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CYTOPLASMIC SIGNALING PATHWAYS THAT REGULATE CARDIAC HYPERTROPHY

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■ **Abstract** This review discusses the rapidly progressing field of cardiomyocyte signal transduction and the regulation of the hypertrophic response. When stimulated by a wide array of neurohumoral factors or when faced with an increase in ventricular-wall tension, individual cardiomyocytes undergo hypertrophic growth as an adaptive response. However, sustained cardiac hypertrophy is a leading predictor of future heart failure. A growing number of intracellular signaling pathways have been characterized as important transducers of the hypertrophic response, including specific G protein isoforms, low-molecular-weight GTPases (Ras, RhoA, and Rac), mitogen-activated protein kinase cascades, protein kinase C, calcineurin, gp130-signal transducer and activator of transcription, insulin-like growth factor I receptor pathway, fibroblast growth factor and transforming growth factor β receptor pathways, and many others. Each of these signaling pathways has been implicated as a hypertrophic transducer, which collectively suggests an emerging paradigm whereby multiple pathways operate in concert to orchestrate a hypertrophic response

INTRODUCTION

Because heart disease remains one of the leading causes of death in all industrialized nations of the world (1–3), much effort has centered on characterizing the intracellular signal transduction cascades that are associated with hypertrophy and cardiomyopathy. Given that adult cardiomyocytes are resistant to cell cycle reentry, many of the same intracellular signaling pathways that regulate proliferation in cancer cells or immune cells instead regulate hypertrophic growth of cardiomyocytes. The hypertrophic growth of cardiomyocytes is initiated by endocrine, paracrine, and autocrine factors that stimulate a wide array of membrane-bound receptors. Their activation results in the triggering of multiple cytoplasmic signal transduction cascades, which ultimately affects nuclear factors and the regulation of

gene expression. In this review, we focus on the intermediate signal transduction cascades that reside within the cytoplasm downstream of membrane-bound receptors and upstream of transcription factors. From this discussion it becomes obvious that no single intracellular transduction cascade regulates cardiomyocyte hypertrophy in isolation, but instead each pathway operates as an integrated component of an orchestrated response between interdependent and cross-talking networks. In this manner, blockade of specific intracellular signaling pathways in the heart can dramatically affect the orchestration of the entire hypertrophic response and effectively diminish heart enlargement. This model predicts that specific activation of any of a number of discrete signal transduction pathways will be sufficient to activate the entire hypertrophic response through effects on other cross-talking signaling networks.

G PROTEINS AND CARDIAC HYPERTROPHY

Heterotrimeric GTP-binding proteins transduce stimulatory or inhibitory signals from agonist-occupied seven-transmembrane-spanning-domain receptors of the rhodopsin superfamily. Within the cardiovascular system, three functional classes of G protein–coupled receptors are of primary importance owing to their acute hemodynamic and chronic myotrophic effects. The functional classes of cardiovascular receptors correspond to the three major classes of G proteins (Figure 1). Thus, β-adrenergic receptors (βAR), which couple primarily to $G_{\alpha s}$, mediate acute enhancement of heart rate and myocardial contractility in response to epinephrine and norepinephrine stimulation (reviewed in 4, 5). The second class of myocardial receptors are the cholinergic receptors, typically coupled to $G_{\alpha i}$, which are activated by acetylcholine. The third class of receptors, coupled primarily to $G_{\alpha q}$, includes angiotensin II, endothelin, and α-adrenergic (αAR) receptors. Activation of these pathways is less important in modulating minute-by-minute cardiac function, but it is likely to play a major role in cardiac hypertrophic responses to pathological stimuli.

All heterotrimeric G proteins consist of separate G_{α} and $G_{\beta\gamma}$ subunits. Agonist occupation of a membrane-bound receptor catalyzes GDP to GTP exchange on the G_{α} subunit and subsequent dissociation of G_{α} from $G_{\beta\gamma}$. Both subunits are then free to modulate the activity of downstream signaling effectors, typically adenylyl cyclase (AC) (modulated by $G_{\alpha s}$ and $G_{\alpha i}$) or phospholipase C (PLC; activated by $G_{\alpha q/11}$) (6). In addition, free $G_{\beta\gamma}$ subunits can directly enhance mitogen-activated protein kinase (MAPK) signaling, phosphatidylinositol 3-kinase (PI3K) activity, and Ras signaling in the heart (7–9; Figure 1). Although multiple G_{α}, $_{\beta}$, and $_{\gamma}$ proteins have been identified, we focus on the function of the three major classes of G_{α} subunits that mediate most receptor-effector coupling pathways in the heart.

$G_{\alpha i}$ in Cardiac Disease

Receptor-mediated activation of the $G_{\alpha i}$ subunit results in the direct attenuation of AC in the heart. AC catalyzes the formation of cyclic AMP, which augments

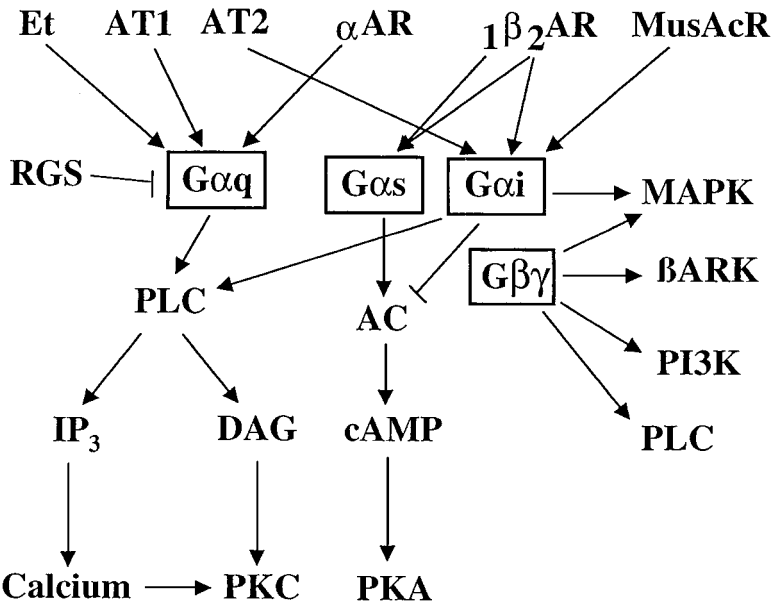

Figure 1 G protein–mediated signaling pathways. Multiple seven-transmembrane-spanning receptors directly couple to G proteins resulting in GDP-GTP exchange, dissociation of the G_α subunit from the $G_{\beta\gamma}$, and activation of effector proteins by both subunits. Abbreviations: Et, endothelin receptor; AT1 and AT2, angiotensin receptors, AR, adrenergic receptor; MusAcR, muscarinic acetylcholine receptor; AC, adenylyl cyclase; PLC, phospholipase C; PKC, protein kinase C; PKA, protein kinase A; MAPK, mitogen-activated protein kinase; βARK, β-adrenergic receptor kinase; PI3K, phosphatidylinositol 3-kinase; IP_3, inositol 3-phosphate; DAG, diacylglycerol.

myocardial contractility, in part, through a protein kinase A-signaling pathway, which directly inhibits phospholamban, promoting increased SERCA2 ATPase activity and augmented calcium handling in the heart (reviewed in 10). Because $G_{\alpha i}$ inhibits AC activity, increased expression of $G_{\alpha i}$ has been predicted to contribute to the pathology of cardiac hypertrophy and heart failure. The first convincing evidence that up-regulation of $G_{\alpha i}$ occurred in human heart failure was published by Neumann et al in 1988 (11). In this small clinical study, three patients with idiopathic dilated cardiomyopathy showed increased $G_{\alpha i}$ and impaired responsiveness to isoprenaline. The hypothesis that increased expression of $G_{\alpha i}$ might be involved in heart failure was subsequently confirmed and extended by Bristow and colleagues in 1991, through a comparative study of myocardial tissue from 12 pairs of failing and nonfailing human ventricles (12). $G_{\alpha i}$ content was increased by ~30% in failing hearts, and basal AC activity was depressed by ~70%.

