The serum response factor (SRF) is a transcriptional regulator required for mesodermal development, including heart formation and function. Previous studies have described the role of SRF in controlling expression of structural genes involved in conferring the myogenic phenotype. Recent studies by us and others have demonstrated embryonic lethal cardiovascular phenotypes in SRF-null animals, but have not directly addressed the mechanistic role of SRF in controlling broad regulatory programs in cardiac cells. In this study, we used a loss-of-function approach to delineate the role of SRF in cardiomyocyte gene expression and function. In SRF-null neonatal cardiomyocytes, we observed severe defects in the contractile apparatus, including Z-disc and stress fiber formation, as well as mislocalization and/or attenuation of sarcomeric proteins. Consistent with this, gene array and reverse transcription-PCR analyses showed down-regulation of genes encoding key cardiac transcriptional regulatory factors and proteins required for the maintenance of sarcomeric structure, function, and regulation. Chromatin immunoprecipitation analysis revealed that at least a subset of these proteins are likely regulated directly by SRF. The results presented here indicate that SRF is an essential coordinator of cardiomyocyte function due to its ability to regulate expression of numerous genes (some previously identified and at least 28 targets newly identified in this study) that are involved in multiple and disparate levels of sarcomeric function and assembly.

Role of the Serum Response Factor in Regulating Contractile Apparatus Gene Expression and Sarcomeric Integrity in Cardiomyocytes*

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The serum response factor (SRF) is a member of the MADS (MCM1, AGAMOUS, DEFICIENS, SRF) box family of transcriptional regulatory proteins. SRF was first identified based on its ability to mediate serum and growth factor activation of the c-fos proto-oncogene (1). Subsequently, it was found that SRF and/or SRF-binding sites (CC(A/T)6GG), termed CArG boxes or serum response elements, regulate expression of a wide variety of inducible genes by various stimuli ranging from growth and transcriptional regulatory factors and proteins required for the maintenance of sarcomeric structure, function, and regulation. Chromatin immunoprecipitation analysis revealed that at least a subset of these proteins are likely regulated directly by SRF. The results presented here indicate that SRF is an essential coordinator of cardiomyocyte function due to its ability to regulate expression of numerous genes (some previously identified and at least 28 targets newly identified in this study) that are involved in multiple and disparate levels of sarcomeric function and assembly.

The amino acid sequence of this protein can be accessed through NCBI Protein Database under NCBI accession number GSE13181.

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2. The abbreviations used are: SRF, serum response factor; MHC, myosin heavy chain; Ad, adenovirus; RT, reverse transcription; GFP, green fluorescent protein; MEF-2, myocyte enhancer factor-2.
ent with animal studies, ultrastructural analysis of SRF-null cardiomyocytes revealed severe defects in the contractile apparatus, including Z-disc and stress fiber formation. To characterize gene expression changes that accompanied this phenotype, we performed cDNA array analysis, reverse transcription (RT)-PCR, and immunofluorescence on SRF-null myocytes. This analysis revealed the attenuation of key cardiac, cytoskeletal, and transcription factor genes in cardiomyocytes lacking SRF. These results demonstrate that not only does SRF regulate structural factors important for muscle function, but significantly that SRF is a central coordinator of numerous genes (some previously identified and at least 28 targets newly identified in this study) involved in multiple and disparate levels of sarcromeric function and assembly.

**MATERIALS AND METHODS**

**Primary Cardiomyocyte Culture and Transduction—SRFf/f mice** were generated by Drs. Naren Ramanan and David Ginty as described previously (16, 17). Ventricular tissue was carefully dissected and digested in 0.41 mg/ml collagenase (Sigma) and 0.30 mg/ml pancreatin previously (16, 17). Ventricular tissue was carefully dissected and dissociated in 0.41 mg/ml collagenase (Sigma) and 0.30 mg/ml pancreatin (Sigma) in Ads buffer (116 mM NaCl, 20 mM HEPES, 0.8 mM NaH2PO4, 1 g/liter glucose, 5.4 mM KCl, 0.8 mM MgSO4, pH 7.35). Cardiomyocytes were enriched on a discontinuous Percoll gradient (40.5 over 58.5%; Sigma) and plated at 50,000 cells/well on a gelatin-coated 24-well plate (Costar). Cultured cardiomyocytes were transduced with Ad-Cre (courtesy of Dr. Beverly Davidson, University of Iowa) or a control virus encoding green fluorescent protein (Ad-GFP, Medical College of Wisconsin Adenoviral Core Facility) at a multiplicity of infection of 25. Each Ad construct drives exogenous gene expression via a cytomegalovirus promoter stabilized with a metallothionein polyadenylation signal.

**Electron Microscopy**—Transmission electron microscopy was carried out at the Medical College of Wisconsin Facility for Electron Microscopy. Five days after transduction, cardiomyocytes were fixed in 1% cacodylate-buffered 2.5% glutaraldehyde, embedded in epoxy resin, sectioned, and imaged on a Hitachi 600 electron microscope. Thin sections (60 nm) were evaluated at magnifications of ×5000, ×25,000, and ×40,000.

**Electrophoretic Mobility Shift Assay**—Total protein was isolated from cardiomyocytes 5 days after transduction in lysis buffer (50 mM HEPES (pH 7.9), 150 mM NaCl, 0.1 mM EGTA, 10 mM Na3VO4, 1.5 mM MgCl2, 10% glycerol, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 1 μg/ml leupeptin). A probe containing the SRF-binding site in the Nppa (atriuretic peptide precursor type A) gene was amplified by PCR using primers 5'-CAGGTGTGAGGTAACTCC-A-3' and 5'-GCTGTCAAAGGCTCCAATA-3'. The probe (50 ng) was labeled with [γ-32P]ATP by T4 polynucleotide kinase, incubated with protein lysate (4 μg), and separated on a 5% nondenaturing polyacrylamide gel. Supershift assays were performed by preincubation of protein lysate with anti-SRF antibody (G-20, Santa Cruz Biotechnology, Inc.).

**Identification of Conserved CArG Boxes—**SRF-binding sites were defined as allowing for one nucleotide mismatch from the consensus CC(A/T)6GG sequence (16). Putative SRF target gene sequence including 5 kilobase pairs upstream and downstream of the predicted transcriptional start site was obtained from the May 2004 Mouse Genome Assembly (available at genome.ucsc.edu/). Conservation was determined by the Jotun Hein (79) method of alignment using the Lasergene suite of genome analysis software (DNASTAR, Inc.).

