioinformatics approach for conserved CArG boxes, several new SRF target genes were defined and shown to be reduced in mutant hippocampal neurons (55). Interestingly, some of the targets encode for guidance receptors (e.g., EPHA4), suggesting that, in addition to regulating the actin cytoskeleton for migration, SRF controls expression of genes required in directing migrating cells to their proper milieu. In the brain, this has enormous ramifications for coordination and learning, and recent data suggest that SRF is indeed intricately tied to memory and learning, at least in mice (28). As with muscle-specific eKOs of SRF, the neuronal eKOs do not exhibit impaired cell proliferation (1, 28, 87). These data strongly endorse SRF as a dispensable transcription factor for growth but an absolutely vital factor for normal cytoskeletal and contractile activities, the biology of which controls such processes as contractile force generation, migration, and learning.

FUTURE DIRECTIONS

It appears SRF’s regulation of a disproportionate number of cytoskeleton/contractile genes was an early innovation to ensure proper cell migration and force generation in those cells and tissues where SRF is particularly abundant (i.e., muscle and brain). Genetic studies from simple eukaryotes to mice clearly illustrate this fact, revealing, somewhat surprisingly, a dispensable function of SRF for growth-related events. Several paths of SRF research should be continued and new ones explored. For example, whether SRF is truly a master regulator of the actin cytoskeleton will require additional eKOs and knockout studies in nonmuscle/nonneuronal cell types. It would be particularly helpful in this context to partner cell biologists with molecular biologists to delve deeper into the SRF-cytoskeleton connection. What role, for example, does SRF play in intracellular trafficking or phagocytosis, both of which require dynamic changes in the actin cytoskeleton? We might also consider adopting additional vertebrate model systems such as zebrafish to study SRF-dependent biological processes in real time (e.g., recruitment of SMC to dorsal aorta). Scores of candidate SRF target genes, including many linked to the actin cytoskeleton (Table 1), require experimental validation, and further interrogation of genomes using ChIP microarray should be done to develop a “CarG dictionary” of SRF-dependent target genes. A goal should be to fully disclose the functional CarGome so we have all target genes in hand for careful study, particularly those that impinge on the actin cytoskeleton. This analysis should not be limited to vertebrates, since the number of validated SRF target genes in invertebrates currently registers only one (13). Identifying all SRF target genes in multiple species will allow for powerful comparative genomic analyses to discover other common regulatory elements and their trans-acting factors that may cooperate with SRF to effect gene transcription. We also need to understand how CArG-SRF is controlling actin cytoskeleton (and other) target genes (Fig. 1). What SRF cofactors (among nearly 50) are utilized and what are the essential SRF protein interfaces necessary for such gene transcription? In this context, an ambitious plan would be to generate an allelic series of SRF mutants to test in transcription-based assays and in vivo using, for example, zebrafish as a high-throughput model system. Finally, inspection of the human SRF locus at the UCSC Browser (http://genome.ucsc.edu/; Human May 2004 Assembly) reveals a single synonymous coding single nucleotide polymorphism (SNP) (G327G); however, there exists a cluster of 3′-untranslated region SNPs that could have some influence on SRF gene expression (5) in human populations. It is possible that hypomorphic alleles of SRF exist (resulting in its attenuated expression) that are linked to human diseases involving the actin-cytoskeleton and/or contractile apparatus.

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