Multiple studies have now confirmed that $G_{\alpha i}$ is up-regulated in different heart failure models from numerous species (13–18). Increased $G_{\alpha i}$ content and impaired AC activity are not limited to failing hearts but have also been demonstrated

in experimental and genetic hypertension characterized by cardiac hypertrophy (14, 15). Up-regulation of $G_{\alpha i}$ and the resulting blunting of AC signaling may thus represent a primary event in cardiac hypertrophy, which can contribute to the transition from compensated hypertrophy to decompensated heart failure. Up-regulation of $G_{\alpha i}$ in cardiac hypertrophy suggests regulation of $G_{\alpha i}$ expression at the molecular level, a notion that is supported by most but not all published studies. Feldman and coworkers were the first to examine $G_{\alpha i}$ mRNA expression in failing human hearts by Northern blot analysis, and they reported increased $G_{\alpha i}$ and $G_{\alpha s}$ gene expression (19). This observation was supported by a subsequent study reporting increased $G_{\alpha i}$ mRNA in heart failure (20). In addition, elevated cyclic-AMP levels were shown to augment $G_{\alpha i2}$ promoter activity, suggesting a plausible mechanism for up-regulation of $G_{\alpha i}$ in heart disease (21). Finally, mice genetically engineered to express a conditional $G_{\alpha i}$-coupled receptor demonstrated a profound decrease in heart rate upon stimulation (22).

$G_{\alpha s}$ in Cardiac Disease

Whereas increased expression of inhibitory $G_{\alpha i}$ is generally accepted as contributing to βAR unresponsiveness in cardiac hypertrophy and heart failure, a role for altered $G_{\alpha s}$ content is less clear because the published data are in conflict over this issue (19, 23–29). Thus, it is not possible to conclude that regulation of $G_{\alpha s}$ occurs or contributes in a meaningful way to the pathophysiology of human heart failure. However, the functional and pathological consequences of increased cardiac $G_{\alpha s}$ expression have been explored in a series of studies using transgenic mice that overexpress $G_{\alpha s}$. In these mice, wild-type $G_{\alpha s}$ was expressed at approximately threefold the endogenous levels, resulting in an increase in $G_{\alpha s}$ activity of 88% without any significant effect on AC activity (30). The major measurable consequence of this level of $G_{\alpha s}$ overexpression on signaling in the heart was an increase in the proportion of βAR coupled to $G_{\alpha s}$. At the functional level, $G_{\alpha s}$ overexpression enhanced isoproterenol-stimulated contractility, but not basal left-ventricular contractility, as measured by echocardiography (31). However, $G_{\alpha s}$ overexpression also increased myocardial collagen content and fibrosis with variable cardiomyocyte atrophy or hypertrophy associated with increased apoptosis (31, 32).

The phenotype of $G_{\alpha s}$-transgenic mice resembles the alterations in cardiac function and pathology associated with exogenously administered catecholamines or catecholamine cardiomyopathies reported in humans (33–36). Deleterious consequences of unregulated cardiac βAR signaling have also been observed in transgenic models of $\beta_1 AR$ and $\beta_2 AR$ overexpression (37–39). It is not clear at this time whether the mechanisms for the deleterious effects of $G_{\alpha s}$ and βAR are mediated through AC or through other signaling pathways.

$G_{\alpha q}$ in Heart Disease

A compelling case supporting a critical role for $G_{\alpha q}$ signaling in cardiac hypertrophy has been established from in vitro and in vivo experimental models and from

inferential clinical data. Most mechanistic information is derived from studies in which cultured neonatal rat cardiac myocytes are stimulated with $G_{\alpha q}$-activating agonists or in which the alpha subunit of $G_{\alpha q}$ is overexpressed. Initial studies by Simpson and colleagues, showed that the αAR agonist norepinephrine, but not the βAR agonist isoproterenol, increased cultured neonatal-rat cardiomyocyte cell size in a dose-dependent manner (40, 41). Using variations of this tissue culture model, hypertrophic effects of phenylephrine, angiotensin II, endothelin, and prostaglandin $F_2\alpha$ have all since been demonstrated (42–45). Each of these structurally diverse hypertrophic agonists stimulates a membrane receptor that activates PLC via the Gq class of GTP-binding proteins, which suggests that Gq and PLC could be hypertrophy-signaling effectors (45–50).

More direct evidence was obtained by the observation that overexpression of Gq-coupled receptors or an activated $G_{\alpha q}$ in cardiomyocytes promotes cellular hypertrophy (51–53). Conversely, inhibition of $G_{\alpha q}$ signaling with microinjected neutralizing antibodies prevents αAR-mediated cardiomyocyte hypertrophy (53). Thus, these studies support an obligatory role for $G_{\alpha q}$ signaling in cardiomyocyte hypertrophy.

Although the studies noted above have demonstrated that cardiomyocyte receptors coupled to $G_{\alpha q}$ can be important transducers of hypertrophy in cultured cardiomyocytes (40–45), there has not, until recently, been general acceptance for the notion that $G_{\alpha q}$-coupled signaling pathways play an important pathophysiological role in vivo. However, indirect evidence for a pathophysiological role of the $G_{\alpha q}$-coupled angiotensin receptor system in heart failure is provided by the favorable results of angiotensin-converting-enzyme inhibitors in modifying the course of heart failure and in regressing cardiac hypertrophy (54–57). In support of this notion, $G_{\alpha q}$ and PLC were shown to be up-regulated in the perinfarct myocardium of experimentally infarcted rats (58).

The necessity for and sufficiency of $G_{\alpha q}$ signaling to stimulate in vivo myocardial growth have been compellingly demonstrated in a series of studies from several laboratories, using genetically manipulated mouse models. $G_{\alpha q}$-overexpressing transgenic mice exhibited a hypertrophy phenotype similar to pressure overload hypertrophy in terms of the extent of cardiac hypertrophy, the pattern of fetal gene expression, and the increase in cardiomyocyte cross-sectional area (59, 60). However, $G_{\alpha q}$ over-expressers also exhibited features distinct from compensated pressure overload hypertrophy, such as eccentric ventricular remodeling, resting sinus bradycardia, and left-ventricular contractile depression (60). This phenotype of hypertrophy and contractile depression in independent lines of $G_{\alpha q}$ over-expressers indicates that signaling events downstream of $G_{\alpha q}$ are sufficient to cause maladaptive cardiac hypertrophy. To evaluate the effects of intrinsic $G_{\alpha q}$ signaling on cardiac responses to pressure overload, $G_{\alpha q}$ over-expressers were subjected to transverse aortic banding (60). Whereas aortic-banded nontransgenic mice developed compensated, concentric, left-ventricular hypertrophy, $G_{\alpha q}$ over-expressers developed eccentric hypertrophy with progressively declining ventricular function, eventually resulting in overt functional decompensation and pulmonary edema.

In support of these findings, overexpression of an activated mutant of $G_{\alpha q}$ also promotes a cardiomyopathic phenotype associated with progressive ventricular dilation (61).

That G_q-mediated signaling induces a cardiomyopathic phenotype was also demonstrated in peripartum $G_{\alpha q}$-transgenic mice (52). In the terminal period of pregnancy or immediately after delivery, these animals rapidly progressed into heart failure characterized by massive biventricular and biatrial-ventricular dilation with pulmonary congestion, pleural effusions, and ascites. Histologically, these hearts exhibited cardiomyocyte apoptosis, but without the inflammatory reaction normally accompanying a necrotic process.

The studies discussed above demonstrate that $G_{\alpha q}$ signaling is sufficient to induce cardiac hypertrophy that makes a transition into heart failure. However, the necessity of $G_{\alpha q}$ signaling in physiologic or pathophysiologic forms of hypertrophy was not, until recently, characterized. Akhter et al demonstrated that transgenic overexpression of a dominant-negative $G_{\alpha q}$ peptide in the heart rendered mice resistant to pressure overload hypertrophy stimulated by acute aortic banding (62). Further supporting evidence was obtained by overexpression of RGS4 in the heart. RGS (regulator of G protein signaling) proteins are GTPase-activating proteins (GAPs) that increase Gα inactivation by promoting GTP hydrolysis. Transgenic overexpression of RGS4 in the heart reduced cardiac hypertrophy in response to pressure overload stimulation (63). Collectively, the above studies demonstrate that $G_{\alpha q}$-coupled signaling responses are both necessary and sufficient for mediating cardiac hypertrophy.