**Indirect Immunofluorescence**—Cardiomyocytes were fixed in 4% paraformaldehyde, permeabilized in 0.5% Nonidet P-40, and blocked in 3% bovine serum albumin. Primary antibodies were used as follows. Rabbit anti-SRF polyclonal antibody (raised against the C terminus of SRF by Proteintech Group Inc.) was used at 1:300 dilution. Anti-phosphohistone H3 antibody (Upstate) was used to label mitotic nuclei at 1:200 dilution. Antibody EA-53 (Sigma) was used to label sarcromeric α-actin at 1:200 dilution. All other antibodies were obtained from the Developmental Studies Hybridoma Bank, University of Iowa: CH-1 (developed by Jim Jung-Ching Lin and used at 1:40 dilution) for labeling sarcomeric tropomyosin, CT3 (developed by Jim Jung-Ching Lin and used at 1:200 dilution) for labeling troponin T, and MF-20 (developed by D. A. Fischman and used at 1:200 dilution) for labeling sarcomeric myosin. The cells were then incubated with a fluorescent secondary antibody (green Alexa 488 or red Alexa 568, Molecular Probes) at 1:200 dilution. The cells were counterstained with 4’,6-diamidino-2-phenylindole (Sigma) to label the nuclei blue or propidium iodide (Sigma) to label the nuclei red and/or with Alexa Fluor 488-conjugated phalloidin (1:20 dilution; Molecular Probes) to label filamentous actin green. Coverslips were mounted on glass slides using Mowiol 4-88 medium (Calbiochem) and visualized using a Nikon Eclipse TE300 inverted fluorescence microscope equipped with ×20/0.45 and ×40/0.60 Plan Fluor objectives. Images were acquired with a SPOT RT digital camera using SPOT RT Version 3.3 imaging software.

**Microarray Hybridization, Statistical Analysis, and Confirmation by RT-PCR—**Mouse 430 2.0 gene arrays (Affymetrix) were used to analyze the transcription profiles of Ad-GFP (control)-transduced and Ad-Cre (SRF-null)-transduced cardiomyocytes. For each array, double-strand cDNA was synthesized from 8 μg of total RNA using oligo(dT) primers, SuperScript II reverse transcriptase, and Escherichia coli DNA polymerase I (Invitrogen). Biotin-labeled antisense cRNA was synthesized using the BioArray HighYield RNA transcript labeling kit (Enzo Biochem, Inc.). The labeled cRNA was purified, fragmented, and subsequently hybridized using the Affymetrix Fluidics Station 400. Raw data were scanned with the Affymetrix Scanner 3000, processed with dChip Version 1.3 software (available at dchip.org), and imported into Microsoft Excel for analysis. A statistical ±1.5-fold change (p < 0.05) was considered significant. For RT-PCR, total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and random hexamer primers (Roche Applied Science). This cDNA provided templates for PCRs that contained dNTPs, [α-32P]dCTP, Taq DNA polymerase, and specific primer pairs. PCR products were separated on 5% acrylamide gels, detected in dried gels using a PhosphorImager (Storm 840, Amersham Biosciences), and quantitated using ImageQuant Version 5.2 software. PCRs were performed at various cycles to ensure linear amplification (data not shown), and minus-RT controls were performed to ensure specific amplification. All numerical quantification is the mean ± S.D. of four independently performed experiments.

**Chromatin Immunoprecipitation—**Ventricular tissue from 3-week-old male CD-1 mice was fixed with 1% formaldehyde and homogenized using 50-μm Medicons in a Medimachine (BD Biosciences). Homogenized tissue was sonicated to obtain 500–800-bp DNA fragments. Immunoprecipitation was performed using a chromatin immunoprecipitation kit (catalog no. 17-295, Upstate) according to the suggested protocol of the manufacturer. For precipitation of SRF, rabbit anti-SRF polyclonal antibody (raised against the C terminus of SRF) was used.3

**RESULTS**

To investigate the role of SRF in cardiac myocyte function, neonatal cardiomyocytes were isolated from homozygous floxed SRF (SRFf/f) animals (16, 17). Excision of the floxed SRF allele was mediated by trans-
**SRF Is Required for Sarcomeric Integrity**

**FIGURE 1.** Transduction with Ad-Cre efficiently mediates excision of floxed SRF alleles in neonatal cardiomyocytes. A, shown is the genomic PCR time course following excision of SRF alleles. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. B, shown is the RT-PCR time course demonstrating depletion of SRF mRNA following Ad-Cre transduction. C, quantification of RT-PCR analysis indicated a 15.7 ± 4.1-fold decrease in SRF mRNA 5 days after transduction with Ad-Cre (n = 4), *p < 0.01 versus Ad-GFP control cells. D, shown is the immunofluorescence (IF) of SRF protein (green) in cardiomyocytes following Ad-Cre treatment. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue). Scale bars = 20 μm. E, mock-transduced cardiomyocytes were 95 ± 2.4% SRF-positive by immunofluorescence compared with 5.2 ± 3.9% 5 days after Ad-Cre transduction (n = 4), *p < 0.01 versus control cells. F, electrophoretic mobility shift assay (EMSA) was performed using the 32P-labeled oligonucleotide probe from the Nppa promoter. Protein lysates from cardiomyocytes were supershifted using SRF-specific antisera to confirm specificity. G, the relative amount of SRF protein was reduced by 27.8 ± 8.2-fold in Ad-Cre-treated cells by day 5 (n = 3), *p < 0.01 versus Ad-GFP-transduced cells.

The efficiency of the Ad-Cre-mediated excision over a 5-day time course was evaluated at 24-h time points via genomic PCR assay using primers specific for the excised region of SRF (Fig. 1A). Unexcised SRFCre alleles were undetectable by this method 5 days after transduction. To ensure that no spurious transcription of the excised SRFCre gene occurred following excision, an RT-PCR time course was carried out using primers specific to the SRF C terminus (Fig. 1B). SRF mRNA was significantly reduced (p < 0.01) by 15.7 ± 4.1-fold in myocytes transduced with Ad-Cre relative to those transduced with the control virus, Ad-GFP (Fig. 1C). GFP is commonly used as a molecular marker and is thought to be biologically inert (18). However, an isolated report has suggested that high levels of GFP expression may be linked to dilated cardiomyopathy in transgenic mice (19). In this study, we did not observe significant differences between cardiomyocytes transduced with Ad-GFP and untreated cells (data not shown).

