Heart failure afflicts about 5 million Americans at an estimated cost to the healthcare system of 40 billion dollars annually.¹,² Despite extensive knowledge of the causes and effects of heart failure, the underlying molecular mechanisms responsible for the disease remain vague. Elucidation of these mechanisms is an essential prerequisite to the development of rational pharmacological approaches to prevent and potentially reverse the pathological changes associated with this catastrophic disease.

Cardiac hypertrophy is an adaptive response of the heart to a wide array of intrinsic and extrinsic stimuli, including hypertension, myocardial infarction, cardiac arrhythmias, valvular disease, endocrine disorders, and contractile abnormalities resulting from mutant sarcomeric proteins. Because cardiomyocytes lose the ability to divide soon after birth, enlargement of the heart during hypertrophy involves an increase in size and mass of individual cardiomyocytes without an increase in cell number. Although initially beneficial, prolonged hypertrophy can become deleterious, resulting in dilated cardiomyopathy, heart failure, and sudden death. Several drugs show efficacy in sustaining cardiac function and prolonging life in heart failure patients, but the 5-year mortality rate for patients with the disease remains nearly 50%, and there is no truly effective pharmacological prevention or cure. The recent creation of several mouse models that mimic aspects of human heart disease represents an auspicious step toward the development of improved drug therapies.

Over the past decade, a multitude of papers have described various signal-transduction pathways that can induce hypertrophy in cultured cardiomyocytes and transgenic mice.³,⁴ However, although it is apparent that a host of signals can cause hypertrophy in experimental systems, which of these really do cause hypertrophy and heart failure in humans remains a fundamental question. In addition, many of the signaling molecules that have been shown to induce hypertrophy in primary cardiomyocytes or transgenic mice have not yet been interconnected with a complete signaling system for transduction of hypertrophic signals from the cytoplasm to the nucleus.

We recently reported that the Ca²⁺/calmodulin (CaM)–dependent protein phosphatase calcineurin can transduce hypertrophic signals in vivo and that inhibition of calcineurin activity in certain situations can block the cellular and molecular events associated with hypertrophy.⁵ These studies led to a model for transduction of hypertrophic stimuli via calcineurin activation and have generated considerable attention because the calcineurin pathway can be inhibited by immunosuppressant drugs already in use in humans. These studies have also raised 2 sets of important questions. (1) Does calcineurin activation represent a final common pathway for transduction of hypertrophic signals from diverse types of pathological stimuli, or is it one of many independent pathways? (2) Might immunosuppressant drugs that inhibit calcineurin activity be useful in treatment of hypertrophy and heart failure? Given that such drugs are used routinely in organ transplant patients, are there any clinical data that address this issue? Here, we describe the calcineurin-dependent pathway for cardiac hypertrophy and consider these questions in the context of existing data from experimental systems and clinical studies.

Reprogramming Gene Expression in the Hypertrophic Heart

In response to hypertrophic stimuli, adult cardiomyocytes reactivate the fetal gene regulatory program and downregulate various adult isoforms.³,⁶ Among the fetal cardiac genes that are activated in hypertrophic cardiomyocytes are those encoding atrial natriuretic factor and B-type natriuretic peptide (ANF and BNP, respectively), which decrease blood pressure by vasodilation and natriuresis. There is also a switch in expression of myosin heavy chain (MHC) isoforms from α to β and from α-cardiac to α-skeletal actin. Although numerous transcription factors have been implicated in reactivation of fetal cardiac genes in response to hypertrophy, how hypertrophic signal transduction pathways are linked to changes in cardiac gene expression has not been explained. It is also unclear whether hypertrophic signals activate one or a few key target genes that initiate the hypertrophic response and lead secondarily to overall reprogramming of cardiomyocyte gene expression or whether all of the genes activated and repressed in the hypertrophic heart respond directly to hypertrophic signals.
Intrinsic Versus Extrinsic Signaling in Hypertrophy

Cardiac hypertrophy can result from intrinsic and extrinsic stimuli. Extrinsic forms of hypertrophy are thought to be mediated by signals generated at the cell membrane. A variety of factors, including angiotensin II (Ang II), the adrenergic agonists phenylephrine (PE) and norepinephrine, endothelin-1 (ET-1), and the cytokine cardiotoxin act as potent hypertrophic agonists.