LOW-MOLECULAR-WEIGHT GTPASES IN CARDIAC HYPERTROPHY (RAS, RHOA, AND RAC1)

A number of in vitro and in vivo studies have implicated a Ras-dependent signaling pathway in the regulation of cardiac hypertrophy. Microinjection of activated Ras protein into cultured cardiomyocytes increased both cell size and atrial natriuretic factor (ANF) expression (64). Transgenic mice that overexpress a constitutively active form of *Ras* in the mouse heart show cardiac hypertrophy and diastolic dysfunction (65). Transfection of expression vectors encoding activated *Raf-1* or *Ras* increased myocyte cell dimensions and augmented expression of hypertrophy-responsive promoters (66). In addition, adenoviral-mediated gene transfer of dominant-negative *Ras* (17N Ras) inhibited the upregulation of protein and mRNA production in response to phenylephrine (PE) treatment in cultured cardiomyocytes (67). However, using the same adenoviral construct, another group concluded that endothelin-1-stimulated cardiomyocyte hypertrophy was not attenuated by dominant-negative 17N Ras (68). Whereas the general consensus is that Ras activation is associated with cardiac hypertrophy, the downstream signaling events that mediate hypertrophy are less defined.

Ras is a low-molecular-weight GTPase and, like the canonical G proteins discussed above, is activated by GDP-to-GTP exchange initiated by membrane-bound receptors. Ras activation can promote activation of Raf-1, PI3K, small GTPase Ral proteins, p120GAP, and p190GAP, leading to Rho activation (reviewed in 69). In addition, Ras activity is known to result in activation of all three MAPK signaling branches [extracellular-signal-regulated kinases 1 and 2 (ERK1 and 2), c-Jun NH_2-terminal kinases (JNKs), and p38], whereas Raf-1 activation is associated only with ERK 1 and 2 activation (Figure 2). Indeed, transgenic mice expressing activated Ras have significant JNK activation in the heart, suggesting that Ras activation may have a broad influence in MAPK signaling responses in the heart (70).

More recently, overexpression approaches in transgenic mice have been used to examine the role of other low-molecular-weight GTPases in cardiac hypertrophy. Overexpression of *RhoA* in the mouse heart caused atrial enlargement and conduction defects, without stimulating ventricular hypertrophy (71). It is unlikely that Rho regulates the actin cytoskeleton in cardiac myocytes, as demonstrated in

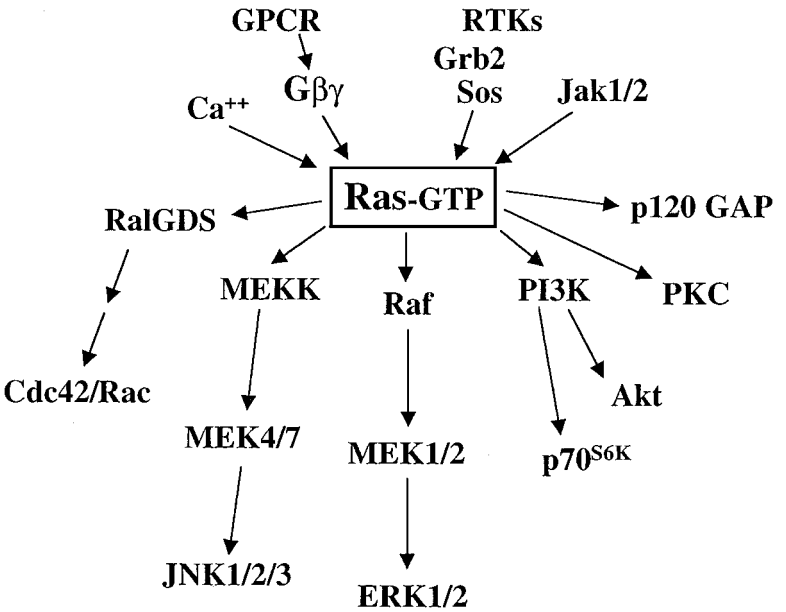

Figure 2 Ras signaling pathways. Ras is activated through G protein–coupled receptors (GPCR), receptor tyrosine kinases (RTK), Janus kinase 1 (Jak), or increases in intracellular calcium resulting in GDP-GTP exchange and the activation of numerous effector proteins. Abbreviations: PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; MEK, mitogen-activated protein kinase kinase; MEKK, mitogen-activated protein kinase kinase kinase; JNK, c-Jun NH_2 terminal kinase; ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein.

other cell types, given that transfection of a Rho inhibitor did not disrupt actin muscle fiber morphology (72). However, angiotensin-II-stimulated RhoA activation in cardiac myocytes did result in the formation of premyofibrils, a function consistent with cytoskeletal organizing capacity (73).

Adenovirus-mediated gene transfer of an activated V12rac1 factor in cultured cardiomyocytes potently induced hypertrophy in a manner indistinguishable from agonist stimulation (74). In addition, adenovirus-mediated gene transfer of a dominant-negative N17rac1 factor attenuated PE-induced morphological hypertrophy in culture (74). More recently, transgenic mice overexpressing an activated V12rac1 factor in the heart produced profound cardiac hypertrophy and ventricular dilation (75). Collectively, these studies indicate that Ras and Rac GTPases are prohypertrophic, whereas RhoA may play only a limited role in the hypertrophic program of cardiomyocytes.

MEK1 AND 2 EXTRACELLULAR SIGNAL-REGULATED KINASE1 AND 2 PATHWAY AND CARDIAC HYPERTROPHY

Two separate ERK isoforms have been described, ERK1 and ERK2, that are coordinately phosphorylated and activated by a wide array of mitogenic stimuli (reviewed in 76). The major upstream activators of ERK1 and 2 MAPKs are two MAPK kinases (MAPKKs), MEK1 and MEK2, which directly phosphorylate the dual site in ERK1 and 2 (Thr-Glu-Tyr) (Figure 3). Directly upstream of MEK1 and 2 in the MAPK-signaling cascade are the MAPKK kinase (MAPKKK) Raf-1, A-Raf, B-Raf, and MEKK1–3 (reviewed in 76).

In response to agonist stimulation or cell stretching, ERK1 and 2 become activated both in cultured cardiac myocytes and in isolated perfused hearts (77–83). These observations have implicated ERK1- and 2-signaling factors as regulators of the hypertrophic response. In support of this notion, transfection of a constitutively active MEK1-encoding construct (immediate upstream activator of ERK1 and 2) augmented ANF promoter activity in cultured cardiomyocytes, whereas a dominant-negative MEK1-encoding construct attenuated activity (84). Using antisense oligonucleotides, Glennon et al demonstrated that ERK signaling is necessary for PE-induced cardiomyocyte hypertrophy in culture (85). Similarly, using the MEK1 inhibitor PD98059, Clerk et al reported that the ERKs were required for sarcomeric organization induced by hypertrophic agonists (83). However, this same study also concluded that PD98059 did not prevent cellular hypertrophy in response to agonist stimulation, suggesting that ERKs play a more specialized role in cardiomyocyte hypertrophy.

Although the Ras-Raf-1-MEK1/2-ERK1/2-signaling pathway may regulate certain intracellular responses to a hypertrophic agonist, a large number of studies have disputed the importance of this pathway in the regulation of cardiac hypertrophy.