We have shown previously that the half-life of SRF protein in fibroblasts is ~12 h (20). To determine whether significant levels of SRF protein persist 5 days after Ad-Cre transduction in cardiac myocytes, we assayed the presence of SRF protein using indirect immunofluorescence (Fig. 1D). We observed that 95.9 ± 2.4% of the myocytes were SRF-positive in untransduced control cells compared with 5.2 ± 3.9% in myocytes 5 days after Ad-Cre transduction (Fig. 1E). To determine whether this represents a significant decrease in overall functional SRF protein, we performed electrophoretic mobility shift assays 5 days after transduction with Ad-Cre or Ad-GFP (Fig. 1F). Quantification of the results from the electrophoretic mobility shift assay experiments (n = 3) revealed a 27.8 ± 8.2-fold decrease in SRF protein 5 days after treatment with Ad-Cre (Fig. 1G). Based on these results, we conclude that transduction with Ad-Cre mediates an efficient reduction of SRF mRNA and protein within 5 days after transduction.

SRF cofactors in the ETS family have been shown to regulate proliferation and to protect human embryonic kidney cells from apoptotic death through an SRF-dependent mechanism (21). Additionally, neonatal mice with inactivated Homeodomain-Only Protein (HOP), an SRF inhibitor, display cardiac hyperplasia (22). In contrast, SRF-null embryonic stem cells are viable and continue to proliferate in culture (23), and cardiac-specific inactivation of SRF during embryonic development appears to have no effect on cardiomyocyte proliferation (13, 14). In light of these incongruous data, we addressed whether SRF is required for cardiomyocyte viability by trypan blue exclusion assay and whether SRF is required for cardiomyocyte proliferation by staining a mitotic indicator, phosphohistone H3. As shown in Fig. 2 (A–C), we observed
SRF is required for structural maintenance of the contractile apparatus in cardiac myocytes. Cardiomyocytes treated with Ad-GFP (A, C, and E) contained clearly identifiable Z-disc sarcomeric banding characteristic of normal cardiac myofibrillar organization (arrows in C). Cells treated with Ad-Cre (B, D, and F) exhibited only sparse pockets of unsubstantial and disorganized myofilaments (indicated by arrow in D). Spiral polysomes were less frequently observed in SRF-null embryonic stem cells (arrows in E and F). Scale bars = 0.5 μm.

To identify SRF-dependent genes responsible for the maintenance of contractile apparatus structure, we performed cDNA expression analysis on SRF-null cardiomyocytes. Microarray technology (Affymetrix GeneChip 430 2.0) was used to define the transcription profile of >34,000 mouse genes in cardiomyocytes treated with Ad-Cre or with Ad-GFP as a control. The number and diversity of gene expression changes in SRF-null cardiomyocytes was striking. There were 388 genes (281 known genes and 107 additional expressed cDNAs) that exhibited in Ad-Cre (5.1 ± 2.0%)- and Ad-Cre (5.6 ± 2.8%)-treated neonatal cardiomyocytes (n = 9).

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Taken together, these observations indicate that SRF is required for structural maintenance of the contractile apparatus in cardiac myocytes.

no difference in cell viability or proliferative capacity in SRF-null cardiomyocytes compared with control cells. The proliferation rates of SRF-null cells (5.6 ± 2.8%) and control myocytes (5.1 ± 2.0%) were consistent with the 3–12% proliferation rate previously reported for rodent neonatal cardiomyocytes (24). Together, these data support the conclusion that SRF is dispensable for cardiomyocyte viability and division.

Examination of control neonatal cardiomyocyte cultures revealed that the majority of the cells were spontaneously beating. However, we observed only isolated rare pockets of weakly beating myocytes 5 days after treatment with Ad-Cre, suggesting that cell morphology and regulation and/or structure of the contractile apparatus may be disrupted in SRF-null cells. Numerous studies have indicated that SRF is important for regulating various aspects of cellular architecture, including cell shape, cell movement, actin dynamics, and actin gene expression. Therefore, we initially examined the effect of SRF on cell morphology by comparing control and SRF-null cardiomyocytes by transmission electron microscopy. Transmission electron microscopy analysis revealed dramatic differences in myofibrillar organization between SRF-null and control cells. In control cells, myofibrils were organized into large parallel stacks, divided into ~1.25-μm sarcomeric units by evenly spaced Z-disks (Fig. 3, A and C). Mitochondria were observed to be frequently aligned and in close association with the myofilaments. In stark contrast, SRF-null cardiomyocytes generally contained a small number of insubstantial and unaligned myofilaments, which were frequently observed to be unassociated with mitochondria (Fig. 3, B and D). In some cells, small bundles of myofilaments were observed in close association with the membrane, reminiscent of the punctate round Z-bodies commonly thought to represent the precursors of Z-discs in developing striated muscle (25). Interestingly, we also observed a reduction in the abundance of spiral polysomes, which are thought to indicate myosin translation, in SRF-null cells (Fig. 3F) compared with control cells (Fig. 3E). Taken together, these observations indicate that SRF is required for proper assembly of the contractile apparatus in cardiac myocytes.

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### TABLE 1
Summary of genes with known function that are attenuated in SRF null cardiomyocytes as determined by gene array analysis.

Genes are functionally grouped. FC, -fold change.

