Cardiomyopathy in transgenic mice with cardiac-specific overexpression of serum response factor

XIAOMIN ZHANG,1 GOHAR AZHAR,1 JIANYUAN CHAI,1 PAMELA SHERIDAN,1 KOICHIRO NAGANO,1 THOMAS BROWN,1 JIHWANG YANG,1 KONSTANTIN KHAPKO,1 ANA M. BORRAS,1 JOEL LAWITTS,1 RAVI P. MISRA,2 AND JEANNE Y. WEI1

1Department of Medicine, Beth Israel Deaconess Medical Center, and Division on Aging, Harvard Medical School, Boston, Massachusetts 02215; and 2Department of Biochemistry, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Received 26 May 2000; accepted in final form 20 November 2000

Zhang, Xiaomin, Gohar Azhar, Jianyuan Chai, Pamela Sheridan, Koichiro Nagano, Thomas Brown, Jihong Yang, Konstantin Khrapko, Ana M. Borras, Joel Lawitts, Ravi P. Misra, and Jeanne Y. Wei. Cardiomyopathy in transgenic mice with cardiac-specific overexpression of serum response factor. Am J Physiol Heart Circ Physiol 280: H1782–H1792, 2001.—Serum response factor (SRF), a member of the MCM1, agamous, deficiens, SRF (MADS) family of transcriptional activators, has been implicated in the transcriptional control of a number of cardiac muscle genes, including cardiac α-actin, skeletal α-actin, α-myosin heavy chain (α-MHC), and β-MHC. To better understand the in vivo role of SRF in regulating genes responsible for maintenance of cardiac function, we sought to test the hypothesis that increased cardiac-specific SRF expression might be associated with altered cardiac morphology and function. We generated transgenic mice with cardiac-specific overexpression of the human SRF gene. The transgenic mice developed cardiomyopathy and exhibited increased heart weight-to-body weight ratio, increased heart weight, and four-chamber dilation. Histological examination revealed cardiomyocyte hypertrophy, collagen deposition, and interstitial fibrosis. SRF overexpression altered the expression of SRF-regulated genes and resulted in cardiac muscle dysfunction. Our results demonstrate that sustained overexpression of SRF, in the absence of other stimuli, is sufficient to induce cardiac change and suggest that SRF is likely to be one of the downstream effectors of the signaling pathways involved in mediating cardiac hypertrophy.

SRF overexpression altered the expression of SRF-regulated genes and resulted in cardiac muscle dysfunction. Our results demonstrate that sustained overexpression of SRF, in the absence of other stimuli, is sufficient to induce cardiac change and suggest that SRF is likely to be one of the downstream effectors of the signaling pathways involved in mediating cardiac hypertrophy.

SRF is a member of the MADS family of transcriptional activators that has been implicated in the transcriptional control of cardiac muscle gene expression (9, 28, 37, 43). SRF regulates target genes by binding to serum response elements (SREs), which contain a consensus CC(A/T)(G)GG (CArG) motif. This cognate binding site of SRF is found in the promoter region of certain immediate-early genes (c-fos) and a number of muscle-specific genes (cardiac α-actin) (50). Mutations in CArG boxes in the promoters of certain muscle-specific genes lead to a loss of their expression in cardiac muscle cells (19, 48). SRF has been reported to have a tissue-restricted pattern of expression in adult mice, where SRF mRNA levels are the highest in skeletal and cardiac muscle, but barely detectable in liver, lung, and spleen tissues (7). During embryogenesis, SRF is expressed preferentially in differentiating cardiac and skeletal muscle cells (13). Targeted disruption of the SRF gene results in embryonic death apparently due to a severe defect in mesoderm formation (5). In addition, the mRNA levels of a number of SRF-regulated genes, including atrial natriuretic factor (ANF), skeletal α-actin, cardiac α-actin, α-myosin heavy chain α-MHC, and β-MHC, have been reported to undergo changes during cardiac development, cardiac hypertrophy, and cardiomyopathy (4, 11, 12, 16). These findings suggest that SRF may play a role in the regulation of genes responsible for cardiac structure and function.