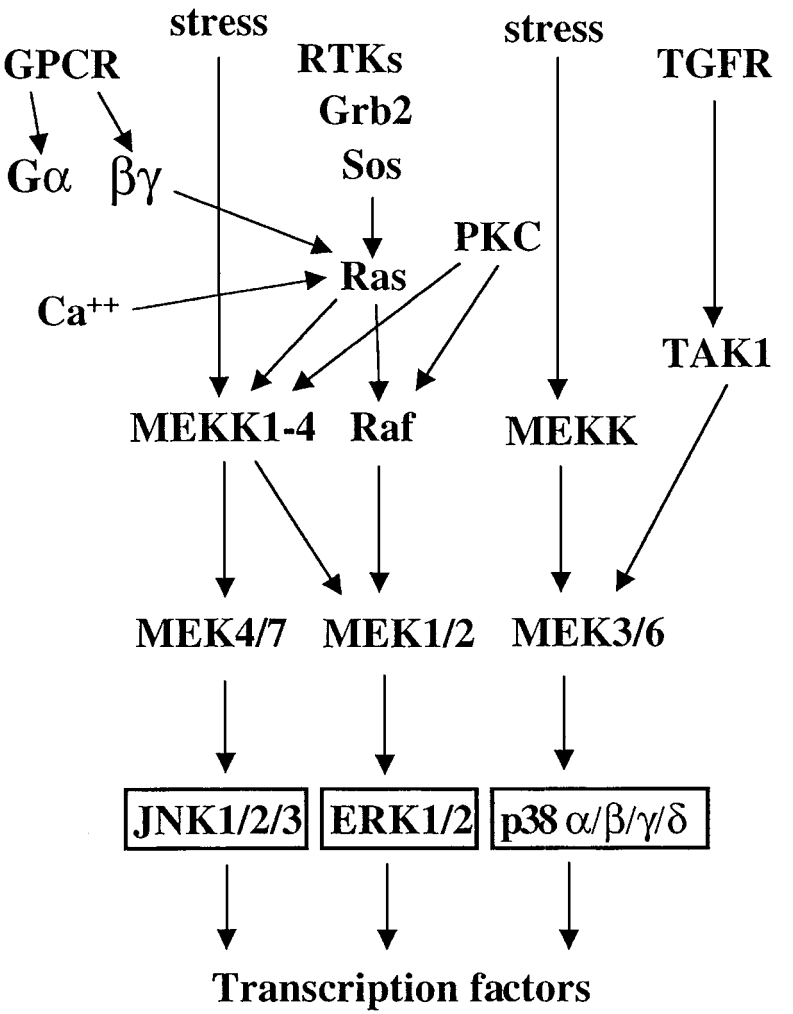

Figure 3 Mitogen-activated protein kinase signaling pathways (MAPK). MAPK signaling pathways are activated in cardiomyocytes by G protein–coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), transforming growth factor β receptor (TGFR), protein kinase C (PKC), calcium, or stress stimuli. These upstream events result in the activation of mitogen-activated protein kinase kinase kinase (MEKK) factors, which leads to the activation of mitogen-activated protein kinase kinase (MEK) factors, and in turn leads to activation of the three terminal MAPK effectors, c-Jun NH_2 terminal kinases (JNK1/2/3), extracellular signal-regulated kinases (ERK1/2), and p38. Abbreviations: TAK1, TGFβ-activated kinase.

Thorburn et al demonstrated that, although Ras-Raf-1-ERK activation was sufficient to augment c-Fos and ANF promoter activity in cardiomyocytes, inhibition of these signaling factors did not antagonize hypertrophic morphology or cytoskeletal organization in response to the agonist (86, 87). However, Post et al reported that neither dominant-negative ERK1 and 2 nor PD98059 were sufficient to block PE-induced ANF promoter activity in cultured cardiomyocytes, suggesting that ERKs are not even important for inducible gene expression. In a subsequent study, transfection of an activated MEK1 encoding expression plasmid was shown to induce c-Fos, but not ANF or myosin light-chain-2V promoter activity in cultured cardiomyocytes (88). More recent studies with the MEK1 inhibitor PD98059 also suggest a minimal role for ERKs in cardiac hypertrophy (70, 89, 90). Intriguingly, one study has even suggested that ERK activation in response to ANF treatment was associated with prevention of cardiomyocyte hypertrophy (91). Although there is clearly a lack of consensus regarding the necessity of ERK signaling as a hypertrophic mediator, overwhelming evidence implicates ERKs as immediate downstream effectors of the hypertrophic response. However, the effector functions of activated ERKs have yet to be fully explored in cultured cardiomyocytes, nor have conclusive genetic or in vivo approaches been used to date. In the future, it will be interesting to examine the association between ERK signaling and cardiac hypertrophy using transgenic or knockout model systems in the mouse.

p38 AND CARDIAC HYPERTROPHY

Four separate p38 MAPK isoforms have been described including p38α, p38β, p38γ, and p38δ (reviewed in 76). p38 MAPKs are activated by a wide array of stress stimuli including chemical stress, physical stress, osmolar stress, radiation stress, and G protein–coupled receptor (GPCR) activation (reviewed in 92). The major upstream activators of p38 MAPKs are two MAPKKs, MKK3 and MKK6, which directly phosphorylate the dual site in p38 MAPKs (Thr-Gly-Tyr) (Figure 3). Less is known of the MAPKKK factors, which lie upstream of MKK3 and MKK6 in cardiomyocytes, although PAK, TAK1, and MLK3 are potential activators (reviewed in 76).

In cardiac myocytes, mechanical deformation, GPCR ligands (angiotensin II, endothelin-1, and PE), and mitogens are potent activators of p38 (reviewed in 93). Activated p38 MAPKs directly phosphorylate serine and threonine residues in a wide array of cytoplasmic proteins and transcription factors to mediate stress-responsive signaling, including MEF2, MAPKAPK2 and 3, ATF-2, ELK-1, Chop, and Max (reviewed in 76). p38α and p38β are thought to be the most important isoforms of p38 expressed in the human heart, whereas p38γ and p38δ are undetectable (94).

The association between p38 MAPKs and the regulation of cardiac hypertrophy has primarily been investigated in cultured neonatal-rat cardiomyocytes. The GPCR agonists PE and endothelin-1 are potent activators of p38 MAPKs in

cardiomyocytes (83). In vivo, p38 MAPK activity is elevated by pressure overload hypertrophy in aortic-banded mice (95) and in human hearts with failure secondary to advanced coronary artery disease (96). Further evidence for p38 as a hypertrophic factor came from the observation that overexpression of an activated MKK3 or MKK6 factor in neonatal cardiomyocytes was sufficient to induce hypertrophy and ANF expression (89, 95, 97). Although there are data supporting a role for p38 activation as a regulator of cardiomyocyte hypertrophy in vitro, its sufficiency and necessity in vivo have not been evaluated.

Investigators have used both the pharmacologic inhibitors SB203580 and SB202190 and dominant-negative constructs to assess the necessity of p38 signaling in cardiac hypertrophy. Pharmacologic inhibition of p38 kinase activity blocked agonist-stimulated cardiomyocyte hypertrophy in culture (83, 89, 97). In addition, adenovirus-mediated gene transfer of a dominant-negative p38β MAPK blunted the hypertrophic response of neonatal cardiomyocytes (95), and pharmacologic or dominant-negative inhibition of p38 signaling significantly reduced agonist-induced b-type natriuretic peptide promoter activity in vitro (98, 99). In contrast, two recent studies have reported that p38 inhibition is not sufficient to attenuate agonist-induced cardiomyocyte hypertrophy under certain conditions, suggesting a more specialized role for p38 MAPK signaling (90, 100). Consistent with this interpretation, inhibition of p38 activity with SB203580 did not affect phenotypic myocyte hypertrophy in response to PE or endothelin-1 within 24 h, yet attenuation was observed by 48 h (83).

c-JUN NH_2 TERMINAL KINASE AND CARDIAC HYPERTROPHY

Three distinct *JNK* or *SAPK* (stress-activated protein kinase) genes have been identified in mammalian cells. Each is activated by the upstream MAPK kinases MKK4 and MKK7, which is turn are activated by MEKK1 or MEKK2 (reviewed in 76). The MKKKKs upstream of MEKK1 and 2 include GLK, HPK1, NIK, MST1, and members of the low-molecular-weight G proteins (Ras), and even Grb2 (reviewed in 76). JNK factors are directly phosphorylated by MKK4 or MKK7 on a dual site consisting of the amino acids Thr-Pro-Tyr (Figure 3). JNK1 and JNK2 have each been shown to be expressed in the heart, whereas JNK3 expression is mostly restricted to the brain (101).

In cultured cardiomyocytes, JNK isoforms become phosphorylated in response to stress stimuli (stretching) or GPCR activation (90, 102, 103). JNK activation has also been associated with load-induced cardiac hypertrophy in the rat, myocardial infarction, and human heart failure (96, 104, 105).

A number of studies have shown that JNK1 and 2 are critical regulators of cardiac hypertrophy in vitro and in vivo. In transfection experiments, an activated MEKK1 or MKK4 induced promoter expression of certain hypertrophy-associated genes and transfection of a dominant-negative MEKK1-encoding expression

vector-attenuated ANF promoter activity (70, 72, 82). In contrast, transfection of MEKK1 was actually reported to attenuate PE-induced sarcomeric organization, suggesting an antihypertrophic effect of the JNK signaling pathway (72). In addition, Nemoto and others demonstrated that MEKK1 and JNK activation blocked ANF expression in cultured cardiomyocytes (97). Although these differing accounts are confusing, they demonstrate both the complexity of MAPK signaling in cardiac myocytes and the variability that is intrinsic to cultured cardiomyocyte hypertrophy assays. Another influencing factor is the cross-regulation between MAPK-signaling pathways because MEKK1 also activates MEK1 and 2 and ERK in addition to JNK (72, 89).