| gene symbol | alias or protein | NCBI ID | probe set ID | FC   |
|-------------|------------------|---------|--------------|------|
| ACTA1       | skeletal alpha actin | M1223 | 1427735_a_at   | -14.6±0.1 |
| ACTA2       | smooth muscle alpha actin | AW214292 | 1444105_a_at   | -4.3±0.2  |
| ACTC1       | cardiac alpha actin | NM_009608 | 1415927_at     | -1.8±0.1  |
| CNN1        | calponin 1 | NM_009922 | 1417917_at     | -7.2±0.2  |
| CSRP1       | cysteine and glycine-rich protein 1 (CRP1) | BF124540 | 1425646_at     | -1.9±0.3  |
| CSRP2       | cysteine and glycine-rich protein 2 (CRP2) | NM_007792 | 1420731_a_at   | -1.5±0.1  |
| DES         | desmin | A156158 | 1426731_a_at   | -1.6±0.1  |
| FH1         | four and a half LIM domains 1 (SLIM1) | U41739 | 1417872_at     | -1.7±0.1  |
| LDB3        | LIM domain binding 3 (Cypher1) | BB479063 | 1433783_at     | -1.6±0.1  |
| MYH6        | myosin heavy chain 6, alpha cardiac | BB481540 | 1417729_at     | -6.1±0.2  |
| MYH7        | myosin heavy chain 7, beta cardiac | NM_080728 | 1448553_at     | -4.5±0.1  |
| MYL3        | myosin, light peptide 3 (MLC1v) | AK002312 | 1428266_at     | -1.5±0.2  |
| MYL4        | myosin, light peptide 4 (MLC1a) | NM_010858 | 1422580_at     | -1.8±0.1  |
| MYL9        | myosin light chain 9, smooth muscle | AK007972 | 1452670_at     | -2.9±0.1  |
| MYO1B       | myosin 1B | AA406997 | 1459679_a_at   | -1.8±0.5  |
| MYOM1       | myomesin 1 (skelemin) | NM_010867 | 1420693_at     | -2.3±0.1  |
| NEBL        | nebulin | BC025863 | 1458184_a_at   | -1.8±0.8  |
| PDLIM7      | PDZ and LIM domain 7 (Enigma) | NM_026131 | 1417959_at     | -4.6±0.1  |
| TAGLN       | transgelin (SM22) | BB114067 | 1423505_at     | -1.5±0.1  |
| TCAP        | titin-cap (telethonin) | AK010167 | 1423145_a_at   | -2.0±0.1  |
| TMD3        | tropomodulin 3 | BB749726 | 1455708_at     | -1.7±0.3  |
| TNNT1       | tropomin C, cardiac / slow skeletal | NM_009393 | 1418370_at     | -6.4±0.1  |
| TPM1        | tropomyosin 1, alpha | AV209969 | 1447713_at     | -2.1±0.1  |
| TPM2        | tropomyosin 2, beta | AK003186 | 1419738_a_at   | -1.8±0.1  |
| TTN         | titin | BC025840 | 1427446_a_at   | -1.5±0.1  |
| BVES        | blood vessel epicardial substance | NM_024285 | 1419003_at     | -1.7±0.1  |
| CFL2        | cofilin 2, muscle | A1323758 | 1418066_a_at   | -2.3±0.2  |
| CMYA1       | cardiomyopathy associated 1 | NM_011724 | 1419220_at     | -1.8±0.1  |
| DMD         | dystrophin | NM_007868 | 1417307_at     | -1.7±0.2  |
| DTNA        | dystrobrevin alpha | AF143543 | 1425292_at     | -1.5±0.3  |
| ENAH        | enabled homolog (MENA) | NM_010135 | 1421624_a_at   | -2.4±0.4  |
| FNB1        | formin binding protein 1 | AI606033 | 1453745_at     | -2.0±0.4  |
| FNB3        | formin binding protein 3 | BBM39901 | 1442171_at     | -2.9±0.2  |
| ITGA9       | integrin alpha 9 | NM_133721 | 1460282_a_at   | -1.8±0.3  |
| ITGB1       | integrin beta 1 | BB236218 | 1430630_a_at   | -1.9±0.8  |
| ITGB1BP2    | integrin beta 1 binding protein 2 (melusin) | AK003906 | 1423238_a_at   | -6.5±0.1  |
| JPH2        | junctophilin 2 | BG870711 | 1455404_at     | -2.1±0.1  |
| KRT1-18     | keratin complex 1, acidic, gene 18 | NM_010664 | 1448169_a_at   | -2.8±0.2  |
| MAPRE2      | microtubule-associated protein (RP1) | BC027056 | 1451989_a_at   | -3.3±0.3  |
| MSN         | moesin | NM_018333 | 1450379_at     | -1.6±0.1  |
| PARD6B      | par-6 | BE953582 | 1423175_a_at   | -3.6±0.1  |
| PCDH7       | protocadherin 7 | BB197591 | 1456214_at     | -1.7±0.2  |
| PCDH18      | protocadherin 18 | BM218630 | 1422890_at     | -1.5±0.6  |
| PCDH22      | protocadherin beta 22 | NM_053147 | 1418941_at     | -1.5±0.5  |
| PKP2        | plakophilin 2 | AA516617 | 1449799_s_at   | -1.7±0.1  |
| SCEL        | scillin | NM_022886 | 1422837_at     | -1.9±0.4  |
| SGCB        | sarcoglycan, beta | AK014381 | 1419668_at     | -1.9±0.2  |
| SVIL        | supervillin | BM203457 | 1460694_a_at   | -1.9±0.1  |
| SYNPO2L     | synaptopodin 2-like | AK004253 | 1428295_at     | -3.4±0.1  |
| TCAML       | testicular cell adhesion molecule 1 | NM_029467 | 1421540_at     | -2.2±0.7  |
| TGBF111     | transforming growth factor beta1 induced 1 | NM_009365 | 1418136_at     | -2.1±0.1  |
| THBS1       | thrombospondin 1 | AI385532 | 1450377_at     | -1.6±0.2  |
| TJP1        | tight junction protein 1 (ZO-1) | NM_009386 | 1417749_a_at   | -1.5±0.1  |
| ANKRD1      | ankyrin repeat domain 1, cardiac (CARP) | AK009959 | 1420991_at     | -1.6±0.1  |
| ANKRD6      | ankyrin repeat domain 6 | BM199054 | 1450227_at     | -1.7±0.4  |
| EGR1        | early growth response 1 | NM_007913 | 1417065_a_at   | -2.6±0.2  |
| LBH         | limb-bud and heart | BC026827 | 1451629_at     | -1.5±0.2  |
| WT1         | Wilms tumor homolog | M55512 | 1425995_s_at   | -1.8±0.2  |
functional contractile apparatus in the heart were significantly down-regulated in SRF-null cardiomyocytes. For example, the expression levels of three α-actin isoforms, calponin, cardiac troponin C, desmin, two MHC isoforms, SM22, and two tropomyosin isoforms were attenuated by ≥3.0-fold. The LIM domain-containing proteins CRP1 and CRP2 were also attenuated in SRF-null myocytes. CRP family members bind to the cytoskeletal and contractile proteins zyxin and actinin (28).

