Gene Expression, Vol. 7, pp. 337–348, 1999
Printed in the USA.

1052-2166/99 $10.00 + .00

Signaling Pathways Mediating the Response to Hypertrophic Stress in the Heart

THOMAS FORCE, ROGER HAJJAR, FEDERICA DEL MONTE, ANTHONY ROSENZWEIG, AND GABRIEL CHOUKROUN[1]

Medical Services, Massachusetts General Hospital, and Department of Medicine, Harvard Medical School, Boston, MA

Cardiac hypertrophy is an increase in the mass of the heart. It is a major risk factor for the development of myocardial infarction and congestive heart failure, diseases that afflict millions of patients worldwide. Hypertrophy can be caused by intrinsic defects of the proteins of the contractile apparatus of the heart, or by extrinsic stimuli such as hypertension. In this review, we will focus on the cytosolic signal transduction pathways that mediate the hypertrophic response to extrinsic stimuli. Although a large number of signaling molecules have been implicated in the hypertrophic response, we will review data that, we believe, suggest there may be only a few molecules that serve as signaling funnels through which many hypertrophic signals must pass on their way to the nucleus. These include the stress response protein kinases (the stress-activated protein kinases or SAPKs, and, possibly, the p38 kinases) and calcineurin. These molecules have as their primary targets transcription factors, many of which have been implicated in the complex yet stereotypic genetic response to hypertrophic stress. In most cases, it is not possible at present to complete the link from hypertrophic stimulus through a specific signaling molecule and a specific transcription factor to the induction of a specific gene that initiates a particular biologic response. We will attempt to identify some of the most important areas where major questions remain in the hopes of stimulating further research into this major cause of death and disability.

Cardiac hypertrophy Stress-activated protein kinases c-Jun N-terminal kinases Heart failure

CAUSES OF HYPERTROPHY

Familial Hypertrophic Cardiomyopathies

Familial hypertrophic cardiomyopathies (HCM) are genetically transmitted diseases with a dominant mode of inheritance [reviewed in (54,67)]. The genes implicated in HCM encode contractile proteins involved in the generation of force by the cardiomyocyte, and include β-myosin heavy chain, cardiac troponin T, α-tropomyosin, myosin-binding protein C, and myosin light chain. These proteins are normally organized into the contractile apparatus of the cell termed the sarcomere. The mutations create what has been termed a "poison peptide," which infiltrates and disrupts organized sarcomeres by interfering with the action of the wild-type protein (22,34). The chronic reduction of force generation stimulates myocyte hypertrophy. In some mouse models of HCM, cytosolic free $[Ca^{2+}]$ is chronically elevated, and this may be a key factor in activating the signaling pathways that mediate the hypertrophic response (see below).

Acquired Hypertrophy

In this review, we will focus on acquired or extrinsic cardiac hypertrophy. Acquired hypertrophy is an adaptation to stress. In its earliest stages, acquired cardiac hypertrophy is a compensatory response to pressure or volume overload, injury, or neurohormonal activation. Hypertrophy is an attempt on the part of the heart to normalize wall tension, which is increased in such conditions as hypertension, abnormalities of the cardiac valves, or following a myocardial infarction in the noninfarcted area. Because wall tension is inversely proportional to the thickness of the wall of the heart, thickening of the heart (hyper-

[1]Address correspondence to Gabriel Choukroun, Massachusetts General Hospital East, Suite 4002, 149 13th St., Charlestown, MA 02129. Tel: (617) 726-9334; Fax: (617) 726-4356; E-mail: gchoukroun@partners.org

trophy) will tend to normalize wall tension and thereby reduce oxygen demand of the myocardium. Adult cardiac myocytes are terminally differentiated and cannot undergo cellular division. Therefore, growth of the heart must occur through an increase in size of the myocytes. Although initially an adaptive response, if the stimulus to hypertrophy persists, the heart often undergoes a transition to contractile failure.

CHARACTERISTICS OF THE HYPERTROPHIC RESPONSE

The phenotypic changes that are the hallmarks of hypertrophy include an increase in cell size and increased protein synthesis. In neonatal rat cardiomyocytes in culture, hypertrophic stimuli also induce enhanced organization of sarcomeres. There are also numerous changes in phenotype brought about by changes in the pattern of gene expression (66). Following initiation of an acute hemodynamic stress (e.g., banding of the aorta), several immediate-early genes are induced, including c-*fos*, c-*jun*, *Egr*-1, and c-*myc*. Following this, several genes encoding proteins of the contractile apparatus are induced. These genes are normally expressed during fetal development, but their expression is renewed following hypertrophic stress. These include skeletal α-actin and ventricular myosin light chain-2 (MLC-2v). In addition, in the rat, there is an isoform shift for myosin heavy chain (MHC), leading to a decrease in α-MHC, the form normally expressed in the adult ventricle, and an increase in β-MHC, expression of which normally disappears early in life. The enhanced expression of β-myosin heavy chain, by improving the economy of contraction, conserves ATP, but it leads to slower contraction and relaxation phases that are characteristic of hypertrophy and can be detrimental. There is also renewed expression of hormones not normally expressed in the adult ventricle. These are atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP). These peptides are vasodilators and diuretics, serving to lower blood pressure and to enhance excretion of excess fluid, and both of these responses will have the beneficial effect of lowering wall tension.

There is also altered expression of genes encoding proteins involved with calcium handling. For example, expression of the sarcoplasmic reticulum calcium pump, SERCA2a, which clears Ca^{2+} from the cytoplasm and allows the myocyte to relax following a contraction, is decreased during hypertrophy, leading to a reduced velocity of relaxation and increased cytosolic-free [Ca^{2+}] (66). Because there is less filling of the sarcoplasmic reticulum with Ca^{2+}, the subsequent contraction is less vigorous. Importantly, this downregulation of SERCA2a expression is not simply a marker of the hypertrophic response. Rather, it likely plays an important role in the progression of hypertrophy to heart failure since we have demonstrated that restoration of SERCA2a levels during the transition to heart failure via adenovirus-mediated gene transfer in vivo can correct the contractile abnormalities (33).

Finally, expression of the cell surface receptor for angiotensin II (Ang II), the AT_{1a} receptor, is increased by hypertrophic stimuli (20). Ang II has been implicated in the progression of hypertrophy and the transition to heart failure, and this may be an important mechanism of that effect.

SIGNALING MOLECULES IMPLICATED IN THE HYPERTROPHIC RESPONSE

Hypertrophic Stimuli

In order to understand the signaling mechanisms governing the hypertrophic response, we must first identify the stimuli that induce hypertrophy. Several stimuli appear to be involved with the development of cardiomyocyte hypertrophy in vitro. Peptide growth factors (e.g., FGF, PDGF, and IGF-1), TGF-β, and some cytokines (cardiotrophin-1 and leukemia inhibitory factor) can cause myocyte hypertrophy in vitro (41,42), though their role in vivo is unclear. Cell stretch is a potent inducer of hypertrophy in cardiomyocytes in-culture, and likely plays a role in the hypertrophic response to pressure overload in the intact animal (48). Cell stretch not only directly activates the hypertrophic response, but also induces the release of one or more neurohormonal agents that are potent stimulators of the hypertrophic response in cardiomyocytes in-culture, and also likely play a critical role in vivo (25,48,50,51,73). These agents, which include Ang II, endothelin-1 (ET-1), and α-adrenergic agents (e.g., norepinephrine, epinephrine, and phenylephrine), not only increase afterload in vivo, enhancing cell stretch, but also via interactions with serpentine receptors coupled to heterotrimeric G proteins of the Gq family, directly activate the hypertrophic response of myocytes independent of their effects on afterload (50,53,73).