In a previous study (53), we demonstrated that the induction of c-fos gene expression in the rat heart in response to hemodynamic stress was reduced with age. We also found that the binding activity of SRF protein to its cognate DNA binding site on the c-fos promoter was increased in old, compared with that in young, rat hearts (56). Moreover, the basal expression of SRF protein was increased in the hearts of old versus young adult rats (33). The consequence of increased SRF expression in the heart remains unclear. To better understand the role of SRF in regulating cardiac function and cardiac gene expression in vivo, we sought to test the hypothesis that increased SRF expression in the heart might be associated with altered cardiac morphology and function. We generated transgenic mice with cardiac-specific overexpression of the human SRF gene and found...
that these mice had heart abnormalities consistent with cardiomyopathy.

MATERIALS AND METHODS

Generation of α-MHC-SRF transgenic mouse lines. The plasmid pCGNSRF was a generous gift from Dr. R. Prywes (Columbia University, New York, NY). The XbaI fragment of the human SRF gene (GeneBank accession number J03161) was released from pCGN5RF, blunt-ended with Klenow fragment, and cloned into the blunt-ended SalI site of a pBluescript II KS(+) plasmid containing the α-MHC promoter (a generous gift from Dr. J. Robbins, The Children’s Hospital and Research Foundation, Cincinnati, OH). The SRF transgenic construct was linearized with NotI and purified for injection into the pronuclear stage zygotes of FVB/N mouse strain according to the standard transgenic procedure of Beth Israel Deaconess Medical Center transgenic facility. At 2–3 wk of age, all animals had a 1-cm portion of tail removed for DNA analysis. The potential transgenic mice were screened twice by the polymerase chain reaction using two different forward primers (SRF1340F: 5′-ACAGGTGCTGACCTTGAGAC-3′ and SRF1434F: 5′-CCATTCAGTGCACCGGC-3′) and one reverse primer (Hgh2073R: 5′-CCATTCAAGTGCACCAGG-3′), which is derived from the c-fos sequence (3′-untranslated region of mouse SRF). This sequence was not present in the construct of the transgenic transgene, and therefore, it was used for measuring the expression of endogenous murine SRF. A double-stranded SRF cDNA fragment from plasmid pCGN5RF was used as probe for examining the mRNA level of total SRF, which represented the endogenous and transgenic SRF.

Electrophoretic mobility shift assays. Whole cell extract of ventricular tissue from transgenic and nontransgenic mice were prepared by a modification of the method described by Tsou et al. (56). Briefly, cardiac ventricular tissue was washed with cold PBS and then suspended in buffer C containing 20 mM HEPES (pH 8.0), 1.5 mM MgCl2, 25% (vol/vol) glycerol, 420 mM NaCl, 0.2 mM EDTA (pH 8.0), 1 mM 1,4-dithiothreitol, and 1× protease inhibitor cocktail (Boehringer-Mannheim Biochemicals). Ventricular tissue was homogenized and then incubated on ice for 30 min before being centrifuged at 10,000 rpm for 15 min. The SRE consensus oligonucleotide was of sequence 5′-GGATGTCCATAGGTGCTGAGCTCAGTCCCATCGGGTCAGCTG-3′, which is derived from the c-fos promoter. The SRE mutant oligonucleotide was of sequence 5′-GGATGTCCATATAACGTACATCTCAG-3′ to which SRF is unable to bind. Oligonucleotides were labeled with [γ-32P]-ATP using T4 polynucleotide kinase. Binding reaction mixtures were incubated at room temperature for 20 min and contained 0.5 ng of DNA probe and 5 μg of protein in the binding buffer with 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, and 1 μg of poly(dl-dC) to inhibit nonspecific binding of the-labeled probe to the ventricular tissue protein. DNA-protein complexes were resolved by electrophoresis through 4% native polyacrylamide gels containing 50 mM Tris, 45 mM boric acid, and 0.5 mM EDTA. The gels were subsequently dried and exposed to Kodak X-Omat film. Gel supershift assays were performed as described above with the exception that subsequent to incubation of oligonucleotide probes with the whole cell extract, 1 μl of anti-SRF antibody (1 μg/μl, Santa Cruz Biotechnology) was added to the reaction mixture and incubated at room temperature for 30 min.

Measurement of protein expression. Protein (50 μg) prepared as described in the electrophoretic mobility shift assays section above was separated by SDS-PAGE on a polyacrylamide gel and transferred to nitrocellulose. The membrane was blocked for 2 h at room temperature in 5% nonfat milk in Tris 20 mM, sodium chloride 137 mM, sodium phosphate 8 mM, pH 7.4. The membranes were subsequently dried and exposed to Kodak X-Omat film. Gel supershift assays were performed as described above with the exception that subsequent to incubation of oligonucleotide probes with the whole cell extract, 1 μl of anti-SRF antibody (1 μg/μl, Santa Cruz Biotechnology) was added to the reaction mixture and incubated at room temperature for 30 min.