Significant attenuation of cytoskeletal (dystrophin and melusin) and cell adhesion (ENAH (enabled homolog), α1 integrin, and ZO-1) proteins was also observed. This is consistent with variations in adhesive interactions among cardiac myocytes that have been implicated in cardiovascular disease (29). For example, disruption of MENA localization at the focal adhesions of murine hearts results in cardiomyopathy (30). Together, these data suggest that SRF is likely to be required for the maintenance of cell junctions in cardiac myocytes. Because cardiomyocytes in culture do not form proper cell-cell junctions, to more definitively address the role of SRF in cell adhesion, it will be important to test cell adhesion characteristics using an in vivo animal model system.

The array data also revealed attenuation of several transcription factors implicated in the regulation of cardiac gene expression (e.g. CARP, EGR1, LBH, and WT1). However, it was surprising that the expression levels of three important cardiac transcription factors (GATA-4, myocardin, and NKX-2.5) appeared unchanged in the gene array analysis because we (13) and others (14) have observed decreased expression of these factors in SRF-depleted tissues using conditional mouse knockout models. To address this apparent paradox, we performed RT-PCR and found that these factors were indeed reproducibly and significantly inhibited in SRF-null cardiomyocytes (Fig. 4). The disparity between these methods may be the result of array probe set cross-hybridization with differentially expressed isoforms of these factors. For example, the expression levels of other members of the GATA family were unchanged in SRF-null cardiomyocytes (e.g. see GATA-6 expression in Fig. 4). Cross-hybridization of GATA family members with the GATA-4 probe set would result in the false negative we observed. Myocardin and NKX-2.5 also have differentially expressed members for their respective families. An additional factor that may contribute to this discrepancy is the generally low level of transcription factor expression. Others have observed similar disparities in liver studies using Affymetrix GeneChip 430 2.0 to detect transcription factors such as hepatocyte nuclear factor-4.4.

It has been reported previously that genes for calcium-handling proteins such as SERCA2 and NCX1 are regulated by SRF (9, 31). We therefore investigated whether SRF is important in cardiomyocytes for expression of genes that encode calcium-handling and calcium-regulated proteins. Inspection of the array data revealed that expression of SERCA2 and calcipressin-1 was down-regulated in SRF-null cells. The SERCA2 protein, encoded by the *Atp2a2* gene, is a central regulator of cytoplasmic Ca²⁺ levels in the heart. Immediately following contraction, SERCA2 pumps return Ca²⁺ to intracellular stores. Calcipressin-1, encoded by the *Dscr1* gene, is an endogenous inhibitor of calcineurin, a Ca²⁺-dependent phosphatase. A recent study demonstrated that calcineurin is localized to the cardiac muscle Z-disk by LIM domain-containing proteins, although the functional significance of this remains unclear (32). The array data were validated using semiquantitative RT-PCR, which confirmed the down-regulation of these genes (Fig. 4).

Other cardiac genes that were found to be significantly attenuated in SRF-null cardiomyocytes include *Ckm* (creatine kinase, muscle), *Nppb* (natriuretic peptide precursor type B), and *Sdc2* (syndecan-2). CKM regenerates ATP needed for sarcomeric contraction. NPPB is a peptide hormone important for the regulation of blood pressure and cardiovascular homeostasis. SDC2 is known to link the cytoskeleton to the extracellular matrix and has been implicated in cardiac remodeling (33).

Together, the results in Fig. 4 and Table 1 demonstrate that SRF controls cardiomyocyte function by regulating expression of genes important for multiple levels of cardiac muscle fiber form and function, including sarcomeric and transcriptional regulatory proteins. It is important to note, however, that not all sarcomeric proteins are deregulated in SRF-null cells. For example, cardiac α-actinin isoforms and cardiac troponin T levels were unchanged by RT-PCR (data not shown),
indicating that SRF is not a general regulator of all sarcomeric genes, but specifically activates the expression of a subset of cardiac genes.

To address whether the cardiac genes identified via array analysis are directly or indirectly regulated by SRF, we first utilized an informatics-based approach to identify conserved CArG box elements. We interrogated 5 kb upstream and downstream of the transcriptional start sites of the 80 putative SRF-dependent genes listed in Table 1. The majority (43 of 80 or 54%) of the putative SRF target genes were found to possess one or more CArG box elements spatially conserved with at least one other vertebrate species. Additionally, we identified conserved CArG elements in the promoter region of these genes.

To directly assess whether SRF regulates the expression of these genes in vivo via direct binding to the promoter region of these genes, we performed chromatin immunoprecipitation assays on a subset of these genes (Fig. 5). Each putative CArG box analyzed by chromatin immunoprecipitation analysis was specifically precipitated using anti-SRF antibody. This is consistent with the hypothesis that SRF directly regulates the expression of these genes in vivo via direct binding to the promoter region of these genes.

As shown in Fig. 5 and Table 2, SRF-null cardiomyocytes displayed significant down-regulation of many genes involved in sarcomeric assembly, maintenance, and function. Although none of the contractile genes examined by RT-PCR showed increased expression in the absence of SRF, the expression of some sarcomeric genes (Actn2, Actn4, and Tnn1) was unaffected. To more carefully assess the expression patterns of these gene products, we used immunofluorescence to characterize the subcellular distribution of protein in SRF-null cells (Fig. 6). Wild-type cardiomyocytes organize actin filaments into long, thin, symmetrically spaced filaments that span the entire length of the myocyte; however, SRF-null cardiomyocytes have dramatically reduced actin filament assembly, as detected by fluorescence to characterize the subcellular distribution of protein in SRF-null cells (Fig. 6). Wild-type cardiomyocytes organize actin filaments into long, thin, symmetrically spaced filaments that span the entire length of the myocyte; however, SRF-null cardiomyocytes have dramatically reduced actin filament assembly, as detected by fluorescently labeled phalloidin toxin (Sigma). The filamentous actin that remains in SRF-null myocytes is punctate, organized into small clumps, and was never observed to span the length of the cell. We also tested if the subcellular distribution of sarcomeric α-actinin protein, a major component of the Z-disk, is disrupted in SRF-null

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cardiomyocytes. Actinin protein in SRF-null cardiomyocytes appeared faint and diffuse compared with its orderly appearance in the well spaced Z-discs of control myocytes. Some SRF-null cells stained positive for αH9251-actinin in a punctate pattern that may represent the residual Z-bodies observed at the ultrastructural level (Fig. 3). The actin-binding protein tropomyosin exhibited an overall decrease in expression in the absence of SRF, similar to that observed in SRF-null cells stained for phalloidin. Immunolabeling of cardiac troponin T, a regulatory subunit in the troponin complex that mediates the tropomyosin response to calcium, was again reduced in SRF-null cells. Because we observed no change in the expression of cardiac α-actinin isoforms and cardiac troponin T in SRF-null cardiomyocytes by RT-PCR (data not shown), the attenuation of α-actinin and troponin T protein may represent protein destabilization or diffuse localization that results in a weak signal resulting from missing cooperative partners in the contractile apparatus.