In addition, perturbation of cardiac contractility due to altered sarcomeric function, as well as mechanical deformation, can result in cardiac hypertrophy. Mutations in MHC, 2 myosin light chains, troponyosin, troponins T and I, myosin binding protein C, and α-cardiac actin have been identified in patients with cardiomyopathies.2–4 Similarly, overexpression, misexpression, or deletion of several sarcomeric and cytoskeletal proteins in the hearts of transgenic mice result in forms of hypertrophy that mimic human heart disease. How perturbation in sarcomeric function is coupled to changes in cardiac gene expression is unclear, although it is well established that cardiomyocytes bearing mutant sarcomeric proteins exhibit alterations in Ca2+ handling and contractility,5–13 which could be coupled to activation of the hypertrophic gene regulatory program.

Ca2+ Signaling as a Potential Inducer of Hypertrophy

Numerous studies support the notion that Ca2+ is a primary signal for cardiac hypertrophy. Hypertrophic agonists such as Ang II, PE, and ET-1 activate [Ca2+]i-dependent signaling systems.14–16 Similarly, myocyte stretch, increased loads on working heart preparations, elevation of extracellular Ca2+, stimulation with Ca2+-channel agonists, treatment with Ca2+-ionophores, and electrical pacing all elevate [Ca2+]i, and induce cardiomyocyte hypertrophy.17–22 Reduced Ca2+ uptake rates by the sarcoplasmic reticulum, increased intracellular basal Ca2+ concentrations, and defects in diastolic Ca2+ sequestration by ventricular cardiomyocytes are also associated with hypertrophy and heart failure in humans and animal models.10–13 At the onset of cardiac hypertrophy, the amplitude of the [Ca2+]i transient increases. However, as hypertrophy progresses to heart failure, the amplitude of the [Ca2+]i transient decreases and is prolonged.23 These alterations in Ca2+ handling result in a significant increase in diastolic Ca2+, manifested as diastolic heart dysfunction at the whole organ level.

The possible existence of a Ca2+-sensitive signaling system for cardiac hypertrophy raises several important questions. How do cardiomyocytes discriminate between elevations in Ca2+ associated with chronic long-term hypertrophic stimuli and normal fluctuations in [Ca2+]i, levels during each phase of contraction/relaxation, in which Ca2+ concentrations vary over several orders of magnitude? If elevated Ca2+ levels are involved in propagation of a long-term hypertrophic signal, the transducing mechanism must be insensitive to these normal fluxes in Ca2+ concentration. It is likely that Ca2+ pools are compartmentalized in cardiomyocytes such that Ca2+ pools that signal the hypertrophic response are distinct from those involved in excitation-contraction coupling in the sarcoplasmic reticulum. The notion that Ca2+ is compartmentalized in cardiomyocytes is supported by the ability of Ca2+ channel blockers, which act at the cell membrane, to attenuate the hypertrophic response. Since changes in Ca2+ concentration at the cell membrane are dramatically less than in the region of the sarcoplasmic reticulum during contraction/relaxation, there must be a specific role for Ca2+ in the vicinity of the cell membrane for hypertrophic signaling.

Linking Cardiac Transcription and Ca2+ Signaling by Interaction of GATA4 and Nuclear Factor of Activated T Cells-3 (NFAT3)

Our approach to uncovering the signaling system for cardiac hypertrophy was to begin at the endpoint for hypertrophic signaling (ie, the genes that are switched on) and attempt to work backward from the nucleus to the cell surface, where hypertrophic signals are initiated. Many of the genes upregulated during hypertrophy are controlled by the cardiac-restricted zinc finger transcription factor GATA4, and recent studies indicated that GATA4 binding sites were important for transcriptional activation of the Ang II type 1a receptor and α-MHC genes in response to pressure-overload hypertrophy in rats.24–26 We therefore used GATA4 as bait in a yeast 2-hybrid screen to identify potential GATA4-interacting proteins that might provide a link to a hypertrophic signal transduction system. From this screen, we discovered that another transcription factor, NFAT3, interacted with high affinity and specificity to the second zinc finger of GATA4.27 This finding was of interest because members of the NFAT family have been studied extensively in T cells, in which they mediate changes in gene expression in response to Ca2+ signaling from the T-cell receptor.27 Thus, the discovery that a Ca2+-regulated transcription factor interacted directly with a cardiac transcription factor involved in activation of hypertrophic responsive genes suggested a possible mechanism that could link alterations in [Ca2+]i, handling to hypertrophic gene activation.