It had been known for some time that antagonists of some of these Gq-linked receptors, particularly ET-1 and Ang II, blocked hypertrophy in the intact animal, suggesting these agents likely played a role in the development of hypertrophy and in the progression of hypertrophy to heart failure in vivo. Recently, Akhter et al. (1) demonstrated that blocking

signaling from Gq-linked receptors by overexpressing in transgenic mice a peptide derived from the carboxy-terminal tail of the αq subunit blocked the development of cardiac hypertrophy induced by aortic banding, lending further support to the idea that signaling from Gq-linked receptors is critical in the hypertrophic response to pressure overload. Which specific receptors are involved in vivo is not clear. Studies with an Ang II type 1a receptor knockout mouse demonstrated that the hearts of these mice readily hypertrophied when exposed to acute pressure overload (16). This does not mean that Ang II is not involved in the hypertrophic response in vivo, however, but merely suggests that with this extreme stress, multiple Gq-linked receptors, which recruit common signaling pathways leading to hypertrophy, are activated.

Signaling Pathways

Because of what appears to be a central role of the neurohormonal mediators in cardiac hypertrophy in vivo, and the findings of Akhter et al. implicating Gq-linked receptors in vivo, we and others have studied the signaling pathways activated by these agents in cardiomyocytes in vitro. These studies have implicated molecules as diverse as the small GTP binding proteins, Ras, Rac, and Rho (21,44,63), all three of the MAP kinase cascades [culminating in the activation of the extracellular signal-regulated kinases (ERK-1 and ERK-2), the stress-activated protein kinases (SAPKs) or c-Jun N-terminal kinases (JNKs), and the p38 family of kinases; reviewed in (56)], the β1 integrins (47), protein kinase C isoforms (4,69), the p70 ribosomal S6 protein kinase (49), and a pathway involving the calcium/calmodulin-dependent serine/threonine phosphatase, calcineurin, and its target, nuclear factor of activated T cells-3 (NF-AT3) (35).

Ras and the ERK Cascade

Many of the signaling molecules listed above were initially identifed as candidate mediators of the hypertrophic response based on the ability of constitutively active mutants of the molecules to induce one or more components of the response either in cardiomyocytes in vitro or when expressed in transgenic animals. Initially, interest focused on the role of Ras in the hypertrophic response. Expression of a constitutively active allele of Ras in the hearts of transgenic mice caused a phenotype resembling hypertrophic cardiomyopathy (21). In addition, expression of constitutively active Ras in cardiomyocytes in vitro was sufficient to induce genetic markers (e.g., ANF expression) and morphological changes of hypertrophy (increase in cell size and development of highly organized sarcomeres) (59,62).

These data implicate Ras in the hypertrophic response, but identifying downstream effectors of the response is made difficult by the fact that Ras has a large number of downstream targets, any one of which could mediate its effects (68). Given their role in mitogenesis and cellular differentiation, and the fact that most hypertrophic stimuli strongly activate the c-Raf-1/ERK pathway, the ERKs were initially postulated to be the mediators of the hypertrophic effects of Ras. Several studies employing either the MEK-1 inhibitor, PD98059, a constitutively active mutant of MEK-1, or dominant inhibitory mutants of c-Raf-1 and the ERKs, have suggested that the ERKs are neither necessary nor sufficient for expression of several components of the hypertrophic response in vitro (43,46,60,61).

Stress Response Protein Kinases

Activity of the SAPKs is increased in the hearts of transgenic mice expressing constitutively active Ras. This observation, and a number of other studies on cardiomyocytes in vitro, suggest that the hypertrophy induced by constitutively active Ras may be mediated via activation of SAPK pathway, or the other stress response pathway, the p38 family (Fig. 1) (3,43,46, 61,63,70,71,74). These studies have demonstrated that overexpression of constitutively active alleles of MEKK-1 (the MAPKKK upstream of the SAPKs) (3,46,63,74), MKK7 (a MEK upstream of the SAPKs) (71), or MKK3 and MKK6 (MEKs upstream of p38) (70,74) induces characteristic hypertrophic responses in cardiomyocytes.

Most studies to date addressing the role of the MAP kinase cascades in myocyte hypertrophy have forced constitutive activation of the MAP kinases by overexpressing activated mutants or wild-type components of these cascades. This results in supra-normal and prolonged activation of normal downstream targets, and, in some cases, activation of MAP kinase cascades or other signaling pathways that are not activated under physiologic conditions, making it difficult to ascribe any effects to one specific pathway [discussed in (8)]. These caveats aside, these important studies do strongly suggest that either the SAPK or p38 pathways are sufficient to induce a hypertrophic response if they are constitutively active over prolonged periods (70,71,74). They do not clarify, however, whether the pathways are critical to the hypertrophic response of cardiomyocytes to physiologically relevant stimuli that produce much less marked activation over much shorter periods of time.

To address this question, several groups have em-

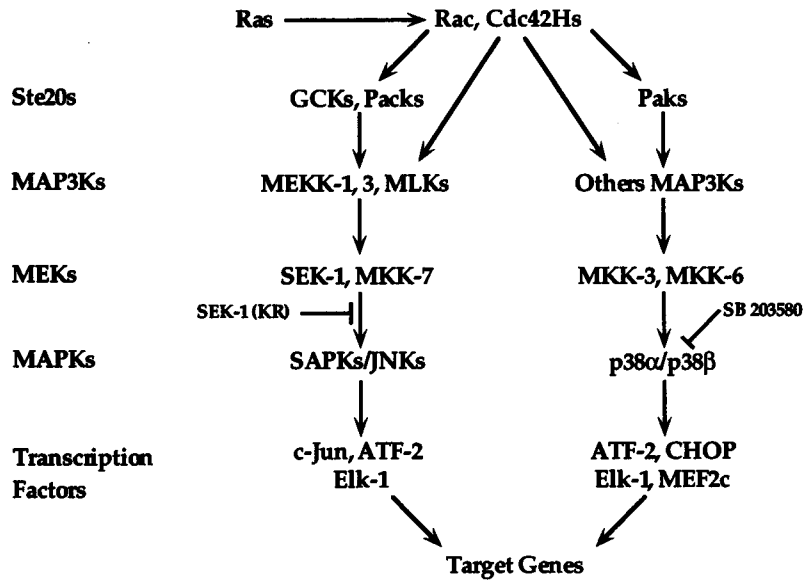

FIG. 1. Stress response MAP kinase cascades. These cascades consist of a three-tiered core module wherein a MAP kinase kinase kinase (MAP3K) activates a MEK, which in turn activates the MAPK. Upstream of the MAP3Ks are small G proteins, Rac, and Cdc42Hs. These appear to be able to activate the cascades via activation of a fourth tier of kinases, the Sterile 20 kinases (Ste20s), or by directly activating MAP3Ks (such as the mixed lineage kinases or MLKs). Once activated, the MAPKs translocate to the nucleus where they recruit a number of transcription factors. Also shown are the sites of action of the inhibitor of SAPK activation [SEK-1(KR)] and the p38 inhibitor (SB203580).

ployed SB203580 and SB202190, two pyridinyl imidazoles that are inhibitors of p38α and p38β (Fig. 1). These studies have suggested vital roles for these p38s in components of the hypertrophic response to neurohormonal mediators. There are two caveats, however. First, while initially touted as specific inhibitors of the p38s, studies have now shown that at concentrations typically employed in the studies of 10 μM or greater, the SB compounds inhibit several SAPK isoforms (72). This points out the need for careful dosage titration. Second, the SB compounds do not inhibit two other p38 family members, p38γ and p38δ. Thus, failure to block a component of the hypertrophic response with the SB compounds does not rule out a role for these p38s in the response.