More recently, evidence has accumulated that strongly implicates JNK activation as a necessary molecular event in the cardiac hypertrophic response, both in vitro and in vivo. Adenovirus-mediated gene transfer of a dominant-negative MKK4 (SEK1) factor significantly attenuated agonist-induced cardiomyocyte hypertrophy in vitro. These observations were extended in vivo by dominant-negative MKK4 adenoviral delivery to the hearts of aortic-banded rats, resulting in reduced cardiac hypertrophy in response to pressure overload (105). Collectively, these studies implicate JNKs as necessary regulators of cardiac hypertrophy in culture and in the adult heart.

PROTEIN KINASE C AND CARDIAC HYPERTROPHY

The protein kinase C (PKC) isoforms, a family of ubiquitous lipid-binding serine-threonine kinases, act downstream of virtually all membrane-associated signal transduction pathways (106). The PKC family consists of $\geq$10 isoenzymes encoded by different genes; each exhibits distinct patterns of tissue-specific expression and agonist-mediated activation. Based on enzymatic properties, PKC isoforms are classified as being conventional (cPKC) or calcium dependent; novel (nPKC) or calcium independent; and atypical (aPKC), which are activated by lipids other than diacylglycerol. An important feature of PKC isoforms is that, when activated, they translocate to distinct subcellular sites.

In cardiac tissue, PKC enzymatic activity is increased after ischemia and acute or chronic pressure overload where it is postulated to mediate ischemic preconditioning and to transduce hypertrophy signaling, respectively (107–110). However, the heterogeneity of PKC isoform expression and differences in PKC isoform regulation and activation in the heart have complicated attempts to precisely define the role of PKC in adaptive cardiac responses and related maladaptive sequelae.

αAR stimulation of cultured rat cardiac myocytes is associated with translocation of PKCβ1 from cytosol to nucleus, PKCβII from fibrillar structures to perinucleus and sarcolemma, PKCε from nucleus and cytosol to myofibrils, and PKCδ to the perinuclear region (110, 111). This differential subcellular compartmentalization of activated PKC isoforms implies distinct substrates and therefore unique cellular functions for each isoform (112, 113). The mechanism for subcellular translocation and activation of PKC isoforms involves binding to

anchoring proteins termed RACKs (receptors for activated C kinases) (111, 114). Each PKC isoform or group of related isoforms binds to a specific RACK through unique binding domains, and interference of PKC-RACK binding with peptide analogs of the RACK-binding domain can inhibit translocation of PKC isoforms (Figure 4).

Unstimulated PKC exists in a folded conformation so that the pseudo-substrate domain occupies the substrate-binding site, rendering the enzyme catalytically inactive. In the presence of phospholipid or calcium (depending on the PKC isoform), the PKC protein unfolds and exposes the substrate and RACK-binding sites, facilitating activation. Biochemically, overexpression of small peptides corresponding to the RACK-binding domain competitively inhibit PKC activity by preventing translocation (109, 115). Conversely, overexpression of pseudo-RACK peptides stimulates unfolding of inactivated PKC isoforms, exposing the catalytic site and RACK association site (116).

The described mechanism of activation of PKC isoforms in cardiomyocytes has been largely pioneered by Mochly-Rosen. Accordingly, a PKCβ C_2 domain peptide was shown to inhibit phorbol ester attenuation of isoproterenol-stimulated calcium channel activity, suggesting that a cPKC (PKCα or β) mediates phorbol 12-myristate 13-acetate (PMA) -induced inhibition of this channel (117). A PKCε V_1 fragment (144 amino acids) or the eight-amino-acid PKCε RACK-binding-site peptide was shown to attenuate PMA or norepinephrine-dependent negative chronotropy and prevented ischemic preconditioning in cultured neonatal cardiac myocytes (109, 115). Finally, specific activation of cardiomyocyte PKCε with an octapeptide pseudo-RACK peptide protected cardiac myocytes from ischemic damage (118).

Whereas associations between PKC isoform activation and specific cardiomyocyte responses are beginning to emerge from in vitro studies, as mentioned above, most in vivo observations of PKC isoform effects have tended to be strictly correlative. For instance, it has been observed that PKCε is selectively translocated to the particulate ventricular fractions during acute or chronic pressure overload (107, 119, 120) and after angiotensin-II stimulation (108). An interesting recent report also noted an association between PKCε activation by chronic ethanol consumption in guinea pigs and ethanol-induced cardioprotection from ischemic reperfusion injury (121). PKCε activation and PKCα up-regulation are two of many cell-signaling events to be described in the genetic $G_{\alpha q}$-mediated hypertrophy model (122). While these associations between PKC activity and different pathological cardiac responses clearly suggest that PKC signaling can contribute mechanistically to these events, gain- and loss-of-function studies are necessary for causality to be established.

Conventional and inducible cardiac-specific transgenesis has been used to explore the direct effects of PKCβ signaling in vivo. Although there is controversy over whether PKCβ is expressed in the adult mouse heart, (123, 124), Wakasaki et al expressed PKCβII under control of a truncated α-myosin heavy-chain promoter and observed a phenotype of hypertrophy, fibrosis, and systolic dysfunction (125). In a follow-up study, PKCβ-mediated phosphorylation of troponin

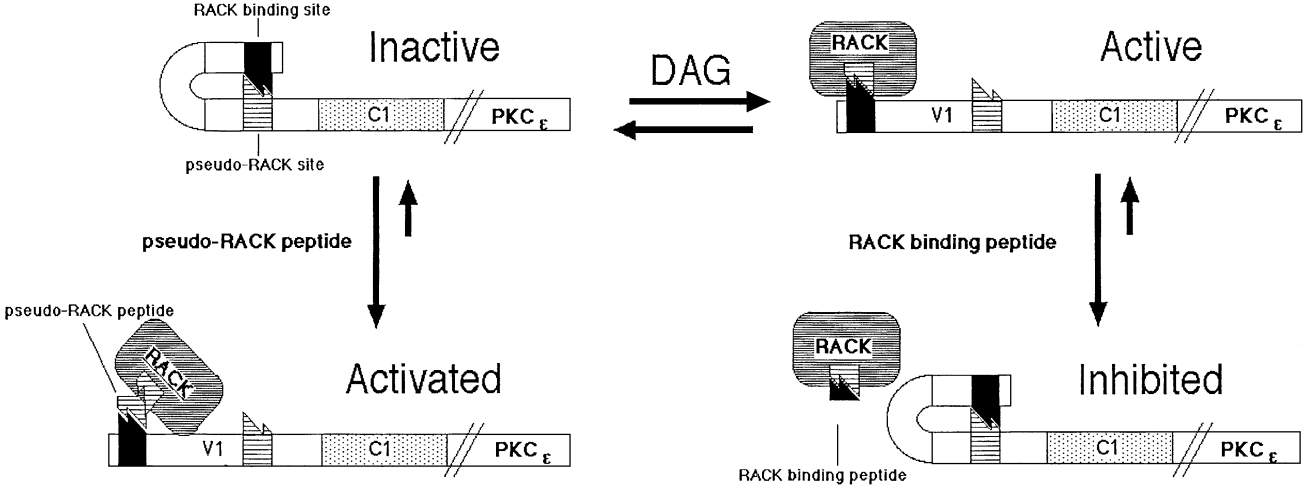

Figure 4 Mechanism of protein kinase C (PKC) activation. The pseudo-RACK binding site normally holds PKC factors in an inactive conformation unless stimulated. Once stimulated by diacylglycerol (DAG) or calcium, PKC factors change conformations, allowing interaction with RACK domain-containing proteins and exposure of the active site.

I was suggested as a mechanism for contractile dysfunction in these mice (126). Using inducible expression of a mutationally activated PKCβ, Bowman and colleagues found hypertrophy when the transgene was induced in the adult heart but a lethal effect of expression in the neonatal mouse (127).

Recent studies are focusing on the more abundant adult cardiac PKC isoforms, such as PKCε. Mochly-Rosen & Dorn have used transgenic techniques to express PKCε-activating and -inhibiting peptides in the mouse heart. The initial description of this model demonstrated that increasing basal translocation of PKCε by approximately 20% was sufficient to exert a powerful protective effect on cardiac contractile function and myocyte integrity in isolated hearts subjected to global ischemia with reperfusion (118). Subsequent studies using the complementary approaches of PKC isoform inhibition and activation demonstrated that PKCϵ activation causes a physiologic form of hypertrophy, whereas inhibition of PKCε translocation with a RACK-binding peptide (εV1) caused the opposite response, that is, thinning of the ventricular walls and lethal heart failure from a dilated cardiomyopathy (128). These studies begin to suggest that PKCε activation is a necessary component of normal trophic growth of cardiomyocytes during postnatal development. It is likely that the approach of in vivo PKC translocation modulation will, in the future, yield similarly useful information about the roles of other naturally occurring myocardial PKC isoforms.