NFAT3 is one of four known NFAT proteins, which have been studied primarily in T cells. NFAT1 (also called NFATp), NFAT2 (also called NFATc), and NFAT4 are expressed at the highest levels in immune cells and skeletal muscle, whereas NFAT3 is expressed in a wide range of tissues, including the adult heart.28–33 NFAT proteins share a common structural organization, with a centrally located DNA binding domain belonging to the Rel family of transcription factors (Figure 1). Another well-known member of this family, NF-κB, also mediates changes in gene expression in response to signaling at the cell membrane. NFAT proteins contain an amino-terminal regulatory domain that controls translocation into the nucleus in response to signal-dependent dephosphorylation.34–36 In resting T cells, NFAT proteins are sequestered in the cytoplasm because of phosphorylation of the amino-terminal regulatory domain, whereas on T-cell receptor activation, [Ca2+]i levels increase, resulting in activation of the cytoplasmic phosphatase calcineurin, which dephosphorylates the regulatory domains of NFATs, allowing them to enter the nucleus. NFAT proteins can bind the DNA sequence GGAAAT as monomers or dimers, but they associate preferentially with activator protein-1 (AP-1) to form a complex that binds a composite DNA sequence in the control
regions of T-cell–responsive genes, such as the IL-2 gene.\textsuperscript{31,37,38} NFAT target genes have been identified in T cells, but little is known of potential target genes in other cell types. A diagram of the calcineurin signaling system as defined in T cells is shown in Figure 2.

Initial deletion studies have shown that the second zinc finger of GATA4 and the carboxyl-terminal portion of the Rel homology domain of NFAT3 are required for interaction of GATA4 and NFAT3 (Figure 1), but we do not yet know the precise amino acid determinants that mediate this interaction. NFAT3 can also interact with GATA5 and GATA6, which are expressed in the heart and share high homology with the zinc fingers of GATA4. Whether other members of the NFAT family also interact with these GATA factors remains to be determined.

Control of Cardiac Hypertrophy by Calcineurin Activation

Calcineurin is a ubiquitously expressed serine- and threonine-protein phosphatase dependent on Ca\textsuperscript{2+} and CaM for activation. The enzyme resides primarily in the cytoplasm and comprises a 59-kDa CaM-binding catalytic A subunit and a 19-kDa Ca\textsuperscript{2+}-binding regulatory B subunit.\textsuperscript{39,40} There are 2 catalytic subunit genes and a single regulatory subunit gene in mice and humans. Only one of the catalytic subunit genes has been knocked out in mice.\textsuperscript{41} These mutant mice are viable and do not show obvious cardiac defects.

Calcineurin has been the focus of intense interest because the immunosuppressant drugs, cyclosporin A (CsA) and FK-506, inhibit enzymatic activity and thereby diminish T-cell receptor signaling.\textsuperscript{36} These drugs interact specifically with the cytoplasmic immunophilin proteins, cyclophilins and FK-506 binding protein-12 (FKBP12), respectively, to form inhibitory complexes that bind the calcineurin A subunit.\textsuperscript{42} Since these drugs do not affect early biochemical events associated with signaling at the cell membrane, such as phosphatidylinositol turnover, Ca\textsuperscript{2+} mobilization, and protein kinase C (PKC) activation,\textsuperscript{43} they can be used to distinguish the roles of such signals from calcineurin activation in cellular responses, such as hypertrophy (see below). Another immunosuppressant, rapamycin, also binds FKBP12, but in contrast to CsA and FK-506, rapamycin does not inhibit calcineurin activity.\textsuperscript{42} Thus, formation of an immunophilin-ligand complex is insufficient to inhibit calcineurin. Interestingly, rapamycin has been shown to selectively inhibit Ang II– and PE-mediated increases in protein synthesis, but not activation of fetal genes in neonatal cardiac myocytes in vitro.\textsuperscript{44,45}