Histological analysis. Portions of the ventricular tissues were placed in 10% neutral-buffered formalin overnight. After fixation, the samples were subjected to a dehydration series and embedded in paraffin. Sections (3–4 μm) were stained using standard hematoxylin and eosin (HE) or Masson trichrome staining protocols (Poly Scientific; Bayshore, NY). The SRF polyclonal antibody and ImmunoCruz System kit (Santa Cruz Biotechnology) were used for immunohistochem-
istry. Photomicrographs were obtained using a Nikon ES400 microscope.

Echocardiography. Mice were anesthetized with intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). The ventral chest was shaved, and the mouse was placed on a thermally controlled foam pad. Echocardiography was performed using a Hewlett-Packard Sonos 5500 ultrasound imaging system equipped with a 10-MHz pulsed array transducer. Electrocardiogram leads (one front paw and two hind paws) were placed. Conventional two-dimensional imaging, M-Mode recordings, and spectral color Doppler evaluations were performed. Cardiac size and shape were determined using M-mode and two-dimensional image recordings. The left ventricular (LV) wall thickness, contractility, and chamber dimensions were determined at end diastole and end systole. All values were based on the average of at least three consecutive beats so as to minimize noise and respiratory variation. Derivative measurements included LV mass, LV volume, and systolic function. Spectral Doppler recordings of mitral inflow patterns were used for evaluation of LV diastolic parameters.

Data analysis. Values were expressed as means \pm SD. Data were analyzed by two independent observers, blind to the transgenic status of the mice. Normality testing was performed on all data, and the nonparametric Mann-Whitney U-test was used to determine the differences between the two groups. A \( P \) value < 0.05 was considered to be statistically significant.

RESULTS

Generation of SRF transgenic mice. We generated transgenic mice to examine the effects of increased SRF expression in the heart. The SRF transgene was under the transcriptional control of the \( \alpha \)-MHC promoter, which has been extensively characterized and used to express a number of transgenes in the mouse heart (46). A plasmid construct containing human SRF cDNA under the control of the \( \alpha \)-MHC promoter, and the human growth hormone gene polyA sequence (Fig. 1A), was injected into pronuclear stage zygotes using a standard microinjection procedure. In this way, five transgenic founder mice (founders A, B, C, G, and J) were obtained for this study (Table 1). The transgene copy number of these transgenic founder mice was

![Fig. 1. Serum response factor (SRF) transgenic construct and measurements of SRF mRNA and protein. A: DNA construct for the generation of SRF transgenic mice. SRF, the full length cDNA of human SRF gene. \( \alpha \)-MHC, \( \alpha \)-myosin heavy chain; Hgh, the polyA sequence of human growth hormone gene. B: mRNA expression of SRF in the ventricles of nontransgenic (NTg) and SRF transgenic mice (Tg). Tg-B, transgenic founder mouse of line B at age 15 wk; Tg-J, Tg mice of line J at age 24 wk. C: Western blot analysis of SRF protein expression in the ventricles of NTg and SRF Tg mice of line J. D: electrophoretic mobility shift assays (representative of three experiments) shows increased serum response element (SRE) binding activity in the heart of SRF Tg mice of line J compared with nontransgenic (NTg) mice at age 24 wk. Electrophoretic mobility shift assay was performed with 5 \( \mu \)g of cell lysate (protein) of ventricular tissue of NTg and Tg mice and 0.5 ng of \( ^{32}P \)-labeled SRE DNA oligonucleotide in the presence of poly(dI-dC). The specificity of binding was determined using competition by 100-fold unlabeled SRE consensus oligonucleotide, the supershift by anti-SRF antibody, and lack of binding to labeled SRE mutant oligonucleotide. \(^{32}P\)-SRE, \(^{32}P\)-labeled consensus oligonucleotide; 100\( \times \) SRE, 100-fold unlabeled SRE consensus oligonucleotide; \(^{32}P\)-SRE mutant, \(^{32}P\)-labeled SRE mutant oligonucleotide.](http://ajpheart.physiology.org)}
CARDIOMYOPATHY IN SRF TRANSGENIC MICE

Table 1. α-MHC-SRF transgenic lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Sex</th>
<th>SRF Transgene Copy Number</th>
<th>Cardiomyopathy</th>
<th>Transgenic Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F</td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>M</td>
<td>3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>F</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>J</td>
<td>M</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Five serum response factor (SRF) transgenic lines with transgene copy numbers ranging from 1 to 6. All lines developed cardiomyopathy (+) and suffered early mortality. Only line J, which harbored one single transgene copy number, was able to produce transgenic progeny. MHC, myosin heavy chain. F, female; M, male.

then determined by Southern blot analysis (Table 1), which demonstrated that founder A had the highest transgene copy number (6 copies), whereas founder J had only one copy of the transgene. Founders B, C, and G were shown to have intermediate numbers of the transgene (4 for founder B, 3 for C, and 2 for G).