There are no adequate inhibitors of the SAPK pathway at this time. Therefore, to determine whether the SAPKs are necesary for various components of the hypertrophic response, it has been necessary to employ transfection of dominant inhibitory mutants of one or more components of the SAPK pathway in an attempt to block signaling down the pathway. Because of the low transfection efficiency that can be achieved in cardiomyocytes, researchers have often had to turn to various surrogates of the hypertrophic response, the most commonly employed being the activity of a reporter construct containing the ANF promoter. These studies have produced conflicting results. Two groups have shown that the SAPKs are necessary for increased ANF reporter activity in response to neurohormonal agents, whereas another group has suggested that the SAPKs inhibit ANF reporter activity (39,46,63). These disparate results, and ones from prior studies examining the role of the ERKs in hypertrophy, suggest that activity of reporter constructs is not an adequate surrogate for hypertrophy. The study of Nemoto et al. also reported that blocking p38 activity with SB202190 blocked activation of the ANF reporter construct, although, again, concentrations of 10–20 μM were employed.

To explore the role of the SAPKs in cardiomyocyte hypertrophy, we have employed adenovirus-mediated gene transfer, which allows one to achieve an efficiency of transduction of cardiomyocytes of greater than 90%, to express SEK-1(KR) (6,13,14). SEK-1(KR) is a kinase inactive mutant of SEK-1, a MEK immediately upstream of the SAPKs (Fig. 1), which functions as a dominant inhibitory mutant of SAPK activation and has been shown to block such responses as apoptosis in response to a number of environmental stresses. Expression of SEK-1(KR) in neonatal rat cardiomyocytes blocked SAPK activation by ET-1 (and Ang II), and abrogated the hypertrophic response as determined by protein synthesis, ANF mRNA expression, and enhanced sarcomere organization (6). In contrast, the MEK-1 inhibitor PD98059 (used at a concentration of 50 μM, which completely blocked ET-1-induced ERK activation), and SB203580 (used at a concentration of 5 μM, which blocked p38 but not SAPK activation) had no

significant effect on the hypertrophic response. These data suggested that the SAPKs, but not the p38s or the ERKs, were necessary for the hypertrophic response to ET-1 and Ang II (unpublished observations) in vitro. The SAPKs are also activated by pressure overload induced by aortic banding, suggesting they could play a role in the hypertrophic response in vivo. We are currently employing a method developed by Hajjar and coworkers (15) of adenovirus-mediated gene transfer to express SEK-1(KR) in the hearts of adult rats in order to explore the role of this pathway in pressure overload-induced cardiac hypertrophy (7).

The role of the p38 pathway is somewhat uncertain at this time. This is due to the fact that no studies using concentrations of inhibitors that specifically inhibit the p38s have demonstrated convincingly that these kinases are necessary for the hypertrophic response in vitro. Furthermore, although Wang et al. reported that p38s were significantly activated by pressure overload, Molkentin et al. reported that they were not activated in the hearts of a transgenic model of hypertrophic myopathy, suggesting activation of the p38s may not be necessary for cardiac hypertrophy in vivo. Although the p38s may not be necessary, given the overlapping substrate specificities of the SAPKs and p38s, it seems likely that they may play a role in amplifying the hypertrophic response in some forms of hypertrophy (see below). In addition, they have been implicated in cardiomyocyte apoptosis based on studies employing gene transfer of constitutively active MKK3, a MEK upstream of the p38s (70).

Interactions of the Stress Response Kinase Pathways and Other Signaling Molecules Implicated in Hypertrophy

How can one reconcile the data suggesting that the SAPKs are necessary for the hypertrophic response with studies implicating numerous other signaling molecules in the response? We believe that for many of those molecules, including Rac1, Ras, and Gq-coupled receptors, studies in other cell systems suggest the SAPKs (and possibly p38s) are downstream components of pathways that are activated by these molecules (Fig. 1).

Rac1. Finkel and coworkers have utilized adenovirus-mediated gene transfer to demonstrate that the small GTPase, Rac1, is necessary for the hypertrophic response to the α-adrenergic agent phenylephrine (44). Constitutively active mutants of Rac1 are capable of activating the SAPKs, suggesting that the SAPKs may be downstream mediators of Rac1 in the hypertrophic response. Furthermore, because activated Ras is capable of activating Rac1 (and the SAPKs), it is possible that the Rac/SAPK pathway is a downstream mediator of Ras-induced hypertrophy. Although this is likely to be the case, there have been no direct demonstrations that activation of the SAPKs by hypertrophic stimuli (or by any stimulus) is dependent on endogenous Rac (29), nor have the intermediates in the pathway been identified.

Heterotrimeric G Proteins of the Gq Class. As noted, some of the strongest evidence to date identifying a class of signaling molecules as mediators of hypertrophy implicate heterotrimeric G proteins of the Gq class. Not only does expression of Gαq induce cardiac hypertrophy in transgenic mice (9,52), but expression of the dominant inhibitory peptide of Gq signaling blocks pressure overload-induced hypertrophy (1). Again, because the neurohormonal mediators with Gq-linked receptors potently activate the SAPKs, and expression of activated mutants of αq activate the SAPKs (19), the SAPKs may be downstream mediators of the hypertrophic response to activated Gq-linked receptors.

Calcineurin and NF-AT3. The other pathway implicated in the hypertrophic response is the calcineurin/NF-AT3 pathway (Fig. 2) (35,57). Calcineurin is a Ca^{2+}-calmodulin-dependent protein phosphatase that is activated when cytosolic calcium concentration increases. Calcineurin then dephosphorylates the transcription factor, NF-AT3 (nuclear factor of activated T cells-3). This exposes a nuclear localization signal on NF-AT3, leading to its import into the nucleus (2). Activity of NF-AT3 appears to be controlled by subcellular localization because cytosolic NF-AT3 is fully DNA binding competent. Cyclosporin A (CsA), a drug that appears to have calcineurin as its only target (24), inhibited the development of hypertrophy in several models of hypertrophic myopathies and in a pressure overload model of hypertrophy at 1 week following aortic banding (57). Although calcineurin has many targets in the cell, the fact that expression of a constitutively active NF-AT3 in the hearts of transgenic mice caused marked hypertrophy (35) suggests NF-AT3 is the relevant target in the hypertrophic response.