The finding that NFAT3 interacted specifically with GATA4 led us to investigate whether aspects of the calcineurin-NFAT signaling system, as characterized in T cells, might function in cardiomyocytes to transduce hypertrophic signals. Our results showed that GATA4 synergized with calcineurin and NFAT3 to activate the BNP promoter in neonatal rat cardiomyocytes and that a high-affinity NFAT binding site within the BNP promoter was required for synergistic activation by GATA4 and NFAT3. Previous studies had shown that activation of BNP transcription in cardiac myocytes required GATA4 binding sites in the promoter.\textsuperscript{46} Thus, at least in the case of this promoter, both NFAT3 and GATA4 must bind nonadjacent sites for transcriptional activation. This type of transcriptional cooperativity is distinct from that of NFAT and AP-1 on T-cell–responsive genes, in which the factors form a heteromeric complex that binds a shared DNA sequence element.\textsuperscript{39}

Compelling evidence for a role of calcineurin as a transducer of hypertrophic signals came from the observation that exposure of cardiomyocytes in tissue culture to CsA or FK-506 blocked their ability to undergo hypertrophy in response to Ang II and PE.\textsuperscript{5} Moreover, Ang II and PE upregulated expression of an NFAT-dependent reporter gene through a CsA/FK-506–sensitive mechanism.\textsuperscript{5} These results indicated that the signaling systems activated by these agonists culminated with NFAT activation in the nucleus and that calcineurin activation was an obligatory step in these signaling pathways. On the basis of these results and studies in transgenic mice (see below), we proposed the model shown in Figure 3 to account for the role of calcineurin in cardiac hypertrophy.

Deletion of the carboxyl-terminal regulatory domain of the calcineurin A subunit releases the enzyme from its requirement for Ca\textsuperscript{2+}/CaM and renders it constitutively active.\textsuperscript{47}
Consistent with studies in primary rat cardiomyocytes, we found that a transgene encoding a constitutively active form of calcineurin under transcriptional control of the α-MHC promoter was sufficient to substitute for hypertrophic signals and induce cardiac hypertrophy that progressed to dilated cardiomyopathy, heart failure, and sudden death in transgenic mice. Electrocardiography has shown that these mice display arrhythmias, which likely account for their high susceptibility to sudden death (R. Shoet and E. Olson, unpublished data, 1998). Pregnant females are especially prone to severe congestive heart failure, evidenced by extensive fluid accumulation due to venous backup (Figure 4).

The cellular, morphological, and molecular changes associated with cardiac hypertrophy in these α-MHC–calcineurin transgenic mice were prevented by systemic administration of CsA or FK-506 at doses shown previously to be immunosuppressive. Importantly, however, normal postnatal growth of the heart was unaffected by these drugs, suggesting that the normal mechanism for cardiac growth is calcineurin independent. Whether physiological hypertrophy in response to exercise involves calcineurin remains to be determined. It should be emphasized that, although high doses of CsA for prolonged periods can be toxic in rodents, under the conditions of our treatment protocol, there was no obvious toxicity or weight loss from CsA administration, when hypertrophy was prevented.

The finding that activated calcineurin can transduce hypertrophic signals in vivo is consistent with an earlier study showing that overexpression of CaM in the heart leads to hypertrophy. Although the mechanism for hypertrophy in that study was not addressed, activation of calcineurin would be a likely downstream step in the CaM pathway.

Recent studies suggest that the unique Ca\(^{2+}\) responsiveness of calcineurin could provide the type of sensitivity and selectivity required for transduction of hypertrophic signals. In contrast to other Ca\(^{2+}\)-sensitive enzymes such as PKC and CaM-dependent protein kinases, which are rapidly and transiently activated in response to brief, high-amplitude Ca\(^{2+}\) pulses, calcineurin is insensitive to such Ca\(^{2+}\) pulses. Instead, calcineurin activation and the resulting nuclear translocation of NFAT require sustained, low-amplitude elevations of Ca\(^{2+}\). Thus, calcineurin would be expected to be unaffected by Ca\(^{2+}\) fluxes during contraction/relaxation of cardiomyocytes. We anticipate that calcineurin may also transduce long-term changes in Ca\(^{2+}\) signaling into transcriptional responses in other excitable cell types, such as skeletal muscle, smooth muscle, and neurons. Indeed, recent studies have shown that calcineurin regulates fiber type–specific gene expression in skeletal muscle, which is known to be dependent on contractile activity and Ca\(^{2+}\) signaling. As a consequence of more frequent neural stimulation, slow skeletal muscle fibers maintain higher levels of [Ca\(^{2+}\)] than fast fibers. Activation of calcineurin selectively upregulates slow fiber–specific skeletal muscle promoters, and conversely, inhibition of calcineurin activity in vivo with CsA results in slow to fast fiber transformation.

