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Partnering Up for Cardiac Hypertrophy

John P. Konhilas and **Leslie A. Leinwand**

From the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder.

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The heart must adapt its mechanical activity to the prevailing hemodynamic demands. When an increased demand is brought about by sustained stimuli such as growth and development, pressure overload, or mutations in sarcomeric proteins, the heart will typically undergo an increase in size caused by myocyte hypertrophy. Underlying this *hypertrophic* response is the coordinated interaction of distinct signaling modules capable of transmitting and executing modifications in gene expression that lead to alterations in myocyte physiology and long-term cardiac adaptation.¹

One of the more intriguing characteristics of the hypertrophic response is that despite the ability of a wide variety of both pathologic and physiologic stimuli to induce cardiac hypertrophy, distinct cytoplasmic signaling cascades that initiate changes in gene expression converge on a common set of nuclear factors. These factors will then transactivate or repress cardiac genes through cis-regulatory elements. One of these transcription factors, myocardin, that appears to be capable of relaying hypertrophic signals to the genome is the subject of the studies performed by Xing et al² detailed below.

An important hypertrophic transcriptional regulator is the zinc finger–containing transcription factor, GATA4. GATA4 is widely expressed and has been identified as a pivotal regulator of developmental and stress-induced changes in cardiac gene expression.^{3,4} GATA4 has been shown to activate numerous genes in the heart by divergent signaling molecules including protein kinase C, calcineurin, and members of the mitogen-activated protein kinase signaling axis.¹ Although GATA4-null embryos arrest before birth,⁵ a point mutation in GATA4 results in septation and coronary vasculature defects,⁶ whereas GATA4 mutations are linked to congenital heart defects in humans.⁷ GATA4 has also been shown to activate a cardiac enhancer/promoter construct in nonmuscle cells.⁸

Another central transcriptional regulator of cardiac gene expression is serum response factor (SRF), which is also widely expressed in many cell types and binds to a DNA consensus sequence known as the CArG (CC[A/T]₆GG) box.^{9,10} SRF belongs to the MADS (MCM1, Agamous, Deficiens, SRF) family of transcription factors that includes myocyte enhancer factor 2 (MEF2).¹¹ Highlighting the importance of SRF is the fact that many cardiac muscle genes require CArG boxes for proper expression,¹² and disruption of SRF DNA binding by cardiac overexpression of SRF that contains 2 mutations located within the domain important for SRF dimerization leads to an early postnatal death (12 days) from a severe dilated

Correspondence to Leslie A. Leinwand, PhD, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Campus Box 347, Boulder, CO 80309-0347. E-mail E-mail: leinwand@stripe.colorado.edu.

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cardiomyopathy.¹³ In addition, cardiac-specific ablation of SRF is embryonic lethal because of cardiac insufficiency,¹⁴ whereas cardiac overexpression of wild-type SRF results in hypertrophic cardiomyopathy.¹⁵ Apart from its role in cardiac development and hypertrophy, SRF also mediates the expression of numerous growth-inducible developmental genes and is critical for smooth muscle development.¹⁶ Moreover, activation of SRF is induced by numerous hypertrophic signals, many of which overlap with GATA4, including calcineurin-dependent signaling¹⁷ and the alpha-adrenergic agonist, phenylephrine (PE).^{18,19} In fact, GATA4 can interact with SRF, and it has been suggested that they may act synergistically through recruitment of each other to bind DNA^{20,21}