Recently, there has been a debate in the literature over the relevance of the calcineurin/NF-AT3 pathway because three other studies have shown that CsA does not prevent pressure overload hypertrophy if the animals are followed for 2 or more weeks instead of the 1-week endpoint used by Molkentin and coworkers (28,34,38). Furthermore, CsA was shown to not prevent cardiac hypertrophy in mice expressing a

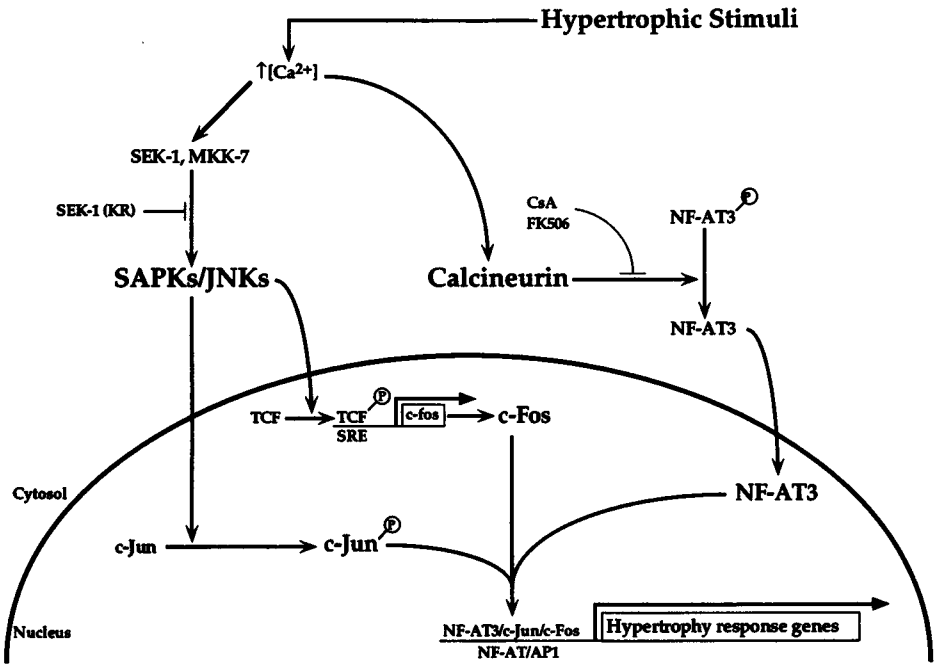

FIG. 2. Signaling pathways implicated in the hypertrophic response: the SAPKs, calcineurin/NF-AT3, and a putative costimulatory pathway. Hypertrophic stimuli (e.g., cell stretch or neurohormonal mediators including Ang II, ET-1, and α-adrenergic agents) lead to an increase in cytosolic-free [Ca^{2+}]. This, together with calmodulin, activates the protein phosphatase, calcineurin, and likely also plays a role in activation of the SAPKs, although the pathway connecting the increase in [Ca^{2+}] to SAPK activation is unclear. Calcineurin dephosphorylates the transcription factor, NF-AT3, exposing a nuclear localization signal and allowing NF-AT3 to be imported into the nucleus. Upon activation, the SAPKs translocate to the nucleus where they phosphorylate and activate the transcription factors, c-Jun, and one or more TCFs (Elk-1 or SAP1). Activated TCFs likely induce transcription of c-*fos*. By analogy with the costimulatory pathway of T cells, the signals from these two parallel pathways may be integrated at the promoters of various hypertrophic response genes by complex formation of c-Fos, c-Jun, and NF-AT3. Also shown are the pharmacologic inhibitors of calcineurin, CsA and FK506, and SEK-1(KR), the dominant inhibitory mutant of the immediate upstream activator of the SAPKs.

constitutively active mutant of the αq subunit of Gq heterotrimeric G proteins (32). These data have led various authors to postulate that a signaling pathway in addition to calcineurin/NF-AT3 must be involved in cardiac hypertrophy. Our data in cardiomyocytes in-culture suggest that this additional pathway might be the SAPK pathway (Fig. 2).

In T lymphocytes, a pathway activated by antigen engagement of the T-cell receptor, the costimulatory pathway, requires signals from both calcineurin/NF-ATs and activated SAPKs for full expression of a number of immune response genes (5,30,55). In some reports, calcineurin appears to be upstream of the SAPKs in T cells because CsA blocked SAPK activation (30,55). We have not found this to be the case in cardiomyocytes in culture, and the current consensus is that the SAPKs and calcineurin are parallel pathways and their signals are integrated at the promoters of relevant genes by interactions of NF-ATs and AP-1 (5). Full induction of the NF-AT-dependent genes requires cooperative interactions between NF-ATs and activated AP-1. Recently, chrystal structure analysis confirmed that NF-AT family members form a complex with AP-1 at specific response elements contained in the promoters of many genes (5). The SAPKs regulate the activity of AP-1, which is often composed of a heterodimer of c-Jun and c-Fos, by phosphorylating two serine residues in the amino-terminal region of c-Jun, thereby increasing transcriptional activating activity of c-Jun (23,45). Our data, and the data implicating calcineurin in the hypertrophic response, suggest that a pathway similar to the costimulatory pathway of T cells may regulate the hypertrophic response in the heart. If so, both pathways would likely be necessary for the full expression of the hypertrophic response to physiologic stimuli, although if either is markedly activated, that may be sufficient by itself to induce hypertrophy. This may explain the "escape" from CsA that several groups have observed. It is important to stress, however, that although it is clear that calcineurin may not be necessary for the hypertrophic response to the extreme stress of pressure overload, the data do not suggest that calcineurin is unimportant, and it may be critically important when the stress is less extreme.

REGULATION OF TRANSCRIPTION BY SIGNALING PATHWAYS

A great deal of work has focused on identifying the promoter elements and transcription factors required for expression of hypertrophic response genes. Relatively little work has been done, however, to connect those transcription factors to signaling pathways, and most of the studies that have been done have relied upon activity of reporter constructs containing portions of the promoter of the gene of interest. Thus, in most cases, definitive proof of the role of a signaling pathway in the induction of a specific hypertrophic response gene is lacking. However, work in other systems allows us to predict the involvement of cytosolic signaling pathways in the induction of some genes.

Upon activation, all MAPKs translocate to the nucleus where they recruit a number of transcription factors. Pulverer et al. (45) first demonstrated that purified p54 MAP kinase (the name given to the SAPKs prior to their cloning) phosphorylated c-Jun at two serine residues in the amino-terminal transcriptional activation domain, and that these phosphorylation events increased the transcriptional activating activity of c-Jun. Others showed that the SAPKs also phosphorylated activating transcription factor-2 (ATF-2) at two residues in the transcriptional activation domain, increasing transcriptional activating activity (12,27,65). We subsequently showed that a heterodimer of c-Jun and ATF-2 likely mediated the induction of c-*jun* in response to another extreme stress, ischemia (37). It is likely that these two transcription factors, regulated at least in part by the SAPKs, also control the induction of c-*jun* in the pressure overloaded heart.