CALCINEURIN AND CARDIAC HYPERTROPHY

Although a great deal of attention has been focused on the elucidation of kinase-signaling pathways as mediators of reactive signaling, the reciprocal pathways of dephosphorylation are less well characterized. Recently, the intracellular phosphatase calcineurin has been implicated as a regulator of the hypertrophic response in conjunction with the transcription factors nuclear factor of activated T cells (NFAT). Calcineurin is a serine-threonine phosphatase that is uniquely activated by calcium-calmodulin (Figure 5). The calcineurin enzyme consists of a 59- to 61-kDa catalytic subunit termed calcineurin A and a 19-kDa calcium-binding EF-hand domain containing protein. The catalytic subunit is encoded by three genes, *calcineurin A*α, *calcineurin A*β, and *calcineurin A*γ. In the adult human, rat, or mouse heart, both *calcineurin A*α and *calcineurin A*β gene products can be detected, but not *calcineurin A*γ (129; JD Molkentin, unpublished results).

The paradigm of calcineurin as a regulator of reactive intracellular signaling through NFAT transcription factors has been firmly established in T cells (130). Activation of the T-cell receptor ultimately results in elevated concentrations of intracellular calcium, which binds calmodulin, resulting in calcineurin activation. Once activated, calcineurin directly dephosphorylates members of the NFAT transcription factor family in the cytoplasm, resulting in their nuclear translocation and the activation of immune response genes (Figure 5). The immunosuppressive drugs cyclosporine A and FK506 are thought to act by inhibiting calcineurin and preventing NFAT nuclear translocation (129).

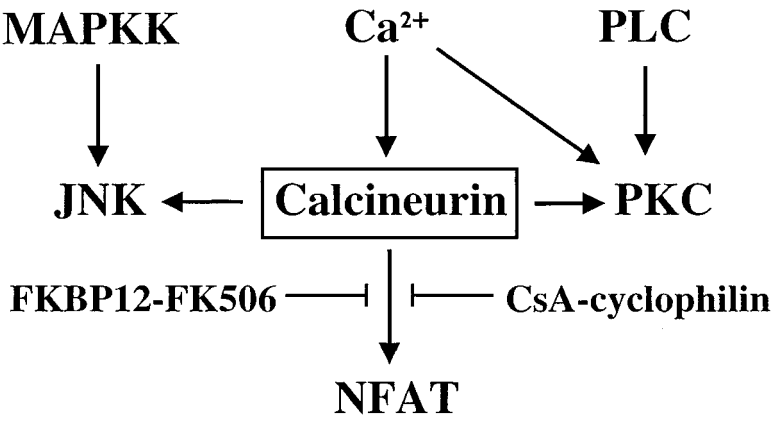

Figure 5 Calcineurin-signaling pathway in cardiomyocytes. Calcineurin is activated by calcium bound to calmodulin, which in turn leads to nuclear factor of activated T cell (NFAT) dephosphorylation and its nuclear translocation. Activated calcineurin has also been shown to promote c-Jun NH_2 terminal kinase (JNK) activation, and certain protein kinase C (PKC) isoforms (120). Abbreviations: CsA, cyclosporine A; MAPKK, mitogen-activated protein kinase kinase; FKBP12, FK506-binding protein.

A conserved role for calcineurin-NFAT signaling was recently identified in the heart (131). The notion of a calcium-activated signal transduction pathway in the myocardium was particularly attractive given the long-standing notion that altered intracellular calcium handling is associated with cardiac hypertrophy and heart failure (reviewed in 132). Overexpression of activated calcineurin in the heart of transgenic mice resulted in profound cardiac hypertrophy that underwent a transition to dilated-heart failure within 2 months (131). Calcineurin was subsequently shown to operate through NFAT3 in the heart because transgenic mice expressing a constitutively nuclear mutant of NFAT3 also demonstrated cardiac hypertrophy. The specificity of the transgenes was demonstrated by the observation that cyclosporine A inhibited cardiac hypertrophy in calcineurin-transgenic mice, but not in NFAT3-transgenic mice (131, 133).

Because cyclosporine A and FK506 are effective inhibitors of calcineurin, many groups pursued pharmacologic approaches to evaluate the necessity of this pathway in various rodent disease models. Pharmacologic calcineurin inhibition attenuated dilated and hypertrophic cardiomyopathy in three different mouse models of heart disease owing to alterations in sarcomeric proteins, and calcineurin also inhibited the development of pressure overload hypertrophy in aortic-banded rats (134). However, immediately after this initial report, four separate studies concluded that calcineurin inhibitors had no effect in blocking pressure overload hypertrophy in rodents (135–138). In contrast, seven additional rodent-based studies have reached the opposite conclusion that calcineurin inhibitors are effective agents for preventing or attenuating cardiac hypertrophy in vivo (61, 133, 139–143). The reason for these conflicting data is unknown, but factors such as

effective drug dosage, differences in the surgical preparations and aortic-banding procedures, or age and sex of animals may underlie the disparities.

Because both cyclosporine A and FK506 have multiple intracellular targets, the mechanism whereby these drugs attenuate cardiac hypertrophy is uncertain. To explore the issue of specificity, a recent report demonstrated that adenovirus-mediated gene transfer of noncompetitive calcineurin-inhibitory-protein domains into cultured cardiomyocytes significantly attenuated agonist-induced hypertrophy (144). These data suggest that cyclosporine A and FK506 mediate their inhibitory effects on cultured cardiomyocytes through a calcineurin-specific mechanism.

In response to hypertrophic stimuli, calcineurin protein content and enzymatic activity are increased (140, 141, 144). However, two other studies reported no change in calcineurin activity in response to pressure overload hypertrophy in the heart (137, 139), whereas a third study reported dramatic down-regulation (138). Once again, the reasons behind these divergent conclusions are uncertain. However, assessment of calcineurin enzymatic activity in cardiac protein extracts is technically difficult given the relatively low calcineurin content in the heart (145), its labile nature and sensitivity to oxidation (146), and the background of other phosphatases that act on the RII peptide substrate.

A lack of consensus also surrounds the studies that have examined calcineurin in failed human hearts. Calcineurin A activity was suggested to be significantly elevated in failed human hearts through the use of a calmodulin co-immunoprecipitation assay (147). More recently, these results were extended by the observation that calcineurin Aβ protein levels and total calcineurin enzymatic activity are each significantly elevated in both hypertrophied and failed human hearts (148; HW Lim & JD Molkentin, unpublished results). In contrast, another group reported that calcineurin A protein content was elevated with one commercial source of antibody but not with another (149). Although it is attractive to suggest that calcineurin may be a disease-predisposing pathway in the human heart, the utility of calcineurin inhibitory agents as potential therapeutics for human heart disease is uncertain given the known deleterious side effects of these drugs, including hypertension (150).