The transgenic founder mice were bred with nontransgenic mice, but four of the five were unable to reproduce transgenic progeny. Among the five founder mice, founder A had developed cardiomyopathy and died at 6 wk of age, before reaching reproductive status. Founders B, C, and G were bred with nontransgenic mice to obtain nine normal-sized litters of neonates (average litter size of 10). However, no transgenic mice were observed in the first generation of mouse offspring (F1) in any of the nine litters, from a total of 90 newborns. In an effort to pursue the possibility of lethality during embryogenesis, four sets of embryos (stages E9-E14) from four different pregnant mice that were mated with founders B and C were screened for the presence of the transgene by polymerase chain reaction. Although no transgenic embryos were identified in any of these embryos, it is still possible that embryonic lethality existed. Founder J, which was shown to have only one copy of the SRF transgene, was the only one of the five transgenic founder mice that successfully reproduced heterozygous transgenic offspring (Table 1). Measurement by Southern blot analysis of the signal of SRF transgene revealed that the line J founder was mosaic (data not shown).

Tissue specificity of SRF overexpression in the transgenic mice was determined by Northern blot analysis. Blots containing the total RNA isolated from several different organs of two different transgenic lines (the founder mouse of line B and F1 transgenic mice of line J) were hybridized to a [α-32P]dCTP-labeled cDNA probe of SRF. The tissues of transgenic mice tested were found to specifically overexpress SRF mRNA in the heart (Fig. 1B). The transgenic animal with higher transgene dosage displayed higher levels of SRF mRNA expression in the ventricle (Fig. 1B).

In nontransgenic mice, Northern blot analysis showed two bands of SRF in cardiac tissue (4.5 kb and 2.5 kb) representing two species of SRF transcripts likely due to alternative splicing, which are the result of differential utilization of two polyadenylation signals for mRNA 3’-end formation (29). The SRF transgene utilized in the present study generates a transcript that consists of part of the α-MHC promoter and the human SRF gene. It is ∼2.5 kb in length and overlaps with the 2.5-kb endogenous SRF transcript. The 2.5-kb band was more intense than the 4.5-kb band in the hearts of the transgenic mice, and this band was also more intense than both bands in the hearts of nontransgenic animals (twofold increase in F1 transgenic mice of line J compared with their littermate nontransgenic mice). The protein expression of SRF was also significantly increased in the ventricles of transgenic mice of line J compared with nontransgenic mice (Fig. 1C).

We then analyzed whether the transgenic mice also overexpress functional SRF protein in the ventricle. We compared the amount of SRF protein in lysate from ventricles of transgenic mice and nontransgenic littermates by electrophoretic mobility shift assays. The whole cell extract from cardiac tissue was isolated from transgenic and nontransgenic mice, and [γ-32P]ATP SRE oligonucleotide corresponding to the c-fos promoter region was used in the binding reactions. Electrophoretic mobility shift assays revealed an increase in the specifically shifted SRF DNA oligonucleotide in cellular extract from transgenic ventricles compared with those of nontransgenic littermates. Moreover, the shifted SRE oligonucleotide was supershifted by an anti-SRF antibody, and it was also specifically competed for by unlabeled consensus SRE oligonucleotide. In addition, there was no binding to the [γ-32P]ATP SRE mutant oligonucleotide (Fig. 1D). These results demonstrate that the transgenic mice displayed cardiac-specific overexpression of functional SRF protein.