The SAPKs, as well as the ERKs and p38s, also target the ternary complex factors (TCFs) including Elk-1. Phosphorylation by any of these MAPKs enhances ternary complex formation of a TCF with serum response factor at serum response elements of various promoters including the c-*fos* promoter. This mechanism likely accounts, in part, for the induction of c-*fos* in the ventricle exposed to pressure overload (Fig. 2). AP-1 is composed of a heterodimer of a c-Jun family member and a c-Fos family member (23). Given the likely effects of the SAPKs on c-Jun transactivating activity and on the induction of the gene encoding c-Fos, it is likely that the induction of any hypertrophic response genes that are regulated by AP-1 will be modulated, in part, by the SAPKs. The hypertrophic response genes that appear to be dependent on AP-1 elements for induction by hypertrophic stimuli include ANF, the AT_{1a} receptor, and two sarcomeric proteins, skeletal α actin and myosin light chain-2v (17,20,26,40).

The ANF gene has been studied more than any other, possibly accounting for the myriad of elements reported to be involved in its induction. Studies employing promoter deletion analysis or cotransfection of transcription factors with promoter/reporter constructs have implicated a number of elements in the control of ANF expression including GATA, Csx/Nkx2.5, SRE, Sp1, NF-AT, and an AT-rich element. Although the SAPKs appear to be important for the induction of ANF in cardiomyocytes (6,31), the precise site of action of the SAPKs is not clear and could be via c-Jun, TCFs, SRF, and/or other transcription factors to regulate induction of ANF (31) (Fig. 3). Other signaling pathways and transcription factors are also involved. The calcineurin/NF-AT3 pathway appears to play an important role in the induction of ANF in cardiomyocytes (35). GATA4 and Csx/Nkx2.5, possibly acting at tandem sites in the ANF promoter, also regulate ANF expression. For these transcription factors, it remains unclear whether post-transcriptional regulation by signaling pathways plays a role in their activation, but the transactivation domain of GATA4 contains a consensus MAP kinase phosphorylation site, suggesting it may be regulated by one of these kinases (36). Because GATA4 also mediates induction of BNP, β-myosin heavy chain, and the angiotensin II receptor, identifying regulators of GATA4 activity would be a major step forward in our understanding of the hypertrophic response (18,20,64).

Although the SAPKs and p38s share several substrates including the ternary complex factors, Elk-1, and the related SAP-2 (11), their substrate specificity is not identical because c-Jun is a SAPK (but not p38) target and the transcription factors SAP-1 and CHOP are p38 (but not SAPK) targets. p38s may also play a role in activation of AP-1, not only via activation of TCFs and induction of c-*fos*, but also via activation of MEF2c and induction of c-*jun* (Fig. 4). p38-mediated phosphorylation of MEF2c enhances its transcriptional activating activity and the c-*jun* gene is one of the targets of MEF2c. The p38 inhibitor, SB203580, blocks induction of c-*jun* in response to some cellular stresses, suggesting that p38 regulates induction of c-*jun* (8). Once c-Jun is translated, the SAPKs enhance its transactivating activity.

p38s also phosphorylate and activate MAPKAP kinase (mitogen-activated protein kinase activated protein kinase)-2 and -3, and via this mechanism the p38 pathway may control activation of CREB and the related ATF-1 (Fig. 4) (58). MAPKAP kinase-2 phosphorylates CREB at Ser 133, increasing transcriptional activating activity of CREB (58). CREB is phosphorylated on Ser 133 following cellular stress. It is likely that this is mediated via activation of p38 and its target, MAPKAP kinase-2, because the

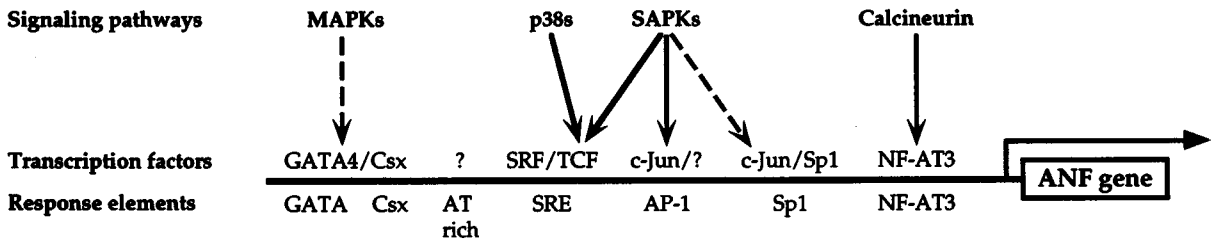

FIG. 3. Integration of signaling pathways and transcription factors at various promoter elements of the ANF promoter. Induction of the ANF gene in response to hypertrophic stress has been reported to be regulated by seven different promoter elements, GATA, Csx, an AT-rich element, SRE, AP-1, Sp1, and NF-AT. Likely regulation by cytosolic signaling pathways of the transcription factors reported to act at these sites (where known) is shown by solid arrows, whereas more speculative regulation is shown by dashed lines. See text for details.

p38 inhibitor, SB203580, markedly inhibits CREB phosphorylation and activation (58). Recently, CREB has been implicated in heart failure because transgenic mice expressing a dominant inhibitory mutant of CREB, in which Ser 133 has been mutated to a nonphosphorylatable residue, die from severe congestive heart failure (10).

CONCLUSIONS

A great deal of progress has been made in identifying signaling pathways that regulate the hypertrophic response in vivo. Agonists with receptors coupled to heterotrimeric G proteins, especially of the Gq class, clearly seem to be involved in pressure overload-induced hypertrophy. Furthermore, it seems

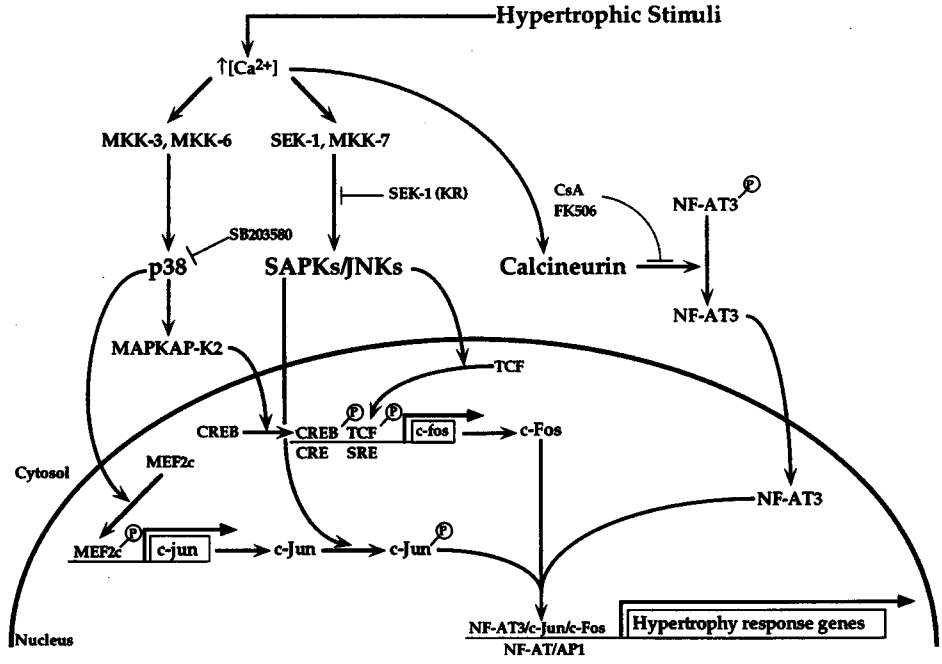

FIG. 4. Signaling pathways implicated in the hypertrophic response: putative roles of p38s. Once activated, p38s translocate to the nucleus where in addition to phosphorylating and activating TCFs (not shown), they also phosphorylate MEF2c, enhancing its transcriptional activating activity. Although the role of MEF2c in the response to hypertrophic stress is not clear, MEF2c does enhance transcription of c-*jun* in response to other cellular stresses, a response that is blocked by the p38 inhibitor, SB203580. p38s also phosphorylate and activate MAPKAP kinase-2, which regulates, via Ser 133 phosphorylation, the activation of CREB. In response to some cellular stresses, CREB regulates the induction of c-*fos*, although no such role for CREB has been documented in the hypertrophic response. In addition to these putative roles for p38s, these kinases have also been implicated in cardiomyocyte apoptosis, raising the possibility that they may play a role in the transition from hypertrophy to heart failure (not shown).