Because calcineurin-NFAT represents a newly appreciated regulatory pathway in heart, a number of critical questions remain to be addressed. The relative importance of NFAT transcription factors as necessary downstream mediators of calcineurin activity is uncertain. Northern blot analysis has demonstrated that at least four different *NFAT* genes are expressed on the heart, suggesting that knockout strategies might not be effective (151–153). Dominant-negative approaches that could globally inhibit NFAT factors or calcineurin in the hearts of transgenic animals will be necessary and are, in fact, in progress.

gp130-SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION AND CARDIAC HYPERTROPHY

Recently, a series of seminal studies have elucidated the role that the interleukin-6 (IL-6) family of cytokines plays in cardiac myocyte maturation and hypertrophy

(reviewed in 154). Cardiotrophin-1 is an important member of the IL-6 cytokine family that interacts with the dimerized membrane receptors gp130 and low-affinity leukemia inhibitor factor receptor (LIFR) (155). Ligand binding to the gp130-LIFR complex results in phosphorylation of Janus kinase (Jak) signaling factors, which in turn phosphorylates gp130, generating a docking site for SH2 domain-containing proteins (156–158; Figure 6). The family of signal transducer and activator of transcription (STAT) are SH2 domain-containing factors that are

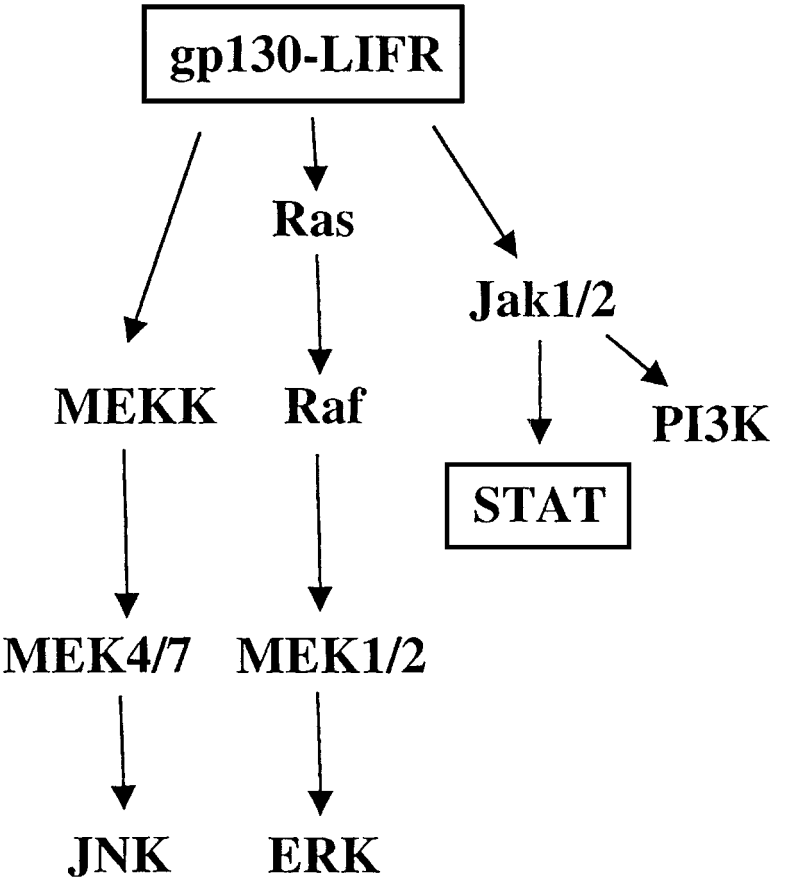

Figure 6 gp130-signal transducer and activator of transcription (STAT) signaling pathway. The gp130 transmembrane receptor associated with the leukemia inhibitory factor receptor (LIFR) is activated by LIF, cardiotrophin, and other members of the interleukin-6 cytokine family. Once activated, this receptor interacts with Janus kinase 1 (Jak1) causing its activation, which in turn leads to STAT phosphorylation promoting dimerization and nuclear entry. This receptor also promotes activation of, phosphatidylinositol 3-kinase (PI3K), Ras, and mitogen-activated protein kinase kinase kinases (MEKK).

recruited to phosphorylated gp130, resulting in their subsequent phosphorylation and homo- and heterodimerization and nuclear translocation (157, 159). In addition, activation of gp130 and/or LIFR has also been shown to lead to MAPK activation through activated Ras (160–162). Finally, the addition of leukemia inhibitor factor (LIF) to cardiomyocytes leads to gp130 activation, which can also lead to PI3K activation by a mechanism involving Jak1-mediated phosphorylation (163). Addition of wortmannin, a PI3K inhibitor, diminished LIF-induced MAPK activation, suggesting that gp130-Jak are interconnected with MAPK signaling pathways through PI3K or enhanced calcium signaling (163, 164).

Cardiotrophin-1–gp130-Jak activity is increased in cardiomyocytes in response to hypertrophic stimulation such as stretching or in response to pressure overload in vivo (165, 166). Cardiotrophin-1 and other members of the IL-6 cytokine family are potent inducers of neonatal cardiomyocyte hypertrophy in culture through gp130-LIFR activation (155, 167, 168). In addition, continuous activation of the gp130-STAT pathway causes cardiac hypertrophy in mice (169), and targeted disruption of *gp130* in the mouse results in embryonic lethality associated with hypoplastic ventricular development (170). Recently, cardiac-specific disruption of *gp130* revealed a critical role for this receptor in cardiac homeostasis and survival after aortic banding. Cardiac-specific *gp130* knockout mice were viable, but they quickly underwent a transition into heart failure after pressure overload stimulation, which was associated with profound apoptosis (171). Downstream, adenovirus-mediated gene transfer of either wild-type or dominant-negative STAT3 was shown to either stimulate or attenuate LIF-induced cardiomyocyte hypertrophy, respectively (172). In addition, transgenic overexpression of STAT3 in the heart induced cardiac hypertrophy (173). These results establish the IL-6 family of ligands, together with gp130-Jak-STAT signaling factors, as sufficient regulators of cardiac development, hypertrophy, and survival.

IGF-1 TRANSDUCTION PATHWAY AND CARDIAC HYPERTROPHY

Insulin-like growth factors (IGFs) I and II (IGF-I and IGF-II) are peptides that convey growth-factor-like signals which promote cellular proliferation and/or differentiation through binding to a specific heterotetrameric receptor with intrinsic tyrosine kinase activity (reviewed in 174). The activated IGF receptor phosphorylates the insulin receptor substrates (IRSs) 1 and 2 (IRS-1 and IRS-2) leading to signal transduction through Crk and Shc and resulting in Grb-2, Sos, and Ras activation (Figure 7). The regulatory subunit of PI3K contains an SH2 domain that interacts with IRS-1, resulting in PI3K activation (175). PI3K then leads to Akt (protein kinase B) activation and p70/p85 S6K through PDK1, which affect diverse intracellular processes such as translational regulation and cell survival (Figure 7).

Numerous studies have implicated IGF signaling in the regulation of cardiac homeostasis and maturation (reviewed in 174). We attempt to briefly review only

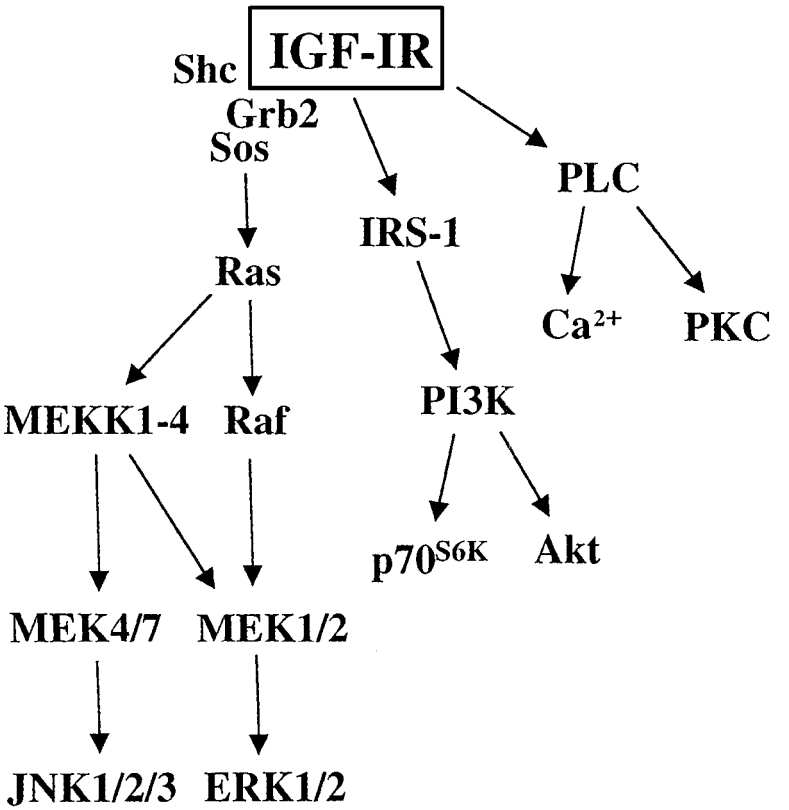

Figure 7 Insulin-like growth factor I receptor (IGF-IR) signaling pathway. IGF-IR activation leads to activation of Ras, phospholipase C (PLC), and insulin receptor substrates (IRS-1) proteins. Abbreviations: MEKK, mitogen-activated protein kinase kinase kinases; MEK, mitogen-activated protein kinase kinases; JNK1/2/3, c-Jun NH_2 terminal kinases; ERK1/2, extracellular signal-regulated kinases; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C.

the data that have implicated IGF-signaling pathways in the control of cardiac hypertrophy. Transgenic mice overexpressing IGF-1 in the myocardium were reported to have increased numbers of total myocytes without hypertrophy of individual muscle fibers (176, 177). In contrast, a recent study reported that overexpression of the local form of IGF-1 in the hearts of transgenic mice was sufficient to induce a hypertrophic phenotype that eventually led to reduced systolic performance (178). There is also a lack of consensus concerning the cardiac phenotype associated with IGF-1 deficiency. Growth hormone and IGF-1 deficiency in humans has been associated with cardiac atrophy and reduced function (179, 180). In contrast, IGF-1-deficient mice were reported to have elevated blood pressure and enhanced cardiac contractility (181).