**DISCUSSION**

The functional unit of cardiac muscle contraction, the sarcomere, is fundamentally composed of alternating thick myosin filaments and thin actin filaments (Fig. 7b) that respond to sarcoplasmic Ca²⁺ influx. Three actin genes are expressed in cardiac muscle. The most abundant isoform in the heart, cardiac α-actin, is expressed by the gene Actc1. The cardiac α-actin promoter contains four conserved CArG boxes (4), to

| Gene       | Conserved CArG box (position relative to start)                                                                 | Reference |
|------------|---------------------------------------------------------------------------------------------------------------|-----------|
| ACTA1      | ccagatattg (-3527), ccatatttg (-1422), ccctagaagg (-1033), ccataaagg (-243), cctcttagg (-192), ccataaatgg (-111) | 69        |
| ACTA2      | ccatatttg (-120), cctgttagg (-70)                                                                            | 70        |
| ACTC1      | ccnaatattg (-1545), ccatatttg (-238), ccctagaagg (-201), ccgaaatgg (-150)                                    | 4         |
| CSR1       | ccnaaagg (-3138), cctatttaa (+2976), ccataatgg (+3198), cctattaag (+3640)                                      | 71        |
| CSR2       | ccatttaag (-1514), ccaatttgg (+1084), ccatttaag (+2913)                                                      | None      |
| DES        | ccataaagg (-3478), acatatgg (+1404), ccatttagg (+707)                                                        | 43        |
| LBD3       | ccatttagg (-81)                                                                                               | None      |
| MYH6       | ccccttagg (-184), ccaatttgg (-66), ccgaataagg (+2432)                                                         | 41        |
| MYH7       | ccatatttg (-2547)                                                                                            | 6         |
| MYL3       | ccatatttg (+544), ccataaatgg (+2677)                                                                         | 27        |
| MYL9       | ccataaatgg (-174), cctgttagg (-112)                                                                            | None      |
| TALGLN     | ccatttagg (-3527), ccataaagg (-271), ccataaatgg (-148)                                                         | 72        |
| TNN1C      | ccatttagg (-1283)                                                                                            | 73        |
| TPM1       | ccattataag (-220), ccataaatgg (-79)                                                                            | 48        |
| TPM2       | ccattataaag (-1940), ccataaatgg (-999)                                                                          | 47        |
| DMD        | cactattag (156), ccatatttg (-130)                                                                              | 7         |
| ENAH       | ccataaatgg (-1200)                                                                                            | None      |
| FNBP1      | cccatttagg (-1670)                                                                                            | None      |
| ITGB1      | ccttaaatgg (-957), ccttttagg (-163)                                                                            | None      |
| ITGB1BP2   | ccaatatgg (-1927), ccttttagg (-135)                                                                            | None      |
| MYH4       | ccataaatg (+3055), ccataaatgg (+3068), ccataaatgg (+4617)                                                      | None      |
| PCDH7      | ccatattag (-3178), acatttag (-1877), ccataaatgg (-1474), ccataaatgg (-1241)                                    | None      |
| PCDH18     | acataaatg (-1408)                                                                                            | None      |
| THBS1      | cctatttagg (-1246)                                                                                            | None      |
| TJ1        | ccatatttaa (-3028), ccaatttag (-1004)                                                                           | None      |
| ANKRD1     | ccttttagg (-2522), cattatttaa (-649), ccatatttag (-559)                                                         | None      |
| EGR1       | ccataaatgg (+460), ccttttagg (+463)                                                                            | 74        |
| GATA4      | ttatttagg (-3831)                                                                                            | None      |
| LBH        | ccataaatgg (-1956)                                                                                            | None      |
| MYOCD      | tccataaatg (-3538), cccataaatgg (-2976)                                                                        | None      |
| NKK2-5     | ccattattg (-4606), ccatttagt (-2974), ccataaatgg (-2544), ccatttagt (+1049)                                    | None      |
| SRF        | ccataaatgg (-81), ccataaatgg (-61), ccatttagg (-38)                                                           | 75        |
| AKT3       | ccattataag (-4729), ccatttagg (-821)                                                                            | None      |
| ATP2A2     | ccataaatgg (-3197), ccatatttg (+1224)                                                                           | 8         |
| CTKM       | ccatttagg (-1234), ccataaatgg (-177)                                                                            | 76        |
| DSCR1      | ccatatttagg (-159)                                                                                            | None      |
| DTR        | ccataaatgg (-188)                                                                                            | None      |
| EREG       | ccttttagg (-4134), ccataaatgg (-849), ccataaatgg (-588), ccataaatgg (-33)                                      | None      |
| FGFR3      | ccaatatgg (-4397), ccatatttag (-2223)                                                                           | 77        |
| IPO8       | ccaatatgg (-506)                                                                                            | None      |
| MUSTN1     | ccatattag (-2605), ccttttagg (-2589)                                                                           | None      |
| NPPB       | ccataaatgg (-3336)                                                                                            | None      |
| PTGS2      | ccttttagg (-2544), ccatatttag (-2224)                                                                           | None      |
| ROBO4      | ccatttagg (-3136), ccatattgg (-1947), ccatattgg (-1769), ccataaatgg (-1220)                                    | None      |
| SDC2       | ccatatttagg (-707), acatttagg (+1044)                                                                           | None      |
| TGFβ2      | ccaatatgg (+4624)                                                                                            | None      |
| TGM2       | ccatatttagg (-4134), ccataaatgg (-849), acataaatgg (-588), ccataaatgg (-33)                                    | None      |

**TABLE 2**

In silico identification of conserved CArG box motifs in putative SRF target genes