likely that one or both of the families of stress response MAP kinases, the SAPKs and p38s, also regulate critical features of the hypertrophic response. But what remains unclear is the identity of the intermediates in the signaling pathways between the heterotrimeric G proteins (or cell stretch) and the SAPKs/p38s. At the downstream end of the pathways signaling hypertrophy, a great deal has been learned about the promoter elements that are critical for the induction of various hypertrophic response genes and, largely by inference, the transcription factors involved. In some cases (e.g., the SAPKs and c-Jun), a connection between cytosolic signaling pathways and the nucleus has been made, but very little is known about the role, if any, of cytosolic signaling pathways in the regulation of other transcription factors, such as GATA4, which appear to be critical to the induction of several genes involved in the hypertrophic response. The precise role of calcineurin and NF-AT3 remains unclear as well. The enthusiasm over the identification of this pathway as a regulator of hypertrophy has only been matched by the disappointment over the finding that the beneficial effects of CsA are short-lived. The latter reports on this subject should not be interpreted as suggesting that calcineurin/NF-AT3 are not involved in the hypertrophic response, but rather as demonstrating the complexity of the response by suggesting the existence of at least two parallel pathways signaling hypertrophy (Figs. 2 and 4). Finally, mechanisms governing the downregulation of SERCA2a and its regulator, phospholamban, remain unknown. This is likely due, in part, to the fact that in vitro models do not seem to recapitulate this downregulation. Because this downregulation may be critically involved in the contractile dysfunction of heart failure, and because we have shown that increasing expression of either SERCA2a or phospholamban via adenovirus-mediated gene transfer partially corrects the abnormalities (13–15,33), it is vital to identify the pathways responsible.

ACKNOWLEDGMENTS

The authors would like to thank John Kyriakis for helpful discussions. This work was supported by U.S. Public Health Service grants HL50361 and HL57623 (to R.H.), HL54202, AI40970, and HL59521 (to A.R.), DK50282 (to T.F.), and by a grant from INSERM and an International Study and Research Grant of the French Lilly Institute (to G.C.). Drs. Rosenzweig and Force are Established Investigators of the American Heart Association.

REFERENCES

1. Akhter, S. A.; Luttrell, L. M.; Rockman, H. A.; Iaccarino, G.; Lefkowitz, R. J.; Koch, W. J. Targeting the receptor–Gq interface to inhibit in vivo pressure overload myocardial hypertrophy. Science 280:574–577; 1998.
2. Beals, C. R.; Clipstone, N. A.; Ho, S. N.; Crabtree, G. R. Nuclear localization of NF-ATc by a calcineurin-dependent, cyclosporin-sensitive intramolecular interaction. Genes Dev. 11:824–834; 1997.
3. Bogoyevitch, M. A.; Gillespie-Brown, J.; Ketterman, A. J.; Fuller, S. J.; Ben-Levy, R.; Ashworth, A.; Marshall, C. J.; Sugden, P. H. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. Circ. Res. 79:162–173; 1996.
4. Bowman, J. C.; Steinberg, S. F.; Jiang, T.; Geenen, D. L.; Fishman, G. I.; Buttrick, P. M. Expression of protein kinase Cβ in the heart causes hypertrophy in adult mice and sudden death in neonates. J. Clin. Invest. 100:2189–2195; 1997.
5. Chen, L.; Glover, J. N. M.; Hogan, P. G.; Rao, A.; Harrison, S. C. Structure of the DNA-binding domains from NFAT, Fos, and Jun bound specifically to DNA. Nature 392:42–48; 1998.
6. Choukroun, G.; Hajjar, R.; Bonventre, J. V.; Kyriakis, J. M.; Rosenzweig, A.; Force, T. Role of the stress-activated protein kinases in endothelin-induced cardiomyocyte hypertrophy. J. Clin. Invest. 102:1311–1320; 1998.
7. Choukroun, G.; Hajjar, R.; Rosenzweig, A.; Force, T. Regulation of cardiac hypertrophy *in vivo* by the stress-activated protein kinases/c-Jun N-terminal kinases. Circulation 98:I-69; 1998.
8. Cohen, P. The search for physiological substrates of mitogen- and stress-activated protein kinases in mammalian cells. Trends Cell Biol. 7:353–361; 1997.
9. D'Angelo, D. D.; Sakata, Y.; Lorenz, J. H.; Boivin, G. P.; Walsh, R. A.; Liggett, S. B.; Dorn, G. W. Transgenic Gαq overexpression induced cardiac contractile failure in mice. Proc. Natl. Acad. Sci. USA 94:8121–8126; 1997.
10. Fentzke, R. C.; Korcarz, C. E.; Lang, R. M.; Lin, H.; Leiden, J. M. Dilated cardiomyopathy in transgenic mice expressing a dominant-negative CREB transcription factor in the heart. J. Clin. Invest. 101:2415–2426; 1998.
11. Gille, H.; Sharrocks, A. D.; Shaw, P. E. Phosphorylation of transcription factor p62TCF by MAP kinase stimulates ternary complex formation at c-*fos* promoter. Nature 358:414–416; 1992.
12. Gupta, S.; Campbell, D.; Derijard, B.; Davis, R. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science 267:389–393; 1995.
13. Hajjar, R. J.; Kang, J. X.; Gwathmey, J. K.; Rosenzweig, A. Physiological effects of adenoviral gene transfer of sarcoplasmic reticulum calcium ATPase in isolated rat myocytes. Circulation 95:423–429; 1997.