Signaling components downstream of the IGF receptor have been implicated in cardiac hypertrophy. Angiotensin-II-induced cardiomyocyte hypertrophy in vitro was shown to induce p70S6K, resulting in greater protein translation (182). Selective inhibition of p70S6K with rapamycin blocked the augmentation of agonist-induced protein synthesis and the ensuing hypertrophic growth of cultured cardiomyocytes (182–184). In vivo, pressure overload stimulation activated both p70S6K and p85S6K, suggesting a role in physiologic hypertrophy (185). More recently, PI3K was also shown to regulate cardiac myocyte hypertrophy in transgenic mice. Overexpression of an activated form of the PI3K catalytic subunit resulted in cardiac hypertrophy, whereas transgenic overexpression of a dominant-negative PI3K produced smaller hearts and individual fibers (186). Finally, Akt activation was also shown to promote cardiomyocyte viability, although its role in hypertrophy was not reported (187). Collectively, these studies indicate that the IGF-PI3K-Akt-p70S6K signaling pathway plays an important role in regulating cardiac hypertrophy, viability, and homeostasis.

FIBROBLAST GROWTH FACTOR 2 AND TRANSFORMING GROWTH FACTOR β IN CARDIAC HYPERTROPHY

Fibroblast growth factor 2 (FGF-2) (also called basic FGF) and transforming growth factor β (TGFβ) are peptide growth factors that can each induce a fetal-like gene program in cultured rat neonatal ventricular myocytes, consistent with induction of the hypertrophic program (188). Since this original description, a number of additional studies have demonstrated the importance of FGF-2 and TGFβ signaling responses as mediators of the hypertrophic program. Both FGF-2 and TGFβ are produced by cardiomyocytes or non-myocytes within the heart, acting as autocrine or paracrine growth factors (reviewed in 189). FGF-2 and TGFβ each bind separate membrane receptors that have intracellular tyrosine kinase activity (FGF) or serine-threonine kinase activity (TGFβ) to elicit further signaling.

In adult cardiac myocytes, pacing induced FGF-2 release from cardiomyocytes, which then acted in an autocrine fashion to stimulate a hypertrophic phenotype and phenylalanine incorporation (190). In vivo, FGF-2 infusion stimulated cardiac hypertrophy in adult rats after myocardial infarction (191), but in a different study by the same group this was not confirmed (192). To definitively examine the role of FGF-2 in cardiac hypertrophy, Schultz et al characterized *FGF-2*-null mice after aortic banding (193). *FGF-2*-null mice demonstrated a significant attenuation of the hypertrophic response after pressure overload stimulation, indicating that this signaling pathway was a necessary regulator of cardiac hypertrophy (193). Although Schultz et al did not examine the downstream consequences on intracellular signaling in *FGF-2*-null mice, another study demonstrated that FGF-2 and its receptor signal, in part, through the MAPK cascade to elicit myocyte hypertrophy (194).

Significantly less is known concerning a role for TGFβ in the hypertrophic response. Although TGFβ1 mRNA is increased in response to pressure overload or norepinephrine infusion in the rat heart, a functional role as a hypertrophic regulator has not been established (195). In vitro, adenovirus-mediated overexpression of TGFβ1 in cultured neonatal cardiomyocytes increased sarcomeric actin, suggesting a growth response (196). In addition, angiotensin-II-induced neonatal myocyte hypertrophy required paracrine TGFβ1 release from non-myocytes in culture (197). Downstream of TGFβ receptor activation (type-I and type-II receptors), two main intracellular-signaling branches involving Smad proteins or TGFβ-activated kinase (TAK1) have been documented (198). Whereas Smad proteins are not known to regulate cardiomyocyte hypertrophy, TAK1 can directly activate MAPKK factors, leading to JNK and/or p38 activation (198). Indeed, TGFβ1 was reported to cause TAK1 activation, leading to p38 phosphorylation and up-regulation of the skeletal α-actin promoter in cultured neonatal cardiomyocytes (199).

OTHER INTRACELLULAR REGULATORS OF CARDIAC HYPERTROPHY

Additional hypertrophic mediators have been implicated, and undoubtedly countless others have yet to be described. For example, Src is activated in pressure-loaded hearts (200), and overexpression of activated Src in neonatal cardiomyocytes induced hypertrophy through a Ras- and Raf-dependent pathway (201). Focal adhesion kinase another nonreceptor tyrosine kinase, was also reported to induce cardiac hypertrophy when overexpressed in transgenic mouse hearts (202). Neuregulin signaling through the ErbB2 or ErbB4 receptors (member of the epidermal growth factor receptor family) can induce neonatal cardiomyocyte hypertrophy in culture through an ERK MAPK signaling pathway (203). In vivo, ErbB2 and ErbB4 mRNA levels were reported to be down-regulated in early-stage heart failure induced by pressure overload, suggesting that lowered ErbB receptor signaling plays a role in loss of hypertrophy and decompensation (204). Signaling through the epidermal-growth-factor-like tyrosine kinase receptor can also induce cardiomyocyte hypertrophy in culture (205).

A number of other less typical signaling pathways may also play a role in cardiac hypertrophy. Thyroid hormone induces cardiac hypertrophy in vitro and in vivo through binding to its intracellular receptor, which then acts as a transcription factor to directly induce expression of cardiac genes (reviewed in 206). More recently, the myosin light-chain kinase was shown to directly regulate sarcomeric organization in cardiac myocytes, a characteristic associated with hypertrophy (207). The cytokine tumor necrosis factor α is also expressed by cardiac myocytes and can activate the tumor necrosis factor receptor, resulting in hypertrophy of cultured cardiomyocytes (208). In addition, transgenic mice overexpressing tumor necrosis factor α specifically in the heart develop dilated cardiomyopathy and heart failure (209, 210).

CONCLUSIONS: Integrated Model of Intracellular Signal Transduction

Estimates derived from the number of kinase and phosphatase genes identified in *Caenorhabditis elegans* predict that, of ~50,000 human genes, ~1100 are kinases and 300 are phosphatases (211). These figures suggest an almost overwhelming complexity in mammalian signal transduction cascades and underscore the potential difficulty in designing pharmacologic agents with both specificity and efficacy in treating various forms of cardiomyopathy. However, lessons from past studies actually suggest an opposite interpretation, that is, that most strategies used today are effective. Numerous studies have demonstrated that inhibition of specific central-signaling pathways can attenuate the hypertrophic response. Indeed, hypertrophy secondary to hypertension in humans can be partially reversed with pleiotropic drugs such as angiotensin-converting enzyme inhibitors, βAR blockers, and calcium channel blockers (212, 213).

In this review, we have discussed a number of studies in animal models of pressure overload hypertrophy, each of which demonstrated attenuation of hypertrophy by inhibiting divergent intracellular signaling pathways. For example, a dominant-negative $G_{\alpha q}$ peptide or a dominant-negative (MKK4) SEK factor each has an impact on the ability of the heart to mount a hypertrophic response in vivo. Furthermore, FGF-2 knockout mice or treatment of rodents with a calcineurin inhibitor (cyclosporine) also attenuates the hypertrophic response in vivo. These seemingly contradictory studies actually support an integrated model of signal transduction in the heart such that multiple pathways are necessary for timely and effective hypertrophy. Specific inhibition of central regulatory pathways likely diminishes the activation of other interdependent signal transduction pathways. Indeed, cyclosporine A-mediated attenuation of hypertrophy was not only associated with inhibition of calcineurin but was also found to lead to inhibition of JNK, PKCα, and PKCθ in pressure-loaded rat hearts (120). Collectively, this model emphasizes the potential for specific inhibition of any of a number of central regulatory pathways as an effective strategy for treating certain forms of hypertrophic disease.

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