**Activated NFAT3 Is Sufficient to Induce Cardiac Hypertrophy and Heart Failure**

Our results from cell culture experiments and in vitro assays predicted that NFAT3 acted downstream of calcineurin in a hypertrophic signaling pathway (Figure 3). Consistent with this model, a deletion mutant of NFAT3 lacking the amino-terminal regulatory domain and therefore rendered constitutively nuclear was also sufficient to induce hypertrophy in transgenic mice, whereas expression of the wild-type NFAT3 protein in the heart did not lead to hypertrophy. The ability of this activated transcription factor to bypass all upstream elements in the hypertrophic signaling pathway suggests that NFAT3 activation alone can trigger the hypertrophic response. This differs from the situation in T cells, in which activation of NFAT-responsive
genes requires activation of calcineurin and a costimulatory pathway leading to AP-1 activation. The apparent autonomy of the NFAT pathway in the heart could indicate that the costimulatory pathway is constitutively active or that NFAT acts together with transcriptional effectors other than AP-1 in the heart. In this regard, the myogenic transcription factor myocyte enhancer factor-2 (MEF2) is a candidate coregulator for NFAT in cardiomyocytes, given its similar role in skeletal muscle.

Although activated NFAT3 is sufficient to induce hypertrophy, we are not yet certain whether all of the effects of calcineurin are mediated by NFAT3 activation. This is a particularly important issue, because it influences whether drug therapies directed specifically at NFAT3 will be effective in preventing hypertrophy. It is formally possible, for example, that the pathway bifurcates immediately downstream of calcineurin activation and that other calcineurin substrates play a role in hypertrophic signaling. Such substrates could be transcription factors or other types of signaling molecules. Of note, calcineurin has been shown to influence Ca\(^{2+}\) fluxes by associating with, and modulating the activities of, voltage-dependent Ca\(^{2+}\) channels and the inositol 1,4,5-triphosphate receptor. Such alterations could play a role in the pathophysiology of heart failure. Of course, even if these types of Ca\(^{2+}\)-regulatory molecules are targets of calcineurin dephosphorylation during hypertrophy, the hypertrophic signal must ultimately be transmitted to the nucleus through a Ca\(^{2+}\)-sensitive transcription factor. Calcineurin has also been shown to activate (probably indirectly) the transcription factor MEF2, which regulates many of the cardiac genes that are upregulated during hypertrophy, the hypertrophic response. The potential hierarchical relationships and interconnections between calcineurin and other intracellular signaling molecules in cardiomyocytes remain to be addressed.

Stimuli such as \(\alpha_1\)-adrenergic agonists, Ang II, ET-1, and physical stretch have all been associated with PKC activation and cardiomyocyte hypertrophy. In vivo studies also suggest a role for PKC-mediated signaling in cardiac hypertrophy. Overexpression of PKC\(\beta\) in the hearts of transgenic mice resulted in alterations in [Ca\(^{2+}\)]\(\text{c}\) handling and cardiac hypertrophy. Activated PKC has been shown to directly activate hypertrophic gene expression by affecting the transcription factors TEF-1, serum response factor, and Sp1 and to indirectly affect cardiac hypertrophy through phosphorylation of Ca\(^{2+}\) channels and cardiac contractile proteins.

CaM kinases have also been implicated in transduction of hypertrophic signals in cultured cardiomyocytes. The predominant isoform of CaM kinase expressed in the heart is CaM kinase II. Expression of the \(\delta\) isoform of CaM kinase II, which is localized to the nucleus, specifically activates the ANF promoter, whereas other isoforms of CaM kinase II that are not localized to the nucleus have no effect on ANF expression. The CaM kinase inhibitors M7 and KN-93 also prevent myocardial cell hypertrophy and upregulation of ANF in response to PE, which indicates that CaM kinase activation is an essential step in PE-mediated hypertrophy. Interestingly, however, overexpression of \(\delta\) CaM kinase activates ANF expression without inducing cellular hypertrophy or myofilament organization. Thus, although CaM kinase activation may be essential for PE-mediated hypertrophy, it is apparently not sufficient and likely requires additional signals for the full hypertrophic response. The downstream targets for CaM kinase phosphorylation that might lead to hypertrophy are presently unknown.