More recently, a novel and potent SRF transcriptional coactivator, myocardin, was discovered and subsequently shown to transactivate CArG box-dependent cardiac promoters.²² Myocardin belongs to the SAP (scaffold-attachment factor A/B, Acinus, PIAS) domain family of nuclear proteins, which are implicated in chromatin remodeling.²³ It has been shown to be necessary and sufficient for both cardiac and smooth muscle differentiation.^{22,24} Interestingly, GATA4 modulates myocardin transcriptional activity either positively on genes such as *Nkx2.5* or negatively such as *ANF*.²¹ Moreover, forced expression of myocardin can induce both cardiac and smooth muscle genes in nonmuscle cells.^{25,26} As mentioned above, GATA4 can activate a transfected cardiac promoter but its ability to activate endogenous genes is unknown. Although GATA4 and SRF null mice have early developmental myocardial defects, myocardin null mice show no myocardial defects at midgestation. However, the role of myocardin in the adult heart has not yet been evaluated because of embryonic lethality caused by vascular abnormalities.²⁷ It will be necessary to evaluate adult cardiac-specific ablation of myocardin before it is possible to determine its role in the adult heart. Inconsistent with the observation in mice is the finding that morpholino knockdowns of myocardin in *Xenopus* embryos inhibit cardiac development, so future experiments need to be aimed at these differences in the two species.²⁶

In this issue of *Circulation Research*, Xing et al² address and confirm a role for myocardin in the hypertrophic response of cardiac myocytes and in the intact heart. In this study, both myocardin mRNA and protein were upregulated by two hypertrophic agonists, fetal bovine serum or PE, in cultured cardiac myocytes. In two mouse models of cardiac hypertrophy, thoracic aortic banding and calcineurin overexpression, myocardin expression levels in the heart were significantly upregulated with concomitant elevations in fetal genes. Finally, the translation of these data to human diseased hearts was validated by demonstrating increased myocardin expression in protein extracts from patients with idiopathic dilated cardiomyopathy. The necessity of myocardin in the hypertrophic response was supported by studies in which myocardin was introduced into cardiac myocytes by adenoviral delivery and subsequently exhibited a hypertrophic phenotype. In contrast, cardiac myocytes expressing a dominant negative myocardin that maintains the ability to interact with SRF but lacks the transcriptional activity domain showed a small decrease in size and were unable to respond to hypertrophic stimuli. The authors demonstrated an additional level of myocardin regulation by the attenuation of myocardin-induced hypertrophy by histone deacetylase, HDAC5.

Given that hypertrophic stimuli initiate a reexpression of fetal gene isoforms in the heart²⁸ and that myocardin is necessary for cardiac development in *Xenopus*, it is tempting to conclude that myocardin causes the reappearance of these fetal genes in the pathological heart. However, the developing heart does not progress to a decompensated state as occurs with persistent pathological stimuli such as hypertension. This fact alone highlights the importance of temporal activation of these signaling intermediates and the necessity for additional “players” that may impart selectivity to gene target activation by posttranslational modification of specific transcription factors and cofactors by intracellular signals. For example, it was recently shown that the transcriptional activity of myocardin is regulated by site-specific phosphorylation by

glycogen synthase kinase-3 β .²⁹ A schematic of the transcriptional regulatory network is illustrated in the Figure. Another looming question is whether myocardin mediates hypertrophy attributable to alternative physiological hypertrophic stimuli such as exercise. Consequently, the emerging picture is that, although distinct hypertrophic and developmental stimuli appear to converge on common transcription factors such as GATA4 and SRF, activation of these factors does not result in indiscriminate expression of GATA4 or SRF-dependent genes. Simultaneous activation of cofactors is required but, in addition, there are many indications that one or more additional levels of regulation exist that allow a cell to further distinguish between different stimuli.