CArG boxes are defined as a CCW₆GG sequence allowing for a 1-bp deviation. Only murine CArG boxes within 5 kb of the transcriptional start site and conserved in at least one other species were included.
which SRF recruits NKX-2.5 (34) and GATA-4 (35) to cooperatively activate expression. In this study, SRF-null cardiomyocytes expressed 4.2 ± 1.8-fold fewer cardiac α-actin transcripts. Consistent with dysregulation of Acta1 contributing to the ultrastructural defects in Fig. 3, cardiac α-actin-deficient mice have myofibrils in disarray (36). We have also shown here that skeletal (Acta1) and smooth muscle (Acta2) α-ac-

| α-SRF 1 | α-SRF 2 | IgG 1 | IgG 2 |
|---------|---------|-------|-------|
| GAPDH   |         |       |       |
| ACTA1   |         |       |       |
| ACTA2   |         |       |       |
| ACTC1   |         |       |       |
| CSRP1   |         |       |       |
| CSRP2   |         |       |       |
| DES     |         |       |       |
| MYH6    |         |       |       |
| MYH7    |         |       |       |
| TPM1    |         |       |       |
| TPM2    |         |       |       |
| DMD     |         |       |       |
| ENAH    |         |       |       |
| ITGB1BP2|         |       |       |
| ITGB1   |         |       |       |
| TJP1    |         |       |       |
| GATA4   |         |       |       |
| MYOCD   |         |       |       |
| NNX2.5  |         |       |       |
| ATP2A2  |         |       |       |
| DSCR1   |         |       |       |
| NPPB    |         |       |       |
| SDC2    |         |       |       |

FIGURE 5. Chromatin immunoprecipitation assays demonstrating in vivo binding of SRF to conserved CArG boxes. Cross-linked chromatin from adult mouse hearts was precipitated with anti-SRF or IgG (as a control) and detected using PCR. Primers for genomic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to confirm the specificity of anti-SRF antibody. IP, immunoprecipitate.

There are two functionally distinct isoforms of MHC in the heart. α-MHC has greater ATPase activity, whereas β-MHC is more efficient and has greater actin filament sliding velocity (37). α-MHC is regulated by a host of cardiac transcription factors, including GATA, myocyte enhancer factor-2 (MEF-2), and myocardin (38, 39). β-MHC transcription is influenced by GATA, MEF-2, and NKX-2.5 (40). Each of the cardiac MHC genes contain at least one functional CArG box within its respective 5′-promoter region (6, 41). The results presented here confirm that SRF is required for expression of the endogenous MHC isoforms in cardiomyocytes. Comparatively mild forms of myofibrillar disarray result from mutations in murine MHC (42), indicating that the attenuation of MHC transcription may be a contributing factor in the contractile apparatus structural defects that are observed in the absence of SRF.

SRF-null cardiomyocytes also exhibit an attenuation of desmin expression, raising the possibility that heart defects seen in SRF-null animals are due to decreased desmin levels. In contrast to other components of the contractile apparatus, desmin is expressed from a single gene. Desmin is an intermediate filament protein that integrates the Z-disk into other intracellular structures. The murine Des promoter contains putative SRF-, GATA-, and MEF-2-binding sites (43). However, the dramatic attenuation of Des transcripts seen here in SRF-null cardiomyocytes is likely insufficient to cause the myofibrillar defects observed in this study because desmin knockout mice properly assemble repeating sarcomeric units in cardiac tissue (44). Desmin-null mice do, however, display muscle degeneration resulting from defects in myofibril alignment (44), indicating that reduced desmin levels may contribute to the misaligned sarcometric structure in SRF-null cells.

Murine transgenic studies indicate that tropomyosin expression levels are important for contractile function. For example, α-tropomyosin-null mice are embryonic lethal due to myofibrillar abnormalities (45), and the forced overexpression of β-tropomyosin results in reduced contractile rates (46). The expression of each isoform was significantly reduced in SRF-null myocytes (Fig. 4). Informatics and chromatin immunoprecipitation analyses suggested direct regulation of tropomyosin by SRF (Fig. 5 and Table 2). These results confirm other recent studies implicating SRF in the control of tropomyosin genes (47, 48).

Of the three subunits that compose the cardiac troponin complex, only troponin C protein, encoded by Tnnc1, is deregulated at the transcriptional level in SRF-null cardiomyocytes. This was unexpected because previous work had shown that cardiac troponin C was unaffected by forced expression of dominant inhibitory SRF in cardiac myocytes (49). One explanation for this discrepancy is that exogenous dominant inhibitory SRF may not have been expressed in sufficient quantities to displace the high level of endogenous SRF in these cells.

Other less well characterized sarcomeric proteins that are attenuated in SRF-null cells include three myosin regulatory light chain genes: myomesin, nebulette, and SM22. The function of myosin light chain protein in cardiac muscle is not well understood. Myomesin is suspected to provide sarcomeric stability by cross-linking the heavy filaments in the M-band (50). Nebulette binds α-actin and sarcomeric α-actinin at the Z-disc in cardiac cells and may play a stabilizing role in sarcomeric structure (51). SM22 localizes to myofibril bundles in myocytes; however, SM22 does not likely contribute to the sarcomeric defects because myofibrils in the SM22 knockout mouse appear normal (52).

Here, we have focused our attention on putative SRF target genes...
SRF Is Required for Sarcomeric Integrity

involved in the structure of the contractile apparatus due to the dramatic sarcomeric phenotype observed in SRF-null myocytes. Other genes may play important roles in these processes. However, due to the complexity of sarcomeric assembly and maintenance at the molecular level, it is difficult to predict which other candidate genes may contribute to this phenotype. Interestingly, recent developmental models of SRF inactivation in striated muscle have yielded less pronounced defects in gene expression and myofibrillar structure (13, 14, 53). We suggest four explanations for these differences. 1) Ad-delivered Cre excision is more efficient than in vivo methods of gene inactivation. For example, treating cultured cardiac myocytes with Ad-Cre resulted in a 15.7 ± 4.1-fold decrease in SRF expression compared with control cells (Fig. 1C). However, upon SM22α-Cre-mediated inactivation of SRF in embryos, we observed a comparatively modest 4.7 ± 1.0-fold decrease in SRF transcripts (13). 2) The presence of non-myocyte cell types in tissues assayed for gene expression changes using in vivo developmental models dilutes and confounds the interpretation of these results. 3) Complex physiological mechanisms may compensate for the depletion of SRF-dependent genes in an SRF-independent manner. 4) The early lethality of in vivo SRF knockout models precludes analysis of complete SRF excision in muscle tissue.