Changes in cardiac gene expression. The effect of overexpression of SRF on the expression pattern of cardiac genes was evaluated in the heart by Northern blotting (Fig. 2). The 11 genes examined, except for c-jun, have been reported to have SREs in the promoter region. In 6-wk-old transgenic animals from line J, the results revealed an upregulation of ANP, β-MHC, skeletal α-actin, c-fos, c-jun, and a downregulation of α-MHC, cardiac α-actin, dystrophin, MLC-2v, SERCA2, and endogenous SRF (Fig. 2A). This pattern of cardiac gene expression occurred at an early time point, long before the onset of increased heart weight or cardiomyopathy. The cardiac mRNA expression in the 6-wk-old transgenic animals was similar to that observed in the 24-wk-old animal when it developed cardiac changes (Fig. 2B). A similar pattern of cardiac gene expression was also observed in the hearts of the other SRF transgenic mice examined, including 10-wk- and 16-wk-old animals of line J, before there was any increase in heart weight.

The alteration of gene expression of several cardiac genes was also measured at the protein level with commercially available antibodies. Western blotting revealed a slight decrease of MLC and dystrophin, but an increase of β-MHC in the hearts of transgenic mice compared with nontransgenic littermates (Fig. 2C).
Development of cardiomyopathy. Examination of the transgenic mice with cardiomyopathy at or near death revealed increased heart size, increased heart weight, and four-chamber dilation (Fig. 3A). The heart weight-to-body weight ratio (mg/g) was significantly increased in the transgenic mice (18.7 ± 2.5, n = 14) compared with age-matched nontransgenic littermates (4.8 ± 1.5, n = 18, P < 0.01) (Fig. 3B). The age of onset and severity of the cardiac phenotype varied in an inversely proportional manner with the transgene copy number. The age of death for the five transgenic founder mice ranged from 6 to 40 wk and also correlated inversely with the copy number of the transgene (Fig. 4A).

Of the five transgenic lines, only line J gave rise successfully to F1 transgenic progeny (Table 1). The development of cardiomyopathy was, therefore, further examined among the progeny of this transgenic line. The heart weight, body weight, and the heart weight-to-body weight ratio were examined in transgenic and nontransgenic mice from 6 wk of age to 27 wk of age. It was found that the heart weight-to-body weight ratio was not different in the transgenic compared with nontransgenic animals until around 20 wk of age when there was progressive development of cardiac hypertrophy and cardiomyopathy in the transgenic animals (Fig. 4B).

Histological examination. In transgenic animals with an increased heart weight-to-body weight ratio, cross-sectional views of the heart at the level of the papillary muscles demonstrated biventricular enlarge-
ment, with greater dilation of the LV chamber. There was also a slight increase in ventricular wall thickness compared with hearts of nontransgenic animals (Fig. 5 A). The cardiac myocytes of the transgenic mice were heterogeneous in size, but most of them were larger in size than those of nontransgenic mice (Fig. 5 B). Mason trichrome staining revealed increased collagen deposition (stained in blue), suggesting significant fibrosis in the heart of the transgenic compared with the nontransgenic animal (Fig. 5 C). Immunohistochemistry confirmed elevated levels of SRF protein (stained in brown) in the myocardium of SRF transgenic compared with nontransgenic mice (Fig. 5 D). Electron microscopy of the heart of a transgenic mouse with end-stage cardiomyopathy showed myofiber degeneration and mitochondrial damage (Fig. 5 E).

Functional cardiac assessment of SRF transgenic animals. To gain a better understanding of the physiological consequences of cardiac-specific overexpression of the SRF gene, detailed measurements of in vivo cardiac structure and function were obtained in young adult transgenic mice using echocardiographic imaging and Doppler techniques. LV diastolic filling parameters were also determined. Echocardiographic studies were attempted in two transgenic founder mice with unsuccessful results. Because the only transgenic line that had progeny was line J, we performed functional studies on young adult F1 progeny of this line.

As shown in Table 2, there was no difference between age-matched nontransgenic and SRF transgenic adult (22–24 wk old) animals by body weight, heart rate, LV stroke volumes, or LV end-diastolic wall thickness. Significant differences were observed between the SRF transgenic and nontransgenic littermates (Fig. 6) in LV mass, LV end-systolic volume, and early diastolic LV filling (peak E wave).

These findings suggest that in the adult SRF transgenic mice, there was subclinical development of cardiac hypertrophy, cardiomyopathy, and evidence of mildly altered cardiac function evidenced by reduced LV end-systolic volume, as well as LV diastolic filling. These mild impairments in LV function are similar to that observed in humans with hypertension and/or diabetes before the development of symptoms of congestive heart failure.