14. Hajjar, R. J.; Schmidt, U.; Kang, J. X.; Matsui, T.; Rosenzweig, A. Adenoviral gene transfer of phospholamban in isolated rat cardiomyocytes. Rescue effects by concomitant gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase. Circ. Res. 81:145–153; 1997.
15. Hajjar, R. J.; Schmidt, U.; Matsui, T.; Guerrero, L.; Lee, K. H.; Gwathmey, J. K.; Dec, G. W.; Semigran, M. J.; Rosenzweig, A. Modulation of ventricular function through gene transfer in vivo. Proc. Natl. Acad. Sci. USA 95:5251–5256; 1998.
16. Harada, K.; Komuro, I.; Zou, Y.; Kudoh, S.; Kijima, K.; Matsubara, H.; Sugaya, T.; Murakami, K.; Yazaki, Y. Acute pressure overload could induce hypertrophic responses in the heart of angiotensin II type 1a knockout mice. Circ. Res. 82:779–785; 1998.
17. Harsdorf, R.; Edwards, J. G.; Shen, Y. T.; Kudej, R. K.; Dietz, R.; Leinwand, L. A.; Nadal-Ginard, B.; Vatner, S. F. Identification of a cis-acting regulatory element conferring inducibility of the atrial natriuretic factor gene in acute pressure overload. J. Clin. Invest. 100:1294–1304; 1997.
18. Hasegawa, K.; Lee, S. J.; Jobe, S. M.; Markham, B. E.; Kitsis, R. N. cis-acting sequences that mediate induction of β-myosin heavy chain gene expression during left ventricular hypertrophy due to aortic constriction. Circulation 96:3943–3953; 1997.
19. Heasley, L. E.; Storey, B.; Fanger, G.; Butterfield, L.; Zamarripa, J.; Blumberg, D.; Maue, R. A. GTPase-deficient Gα16 and Gαq induce PC12 cell differentiation and persistent activation of c-Jun NH2-terminal kinases. Mol. Cell. Biol. 16:648–656; 1996.
20. Herzig, T. C.; Jobe, S. M.; Aoki, H.; Molkentin, J. D.; Cowley, A. W.; Izumo, S.; Markham, B. E. Angiotensin II type 1a receptor gene expression in the heart: AP-1 and GATA-4 participate in the response to pressure overload. Proc. Natl. Acad. Sci. USA 94:7543–7548; 1997.
21. Hunter, J. J.; Tanaka, N.; Rockman, H. A.; Ross, J., Jr.; Chien, K. R. Ventricular expression of the MLC-2v-ras fusion gene induces cardiac hypertrophy and selective diastolic dysfunction in transgenic mice. J. Biol. Chem. 270:23173–23178; 1995.
22. James, J.; Robbins, J. Molecular remodeling of cardiac contractile function. Am. J. Physiol. 273:H2105–H2118; 1997.
23. Karin, M. The regulation of AP-1 activity by mitogen-activated protein kinases. J. Biol. Chem. 270:16483–16486; 1995.
24. Klee, C. B.; Ren, H.; Wang, X. Regulation of the calmodulin-stimulated protein phosphatase, calcineurin. J. Biol. Chem. 273:13367–13370; 1998.
25. Komuro, I.; Yazaki, Y. Control of cardiac gene expression by mechanical stress. Annu. Rev. Physiol. 55:55–75; 1993.
26. Kovacic-Milivojevic, B.; Wong, V. S. H.; Gardner, D. G. Selective regulation of the atrial natriuretic peptide gene by individual components of the activator protein-1 complex. Endocrinology 137:1008–1117; 1996.
27. Livingstone, C.; Patel, G.; Jones, N. ATF2 contains a phosphorylation dependent transcriptional activation domain. EMBO J. 14:1785–1797; 1995.
28. Luo, Z.; Shyu, K.-G.; Gualberto, A.; Walsh, K. Calcineurin inhibitors and cardiac hypertrophy. Nat. Med. 4:1092–1093; 1998.
29. Mackay, D. J. G.; Hall, A. Rho GTPases. J. Biol. Chem. 273:20685–20688; 1998.
30. Matsuda, S.; Moriguchi, T.; Koyasu, S.; Nishida, E. T lymphocyte activation signals for interleukin-2 production involve activation of MKK6-p38 and MKK7-SAPK/JNK signaling pathways sensitive to cyclosporin A. J. Biol. Chem. 273:12378–12382; 1998.
31. McDonough, P. M.; Hanford, D. S.; Sprenkle, A. B.; Mellon, N. R.; Glembotski, C. C. Collaborative roles for c-Jun N-terminal kinase, c-Jun, serum response factor, and Sp1 in calcium-regulated myocardial gene expression. J. Biol. Chem. 272:24046–24053; 1997.
32. Mende, U.; Kagen, A.; Cohen, A.; Aramburu, J.; Schoen, F. J.; Neer, E. J. Transient cardiac expression of constitutively active Gαq leads to hypertrophy and dilated cardiomyopathy by calcineurin-dependent and independent pathways. Proc. Natl. Acad. Sci. USA 95: 13893–13898; 1998.
33. Miyamoto, M. I.; Guerrero, J. L.; Schmidt, U.; Gwathmey, J. K.; Dec, G. W.; Rosenzweig, A.; Hajjar, R. J. Adenoviral gene transfer of SERCA2a improves LV function in aortic-banded rats in transition to heart failure. Circulation 98:I-731; 1998.
34. Molkentin, J. D. Calcineurin inhibition and cardiac hypertrophy [response to letter]. Science 282:1007; 1998.
35. Molkentin, J. D.; Lu, J. R.; Antos, C. L.; Markham, B.; Richardson, J.; Robbins, J.; Grant, S. R.; Olson, E. N. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 93:215–228; 1998.
36. Molkentin, J. D.; Olson, E. N. GATA4: A novel transcriptional regulator of cardiac hypertrophy? Circulation 96:3833–3835; 1997.
37. Morooka, H.; Bonventre, J. V.; Pombo, C. M.; Kyriakis, J. M.; Force, T. Ischemia and reperfusion enhance ATF-2 and c-Jun binding to cAMP response elements and to an AP-1 binding site from the c-jun promoter. J. Biol. Chem. 270:30084–30092; 1995.
38. Muller, J. G.; Nemoto, S.; Laser, M.; Carabello, B. A.; Menick, D. R. Calcineurin inhibition and cardiac hypertrophy [letter]. Science 282:1007; 1998.
39. Nemoto, S.; Zelin, S.; Lin, A. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. Mol. Cell. Biol. 18:3518–3526; 1998.
40. Paradis, P.; MacLellan, W. R.; Belaguli, N. S.; Schwartz, R. J.; Schneider, M. D. Serum response factor mediates AP-1-dependent induction of the skeletal a actin promoter in ventricular myocytes. J. Biol. Chem. 271:10827–10833; 1996.
41. Parker, T. G.; Schneider, M. D. Peptide growth factor can provoke 'fetal' contractile protein gene expression in rat cardiac myocytes. J. Clin. Invest. 85:507–514; 1990.
42. Pennica, D.; Shaw, K. J.; Swanson, T. A.; Moore, M. W.; Shelton, D. L.; Zioncheck, K. A.; Rosenthal, A.;