Genes known to play significant roles in cytoskeletal organization were also identified by our gene array analysis (Table 1). Prominent among these were dystrophin and melusin. We have identified conserved CArG box sequences in these genes and have confirmed that at least one of these cis-regulatory sites binds SRF in vivo. Dystrophin, encoded by the Duchenne muscular dystrophy gene Dmd, links the internal cytoskeleton to the extracellular matrix. Melusin, encoded by Itgb1bp2, is thought to mediate the interaction between myofibrils and the extracellular matrix through β1 integrin (54). In SRF-null myocytes, reduced interaction of the cytoskeleton and/or myofibrils with the extracellular matrix due to reduced levels of melusin and dystrophin may result in the reduced stability of the sarcomere during contraction.

SRF acts with the cardiac factors GATA-4, myocardin, NKX-2.5, and TBX-5 in a mutually reinforcing regulatory network to control cardiac gene expression. The results presented here have begun to elucidate this complex regulation.

GATA-4 is the principal myocardial isoform of the GATA transcription family in the heart (55). Targeted deletion of GATA-4 in mouse embryos results in cardiac looping, septation, and trabeculation defects (56). GATA-4 is known to regulate a number of cardiac proteins, including α-actin, MHC, and NPPB (35, 57, 58). We observed a 15.2 ± 4.9-fold decrease in GATA-4 expression in SRF-null myocytes by RT-PCR (Fig. 4). Recent work in Danio rerio has identified putative GATA-, NKX-2.5-, MyoD-, and TBX-5-binding sites in the gata-4 promoter (59). However, until this study, SRF had not been implicated in the direct regulation of GATA-4. We have identified a conserved CArG box 3830 bp upstream of the Gata-4 transcriptional start site (Table 2) to which SRF binds in vivo (Fig. 5). However, we have not ruled out the possibility that loss of GATA-4 in SRF-null myocytes is a secondary result of the coincident TBX-5 and/or NKX-2.5 attenuation.

Myocardin is a recently discovered transcription factor that regulates cardiac genes such as Acta2, Myh6, and Nkx-2.5 (39). Myocardin is enriched in smooth muscle tissue and also regulates smooth muscle genes (60). In this study, we observed a 5.0 ± 1.3-fold decrease in Myocd expression in SRF-null cardiomyocytes and identified two conserved CArG boxes in the Myocd gene (Table 2), at least one of which (at −3538) binds SRF protein in vivo (Fig. 5). Together, these data suggest that SRF directly regulates myocardin expression in cardiomyocytes.

Expression of the cardiac-specific homeobox protein NKX-2.5 in early (embryonic day 7.5) cardiac progenitor cells of the mesoderm is required for heart development and believed to be induced by bone morphogenetic protein and fibroblast growth factor signals from the adjacent endoderm (61). NKX-2.5 is known to physically interact with SRF independent of DNA binding and to activate sarcomeric genes such as Actc1 (34). Here, we have shown by RT-PCR analysis that Nkx-2.5 transcripts were reduced by 11.2 ± 5.6-fold in SRF-null cardiomyocytes. We have identified four conserved CArG boxes within 5 kb of the murine Nkx-2.5 transcriptional start site (Table 2), at least one of which (at −3544) binds SRF in vivo (Fig. 5). These data suggest that SRF is required to maintain Nkx-2.5 expression in cardiomyocytes. Therefore, the expression of both Nkx-2.5 and GATA-4 appears to be dependent on SRF in cultured cardiac myocytes (this study) and in the developing heart (13, 14), but surprisingly not in embryonic stem cells (27).

The TBX family of transcription factors is also important for regula-
tion of cardiac genes. NKX-2.5 contributes to expression of TBX-5 (62), and TBX-5 is necessary for the myocardial expression of SRF (63). TBX-5 physically interacts with NKX-2.5 to synergistically drive cardiomyocyte differentiation and increased expression of NKX-2.5, GATA-4, and MEF-2c (64). Because we did not observe a conserved CArG box in the \( Tbx-5 \) promoter, we propose that the 5.3 \( \pm \) 0.5-fold decrease of \( Tbx-5 \) transcripts in SRF-null cardiomyocytes is a secondary consequence of decreased NKX-2.5. However, a functional CArG box may lie outside the 10 kb of genomic sequence that we interrogated.

We also observed a modest yet reproducible decrease (1.5 \( \pm \) 0.2-fold) in MEF-2c expression in SRF-null cardiomyocytes. MEF-2c attenuation may be a secondary effect of reduced NKX-2.5 because there are no conserved CArG boxes within 5 kb of the MEF-2c transcriptional start site (Table 2), and MEF-2c expression is stimulated by NKX-2.5 in differentiating P19 cells (65). Reduced expression of MEF-2c may contribute to the attenuation of \( Des \) and \( Myh7 \) in SRF-null cells (66, 67).

A subset of the SRF target genes identified in this study have been implicated previously as playing a key role in SRF-dependent differen-
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21. In contrast, Niu et al. suggested that neither NKX-2.5 nor GATA-4 is SRF-dependent in differentiating embryonic stem cells or in a conditional SRF mouse knockout model. These differences in SRF-dependent gene regulation of key transcriptional regulators reinforce the idea that SRF regulates distinct programs of expression at different times during heart and cardiomyocyte development. Finally, Niu et al. posited a model for SRF in controlling cardiomyocyte apoptosis. We did not find that loss of SRF results in significant loss of cardiomyocyte number in cell culture. This suggests that loss of SRF per se does not lead to apoptosis and argues against a central role for wild-type SRF in controlling intrinsic apoptotic pathways in cardiomyocytes.

In summary, we have shown SRF to be a central component of the genetic transcriptional network of cardiac muscle cells. Inactivation of SRF disrupts the expression of both genes important for sarcomeric structure and key cardiac transcription factors, thereby resulting in the attenuation of sarcomeric proteins necessary for contractile apparatus maintenance. Our results indicate that SRF regulates cardiomyocyte function by virtue of its ability to control expression of genes involved in multiple levels of sarcomeric structure and function. As depicted in Fig. 7a, observations from this study and previous findings (68) demonstrate that there is a complex interrelationship of SRF-dependent genes that confer the cardiac phenotype. Notably in this model, a hierarchical cascade of cardiac-specific gene transcription is apparent in which SRF both directly and indirectly regulates the expression of genes required for contractile apparatus Assembly.