DISCUSSION

In the present study, we demonstrated that cardiac-specific overexpression of SRF transgene in mice resulted in the dysregulation of several cardiac genes for which, most likely, SRF is an important transcription regulator. In addition, sustained overexpression of SRF, even in very low copy numbers (as low as 1), was apparently sufficient to induce a phenotype of cardiomyopathy and early mortality. Five transgenic founder mice were obtained for the study, but none of them had more than six copies of the transgene. Among these transgenic founder mice, only founder J, which harbored only one single copy of the transgene, was able to produce live transgenic progeny. These results suggest that SRF overexpression is not well tolerated during embryonic development.

The effect of SRF overexpression on cardiac gene expression in vivo. The SRF binding site has been found in the promoter regions of a number of genes, including those of α-MHC, β-MHC, MLC, cardiac α-actin, skeletal α-actin, SERCA2, ANF, and dystrophin. The role of SRF in the regulation of these genes has been mainly characterized in vitro (4, 6, 23, 24, 27, 28, 30, 35, 37, 40, 55). Although the in vivo role of SRF in the regulation of its target genes remains incompletely established, changes in the expression level of a number of SRF-regulated genes have been reported in cardiac hypertrophy or cardiomyopathy. This includes the upregulation of ANF, β-MHC, and skeletal α-actin and downregulation of α-MHC, cardiac α-actin, and SERCA2 (16, 44). The molecular regulatory mechanism(s) underlying these changes remain(s) unclear, but it is considered to be a result of the adaptation of the heart in response to physiological and pathological conditions (16, 36).
Fig. 5. Histological examination of hearts from NTg and 15-wk-old SRF Tg mouse of line B with end-stage cardiomyopathy. A: cross section of the ventricles at the level of papillary muscle stained with hemotoxylin and eosin. The SRF Tg heart is dilated in both the right (RV) and left ventricle (LV) compared with that of NTg mouse. B: LV cardiac myocytes of NTg and SRF Tg mice (HE stain). Most of the cardiac myocytes of Tg are larger than those of NTg mice. Magnification of ×400. C: Masson trichrome staining of the LV section of NTg and Tg mice. There is interstitial fibrosis (collagen stains blue with trichrome) in the heart of Tg. Magnification of ×200. D: SRF immunoperoxidase staining (in brown) of ventricular section of NTg and SRF Tg mice. Magnification of ×200. E: electron microscopic view of LV cardiac myocytes of a NTg littermate and a SRF Tg mouse (founder of line B) with end-stage cardiomyopathy. There is mitochondrial damage and myofiber degeneration in Tg.
Through the transgenic approach, we were able to evaluate the in vivo effect of SRF overexpression on cardiac gene expression. Eleven genes were selected as cardiac markers for the evaluation. Among them, c-fos, c-jun, and a-MHC are immediate early genes that are early markers for the cardiac hypertrophic response, whereas ANP, c-actin, skeletal c-actin, c-MHC, b-MHC, and SERCA2 genes are late markers for cardiac hypertrophy (8, 31, 45, 57). MLC2v and dystrophin were selected because of their important roles in cardiac contraction and structure, respectively (17, 39). All of these genes, except c-jun, have SRF cognate binding sites in their promoter region (4, 6, 24, 27, 30, 35, 37, 40, 51). Because the SRF gene itself has two SRF binding sites in the promoter region (51), the mRNA level of endogenous SRF was also analyzed in the transgenic animals.

The expression of these genes in the heart was examined at two different time points in the line J transgenic animals: before and after the development of cardiac changes. The mRNA expression, examined in the hearts of 24-wk-old transgenic animals that had begun to develop mild cardiac changes, revealed multiple changes among the genes examined. Interestingly, the changes, such as the upregulation of ANF, skeletal c-actin, and b-MHC and the downregulation of c-MHC as well as cardiac c-actin, reflected similarity to the fetal gene program, which has been observed to be reinduced in some instances of cardiac hypertrophy, cardiomyopathy, and congestive heart failure (12, 16, 20, 32). We also found that the altered pattern of cardiac gene expression was present by as early as 6 wk of age, before there was any evidence of increased heart weight or cardiomyopathy in these animals. The altered pattern of cardiac gene expression observed in the SRF transgenic mice in the present study, including the changes resembling the fetal gene program, is likely to be the result, either direct or indirect, of SRF overexpression in vivo. Further experiments to test this notion, such as the generation of antisense-SRF transgenic mice, are currently underway and will be helpful in elucidating the role of SRF in the regulation of cardiac gene expression.