Treatment of cultured cardiomyocytes with hypertrophic agonists has also been shown to result in activation of MAPK signaling pathways. MAPK signaling consists of 3 separate phosphorylation cascades that result in activation of extracellular signal–regulated protein kinases (ERKs) 1 and 2, JNKs, or p38 kinases. In vitro studies have suggested a role for ERK1 and ERK2 in mediating adrenergic receptor–stimulated hypertrophy. However, activation of ERK1 and ERK2 is not sufficient to stimulate sarcomeric assembly by cardiomyocytes in vitro. More recently, 2 independent studies have implicated p38-MAPKs, and the upstream activator MAPK kinase-6, as necessary and sufficient mediators of hypertrophy in cultured cardiomyocytes. Ang II– and adrenergic-mediated hypertrophy were shown to be associated with JNK activation, and expression of a constitutively active MAPK kinase-7 mutant (or JNK kinase-2), a specific activator of only JNK, was shown to be sufficient to mediate hypertrophy in cultured cardiomyocytes. However, JNK was also reported to be a suppressor of ANF gene expression in cultured cardiomyocytes, suggesting that responsiveness of cardiomyocytes to JNK may be modulated by other cellular factors.

Although numerous studies in cultured cardiomyocytes have suggested an association between MAPK signaling molecules and cardiac hypertrophy, very few studies have examined these associations in vivo. Transgenic mice over-

### Coordination of Calcineurin With Other Intracellular Signaling Pathways

An important issue for the future will be to analyze the role of calcineurin as a mediator of cardiac hypertrophy in the context of previously identified hypertrophic signaling pathways. In vitro studies with cultured cardiomyocytes have demonstrated a role for PKC, protein kinase A, CaM kinases, and members of the mitogen-activated protein kinase (MAPK) family such as c-Jun N-terminal kinase (JNK), p38-MAPK, p42-MAPK, and p44-MAPK in the hypertrophic response. The potential hierarchical relationships and interconnections between calcineurin and other intracellular signaling molecules in cardiomyocytes remain to be addressed.
expressing p21ras, an upstream activator of Raf in the MAPK cascade, develop cardiac hypertrophy and diastolic dysfunction. Other in vivo associations that have been described include p38 activation with pressure-overload hypertrophy, JNK activation with myocardial infarction, and ERK and JNK activation with heart disease in stroke-prone rats.

In-depth studies of \( \beta \)-adrenergic receptor signaling and cardiac hypertrophy have suggested a link between cAMP- and Ca\(^{2+} \)-dependent signaling pathways. Heart failure is associated with a decrease in \( \beta \)-adrenergic receptor signaling presumably due to \( \beta \)-adrenergic kinase (\( \beta \)ARK)–dependent phosphorylation and desensitization of \( \beta \)-adrenergic receptors. Transgenic mice that overexpress \( \beta \)ARK in the heart were shown to have decreased inotropy, while transgenic mice overexpressing a peptide inhibitor of \( \beta \)ARK were shown to have increased basal contractility. Expression of the \( \beta \)ARK inhibitory fragment in hearts of mice lacking the muscle lim domain protein, which normally develop dilated cardiomyopathy, results in restoration of normal cardiac function. These studies suggest that \( \beta \)-adrenergic signaling antagonizes hypertrophic signaling, presumably by elevating adenylate cyclase activity and cAMP levels. This mechanism of action also suggests that \( \beta \)-adrenergic signaling antagonizes Co2+ signaling pathways, such as those utilizing PKC or calcineurin.

Studies published to date suggest an integrated model of intracellular hypertrophic signaling. This integrated model predicts interconnections between calcineurin, PKC, CaM kinase, and MAPK signaling pathways. Consistent with this notion, recent studies in T cells suggest that the MAPK and calcineurin pathways may indeed be interconnected. For example, ERK2 synergizes with NFAT4 in promoting T-cell immediate-early transcriptional responses, and NFAT factors directly interact with AP-1 to regulate inducible gene expression in T cells. This is particularly relevant given that JNK, a member of the MAPK pathway, directly activates JNK activity and expression of the interleukin-2 promoter. Calcineurin has also been shown to synergize with Ras and CaM kinase IV to induce AP-1-dependent gene expression in T cells. Calcineurin and CaM kinase IV cooperatively activate MEF2 (B. Nicol and E. Olson, unpublished data, 1998). Together, these studies suggest interconnections between PKC, CaM kinase, and MAPK cascades, and calcineurin-mediated signaling.