Taga, T.; Paoni, N. F.; Wood, N. I. Cardiotrophin-1. Biological activities and binding to the leukemia inhibitory factor receptor/gp130 complex. J. Biol. Chem. 270:10915–10922; 1995.
43. Post, G. R.; Goldstein, D.; Thuerauf, D. J.; Glembotski, C. C.; Brown, J. H. Dissociation of p44 and p42 mitogen-activated protein kinase activation from receptor-induced hypertrophy in neonatal rat ventricular myocytes. J. Biol. Chem. 271:8452–8457; 1996.
44. Pracyk, J. B.; Tanaka, K.; Hegland, D. D.; Kim, K. S.; Sethi, R.; Rovira, I. I.; Blazina, D. R.; Lee, L.; Bruder, J. T.; Kovesdi, I.; Goldschmidt-Clermont, P. U.; Irani, K.; Finkel, T. A requirement for the rac1 GTPase in the signal transduction pathway leading to cardiac myocyte hypertrophy. J. Clin. Invest. 102:929–937; 1998.
45. Pulverer, B.; Kyriakis, J. M.; Avruch, J.; Nikolakaki, E.; Woodgett, J. R. Phosphorylation of c-*jun* mediated by MAP kinases. Nature 353:670–674; 1991.
46. Ramirez, M. T.; Sah, V. P.; Zhao, X. L.; Hunter, J. J.; Chien, K. R.; Brown, J. H. The MEKK-JNK pathway is stimulated by α1-adrenergic receptor and Ras activation and is associated with in vitro and in vivo cardiac hypertrophy. J. Biol. Chem. 272:14057–14061; 1997.
47. Ross, R. S.; Pham, C.; Shai, S. Y.; Goldhaber, J. I.; Fenczik, C.; Glembotski, C. C.; Ginsberg, M. H.; Loftus, J. C. β_1 integrins participate in the hypertrophic response of rat ventricular myocytes. Circ. Res. 82: 1160–1172; 1998.
48. Sadoshima, J.; Izumo, S. The cellular and molecular response of cardiac myocytes to mechanical stress. Annu. Rev. Physiol. 59:551–571; 1997.
49. Sadoshima, J.; Izumo, S. Rapamycin selectively inhibits angiotensin II-induced increase in protein synthesis in cardiac myocytes in vitro. Circ. Res. 77:1040–1052; 1995.
50. Sadoshima, J.; Xu, Y.; Slayter, H. S.; Izumo, S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 75:977–984; 1993.
51. Sakai, S.; Miyauchi, T.; Kobayashi, M.; Goto, K.; Sugishita, Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. Nature 385:353–355; 1996.
52. Sakata, Y.; Hoit, B. D.; Liggett, S. B.; Walsh, R. A.; Dorn, G. W. Decompensation of pressure-overload hypertrophy in Gαq-overexpressing mice. Circulation 97: 1488–1495; 1998.
53. Shubita, H. E.; McDonough, P. M.; Harris, A. N.; Knowlton, K. U.; Glembotski, C. C.; Brown, J. H.; Chien, K. R. Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly, and cardiac gene expression in ventricular myocytes. J. Biol. Chem. 265:20555–20562; 1990.
54. Spirito, P.; Seidman, C. E.; McKenna, W. J.; Maron, B. J. The management of hypertrophic cardiomyopathy. N. Engl. J. Med. 336:775–785; 1997.
55. Su, B.; Jacinot, E.; Masahiko, H.; Kallunki, T.; Karin, M.; Ben-Neriah. JNK is involved in signal integration during costimulation of T lymphocytes. Cell 77:727–736; 1994.
56. Sugden, P.; Clerk, A. Stress-responsive mitogen-activated protein kinases (c-Jun N-terminal kinases and the p38 mitogen-activated protein kinases) in the myocardium. Circ. Res. 83:345–352; 1998.
57. Sussman, M. A.; Lim, H. W.; Gude, N.; Taigen, T.; Olson, E. N.; Robbins, J.; Colbert, M. C.; Gualberto, A.; Wieczorek, D. F.; Molkentin, J. D. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. Science 281:1690–1693; 1998.
58. Tan, Y.; Rouse, J.; Zhang, A.; Cariati, S.; Cohen, P.; Comb, M. FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. EMBO J. 15:4629–4642; 1996.
59. Thorburn, A.; Thorburn, J.; Chen, S. Y.; Powers, S.; Shubeita, H. E.; Feramisco, J. R.; Chien, K. R. H-Ras dependent pathways can activate morphological and genetic markers of cardiac hypertrophy. J. Biol. Chem. 268:2244–2249; 1993.
60. Thorburn, J.; Carlson, M.; Mansour, S. J.; Chien, K. R.; Ahn, N. G.; Thorburn, A. Inhibition of a signaling pathway in cardiac muscle cells by active mitogen activated protein kinase kinase. Mol. Biol. Cell 6:1479–1490; 1995.
61. Thorburn, J.; Frost, J. A.; Thorburn, A. Mitogen-activated protein kinases mediate changes in gene expression, but not cytoskeletal organization associated with cardiac muscle cell hypertrophy. J. Cell Biol. 126: 1565–1572; 1994.
62. Thorburn, J.; McMahon, M.; Thorburn, A. Raf-1 kinase activity is necessary and sufficient for gene expression changes but not sufficient for cellular morphology changes associated with cardiac myocyte hypertrophy. J. Biol. Chem. 269:30580–30586; 1994.
63. Thorburn, J.; Xu, S.; Thorburn, A. MAP kinase- and Rho-dependent signals interact to regulate gene expression but not actin morphology in cardiac muscle cells. EMBO J. 16:1888–1900; 1997.
64. Thuerauf, D. J.; Hanford, D. S.; Glembotski, C. C. Regulation of rat brain natriuretic peptide transcription. J. Biol. Chem. 269:17772–17775; 1994.
65. van Dam, H.; Wilhelm, D.; Herr, I.; Steffen, A.; Herrlich, P.; Angel, P. ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. EMBO J. 14: 1798–1811; 1995.
66. Van Heutgen, H. A. A.; Lamers, J. M. J. Changes in cardiac phenotype in hypertrophy and failure: From receptor to gene. Heart Failure Rev. 2:95–106; 1997.
67. Vikstrom, K. L.; Leinwand, L. A. Contractile protein mutations and heart disease. Curr. Opin. Cell Biol. 8: 97–105; 1996.
68. Vojtek, A. B.; Der, C. J. Increasing complexity of the Ras signaling pathway. J. Biol. Chem. 273:19925–19928; 1998.
69. Wakasaki, H.; Koya, D.; Schoen, F. J.; Jirousek, M. R.; Ways, D. K.; Hoit, B. D.; Walsh, R. A.; King, G. L. Targeted overexpression of protein kinase C β2 isoform in myocardium causes cardiomyopathy. Proc. Natl. Acad. Sci. USA 94:9320–9325; 1997.
70. Wang, Y.; Huang, S.; Sah, V. P.; Ross, J.; Brown, J.

H.; Han, J.; Chien, K. R. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J. Biol. Chem. 273:2161–2168; 1998.

71. Wang, Y.; Su, B.; Sah, V. P.; Brown, J. H.; Han, J.; Chien, K. R. Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells. J. Biol. Chem. 273:5423–5426; 1998.
72. Whitmarsh, A. J.; Yang, S.-H.; Su, M.-S.; Sharrocks, A. D.; Davis, R. J. Role of p38 and JNK mitogen-activated protein kinases in activation of ternary complex factors. Mol. Cell. Biol. 17:2360–2371; 1997.
73. Yamazaki, T.; Yazaki, Y. Is there a major involvement of the renin-angiotensin system in cardiac hypertrophy. Circ. Res. 81:639–642; 1997.
74. Zechner, D.; Thuerauf, D. J.; Hanford, D. S.; McDonough, P. M.; Glembotski, C. C. A role for the p38 mitogen-activated protein kinase pathway in myocardial cell growth, sarcomeric organization, and cardiac-specific gene expression. J. Cell Biol. 139:115–127; 1997.