Two SRF isoforms, 4.5 kb and 2.5 kb in length, were previously found to be expressed in the mouse heart (7). These two transcripts may result from differential utilization of two polyadenylation signal sequences for the mRNA 3’ end formation. The 4.5-kb transcript contains first and second polyadenylation signal sequences, whereas the 2.5-kb transcript only contains the first polyadenylation signal sequence (7). Recently, Kemp and Metcalfe (29) reported on four SRF isoforms termed SRF-L, SRF-M, SRF-S, and SRF-I. SRF-M is expressed at a similar level to SRF-L in differentiated vascular smooth muscle cells and skeletal muscle cells, whereas SRF-L is the predominant form in many other tissues. SRF-S expression is restricted to vascular smooth muscle, and SRF-I expression is restricted to the embryo. The two SRF RNA species 4.5 kb and 2.5 kb that were observed in the present study differ by 2 kb, whereas the two isoforms SRF-L and SRF-M that were reported by Kemp and Metcalfe (29) differ only by 192 base pairs, which is the length of exon 5. More sequencing analyses will be needed to determine whether there might be an association of the 2.5-kb SRF transcript with the SRF-M isoform.

It would be of interest to understand better the mechanism(s) of how some genes were upregulated, whereas others were downregulated by SRF overexpression, although they were all SRF targets and had SREs in their promoter regions. It is possible that there might be a differential effect of SRF on the promoter regions of these genes, depending on the presence of other transcription factors and/or depending on the binding of different SRF proteins that are encoded by the various SRF mRNA transcripts, which may either activate or repress the promoters of genes (29). Further studies to evaluate the possible inhibitory effect of SRF isoforms on the downregulated genes and/or the potential effect of the other binding proteins

### Table 2. Echocardiographic findings

<table>
<thead>
<tr>
<th></th>
<th>Nontransgenic</th>
<th>Transgenic</th>
<th>P</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, g</td>
<td>0.09 ± 0.01</td>
<td>0.15 ± 0.02</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>LVDd,m, mm</td>
<td>3.8 ± 0.32</td>
<td>4.04 ± 0.34</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LVEd,m, mm</td>
<td>2.45 ± 0.32</td>
<td>2.73 ± 0.47</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>EFS, %</td>
<td>39.6 ± 0.6</td>
<td>32.1 ± 8.0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Peak E, m/s</td>
<td>0.61 ± 0.13</td>
<td>0.38 ± 0.12</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Peak A, m/s</td>
<td>0.16 ± 0.06</td>
<td>0.18 ± 0.12</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>VEs,m, mm</td>
<td>55.2 ± 15.9</td>
<td>68.2 ± 16.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Ves,m, mm</td>
<td>11.7 ± 3.9</td>
<td>25.2 ± 6.6</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>PWd,m, mm</td>
<td>0.75 ± 0.13</td>
<td>0.87 ± 0.10</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>AWd,m, mm</td>
<td>0.79 ± 0.13</td>
<td>1.03 ± 1.1</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice. LVDd, left ventricular diastolic dimension; LVEd, left ventricular systolic dimension; EFS (%), endocardial fractional shortening; Peak E, maximal early diastolic (atrial contraction induced) transmitral flow velocity; Peak A, maximal late diastolic (atrial) transmitral flow velocity; Ves, end-systolic volume; PWd, posterior wall thickness (diastolic); LVDs, left ventricular systolic dimension; EFS, %, endocardial fractional shortening; Peak A, maximal late diastolic (atrial) transmitral flow velocity; Peak E, maximal early diastolic (atrial contraction induced) transmitral flow velocity; Ves, end-systolic volume; PWd, posterior wall thickness (diastolic); AWd, anterior wall thickness (diastolic); NS, not significant.

Fig. 6. Representative M-mode echocardiographic tracings from NTg littermates and 24-wk-old SRF Tg mice of line J. Functional parameters are shown in Table 2. PW, posterior wall.
that might be influenced by SRF overexpression will be helpful. It would also be important to know how SRF overexpression could reinduce a pattern of fetal gene program in the transgenic mice before there was any evidence of cardiomyopathy. Determination of the expression of SRF and SRF-target genes in other animal models of cardiac hypertrophy and cardiomyopathy would serve to delineate the role of SRF in the regulation of genes responsible for cardiac structure and function.