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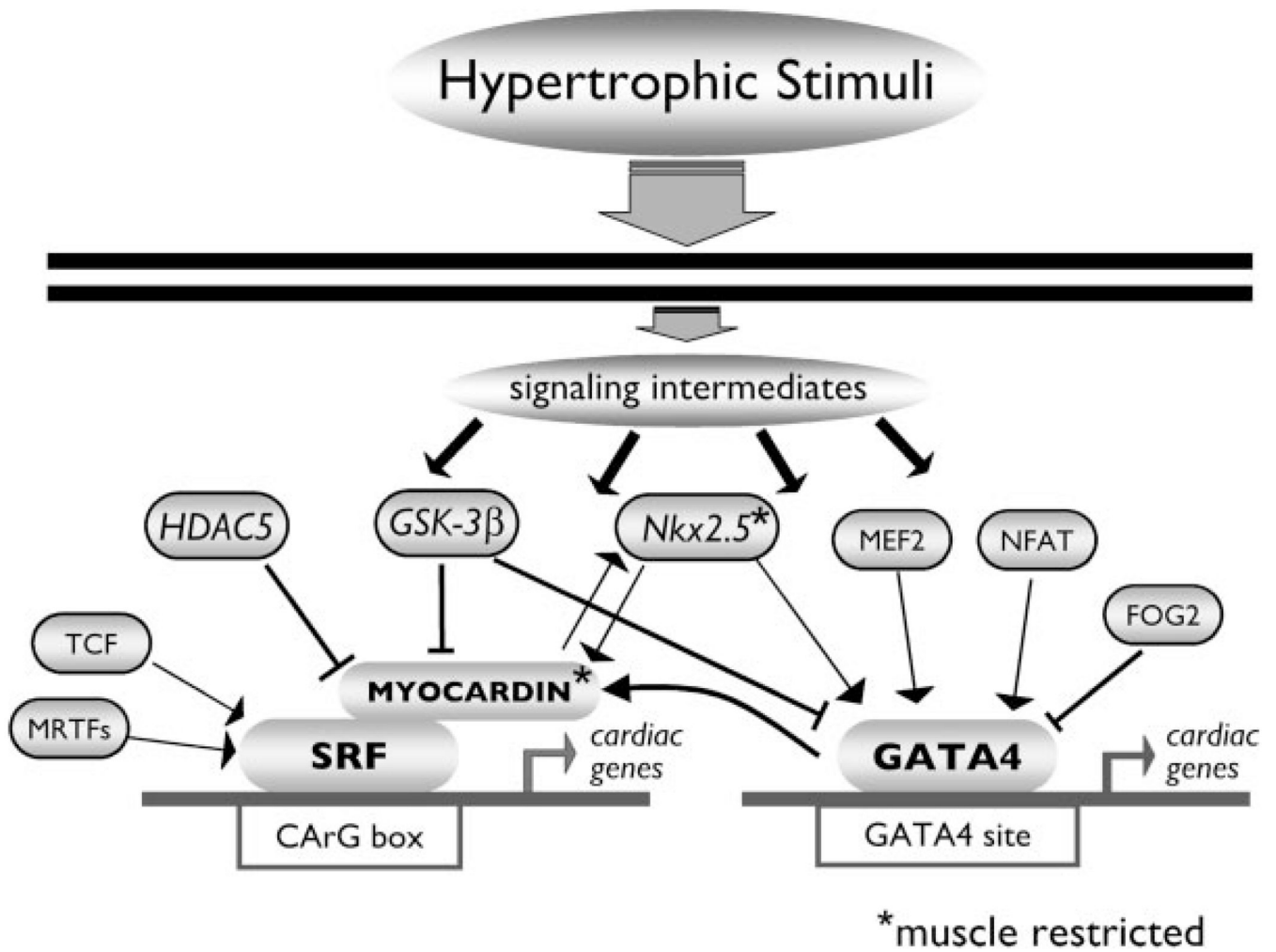


Figure 1.

Schematic representation of GATA4- and SRF-dependent gene regulation. Hypertrophic stimuli initiate a signaling cascade that converges on the transcriptional regulators GATA4 and SRF in the heart. The activation and/or repression of gene expression by GATA4 and SRF depend on the interaction with specific cofactors that are spatially and temporally activated. MRTF indicates myocardin-related transcription factor; TCF, ternary complex factor; HDAC5, histone deacetylase 5; GSK-3 β , glycogen synthase kinase 3 β ; MEF2, myocyte enhancer factor 2; NFAT, nuclear factor of activated T-cells; FOG2, friend of GATA2.