**Overexpression of SRF results in cardiomyopathy.** In the present study, the SRF transgenic mice displayed impaired cardiac function and cardiomyopathy when they developed increased heart weight (cardiomyopathy). The impact of SRF overexpression on cardiac function is likely to be related to the function of SRF-regulated genes, many of which are essential for the maintenance of cardiac structure and function. Point mutations or deletions in the genes of both cardiac and skeletal actin, dystrophin, β-MHC, and MLC have been associated with either hypertrophic or dilated cardiomyopathy (10, 17, 54). In addition, the experimental introduction of an Arg403Gln mutation into the cardiomyopathy (10, 17, 54). In addition, suppression of cardiac actin and induction of skeletal actin gene in the SRF transgenic mice could change the structure and function of the cardiac myocyte. Therefore, increased expression of SRF, even at low levels, could have deleterious effects, through its influence on the many other genes important in the heart. For instance, in normal mature cardiac myocytes, cardiac actin comprises about 80% of total actin protein, with skeletal actin comprising the remaining 20%. Actin dysfunction may lead to heart failure (42). Suppression of cardiac actin and induction of skeletal actin gene in the SRF transgenic mice could change the normal ratio of the two actin isoforms and thereby impair cardiac function. Myosin is a chemomechanical motor that converts chemical energy into the mechanical work of muscle contraction (58). α-MHC confers a higher ATPase activity and higher shortening velocity to the heart compared with β-MHC (39). A shift from α-MHC to β-MHC in the heart could potentially lead to slower ventricular contraction (49) and even systolic dysfunction (41).

In addition, suppression of MLC may impair its ability to regulate muscle contraction and/or sarcomere organization during hypertrophy (2, 39). Dystrophin is a cytoskeletal protein in muscle fibers. Mutations in this gene can lead to human Duchenne muscular dystrophy (14). In those patients with the most severe cardiac phenotype of this disorder, the cardiac muscle is unable to produce dystrophin due to a defect in the transcription of the gene (17). It is possible that suppression of dystrophin in the SRF transgenic mice in the present study could have contributed to the cardiac dysfunction, resembling human cardiomyopathy due to the loss of dystrophin. SERCA2 contributes in an important manner to lowering diastolic Ca²⁺ and to relaxation of the heart (15). It is also possible that suppression of SERCA2 could result in abnormal calcium handling (3) and altered myocardial relaxation (34). The cardiac muscle dysfunction observed by echocardiography in the SRF transgenic animals is likely due to the dysregulation of one or more of these SRF-regulated genes.

Recently, Huang et al. (26) reported that overexpression of green fluorescent protein caused cardiomyopathy in transgenic mice, but no cardiac fibrosis was observed. In the SRF transgenic mice in the present study, along with the altered cardiac gene expression, collagen deposition and interstitial fibrosis were also found. These observed changes could have resulted from the modulatory effect of SRF on the expression of matrix metalloproteinases, collagenases, and/or their inhibitors.

The SRF-containing complex is one of the downstream targets of the intracellular signaling pathways of the mitogen-activating protein (MAP) kinase and Rho GTPase families (1, 59). Several studies have shown that both pathways play a role in mediating cardiac hypertrophy (22, 25). The contribution of SRF to the hypertrophic response was previously studied in cardiomyocytes, in which the activation of a cascade of p21ras, protein kinase C, and MAP kinase, as well as the induction of c-fos, were observed after mechanical loading (47). Although there is as yet no established evidence of SRF being directly involved in causing cardiac hypertrophy mediated by the Rho signaling pathway, the possible participation of SRF in the RhoA-induced cardiac hypertrophy was recently proposed (18).

In conclusion, certain SRF-regulated genes play an important role in the maintenance of cardiac structure and function. Sustained overexpression of SRF, even in very low copy numbers, is sufficient to cause dysregulation of several SRF target genes and induce cardiomyopathy in the transgenic mice. Taken together, these findings indicate that SRF might be an important downstream regulator in those pathways involved in mediating cardiac hypertrophy and cardiomyopathy.

We are deeply grateful to Drs. R. Prywes and A. Usheva-Simidjievski for critical reading of the manuscript. We thank Dr. R. Prywes for a generous gift of the pCNSRF plasmid and Dr. J. Robbins for a generous gift of the α-MHC promoter. We thank Dr. J. M. Laplante and C. Huang for invaluable discussions, Dr. P. S. Douglas, K. Converso, and L. J. Ma for echocardiographic assistance, and Dr. Z. Wang for histological analysis.
This work was supported in part by National Institutes of Health Grants AG-00294, AG-08812, AG-13514, AG-18388, and AG-00251.

REFERENCES