**Calcineurin-Dependent Induction of Cardiac Hypertrophy in Response to Sarcomeric Dysfunction**

Over the past 5 years, numerous transgenic mouse models of cardiac hypertrophy and cardiomyopathy have been generated and shown to mimic aspects of human heart disease. Several of these transgenic models have recently been tested for their sensitivity to CsA and FK-506 in an effort to determine whether calcineurin activation is responsible for the associated cardiac pathology.

Transgenic mice that overexpress the actin capping molecule tropomodulin in the heart develop severe dilated cardiomyopathy. CsA and FK-506 given to tropomodulin-overexpressing transgenic mice prevented cardiac dilation when administered before signs of disease arose (Figure 5). Moreover, calcineurin phosphatase activity was markedly increased in the hearts of these transgenic mice, and activity was inhibited by CsA. Treatment with CsA of mice that develop dilated and hypertrophic cardiomyopathy due to cardiomyoverexpression of fetal \( \beta \)-tropomysin or a nonphosphorylatable myosin light chain-2v protein also prevented the morphological, histological, and molecular manifestations of cardiomyopathy. However, a mouse model of cardiac hypertrophy caused by overexpression of a constitutively active retinoic acid receptor was not responsive to CsA treatment. The failure of the latter model to respond to calcineurin inhibitors demonstrates that hypertrophy can occur in the absence of calcineurin activation. However, the precise mechanism whereby the retinoic acid receptor leads to hypertrophy and whether it might function downstream of calcineurin in a hypertrophic signaling pathway are unknown.

Expression of an activated form of the G protein Goq in the heart, under control of the \( \alpha \)-MHC promoter, also results in hypertrophy through activation of downstream Ca\(^{2+} \) signaling pathways. Treatment of this line of transgenic mice with CsA blunted but did not prevent hypertrophy. These findings suggest that Goq activation, which normally occurs in response to \( \alpha \)-adrenergic, Ang II, and ET-1 receptor occupancy, leads to activation of calcineurin, but that other signaling pathways are sufficient to induce hypertrophy in the absence of calcineurin activation.

The 3 sarcomere-based models of cardiomyopathy described above mimic aspects of human heart disease. Cardiomyopathies that arise from intrinsic defects in contractile protein genes are thought to affect about 1 in 500 young adults. Prevention of disease in these transgenic models of cardiomyopathy suggests that calcineurin may be involved in the pathogenesis of certain intrinsic forms of heart disease and raises the possibility of a novel approach for treating various forms of heart disease by
inhibiting calcineurin. However, it will be important to test other such mouse models for their sensitivity to CsA before it can be concluded that this represents a general mechanism for hypertrophy in response to sarcomeric dysfunction.

**Hypertrophy in Response to Pressure Overload**

Pressure overload in response to aortic constriction is one of the most potent stimuli for hypertrophy. The pressure-overload stimulus initially increases ventricular wall tension, which is thought to activate multiple signaling pathways, a consequence of which is increased intracellular diastolic Ca$_{2+}$. Several recent studies have investigated whether calcineurin activation is required for hypertrophy in response to aortic banding. The results have been confusing.

Sussman et al.$^{105}$ reported that CsA prevented hypertrophy in response to pressure overload in aortic-banded rats and that failure to mount a hypertrophic response resulted in death within 6 days. Others have also observed complete (I. Komuro, personal communication, January 1999) or partial$^{106}$ inhibition of pressure-overload hypertrophy by CsA. However, other studies found that CsA did not block hypertrophy 2 to 6 weeks after banding of the aorta.$^{99-101}$ CsA also failed to prevent left ventricular hypertrophy in spontaneously hypertensive rats.$^{101}$ The basis for these different results is unclear, but it seems likely that differences in banding procedures or drug regimens could account, at least in part, for the different findings. Interpretation of the results of these studies is also confounded by the sensitivity of rats to CsA toxicity, presumably caused by renal dysfunction, which may lead to secondary hypertrophy due to hypertension. Given the multiple signaling systems implicated in hypertrophy, it also seems likely that a stimulus as potent as pressure overload would activate multiple intracellular signaling pathways. An unequivocal test of the role of hypertrophy in pressure-overload hypertrophy will require genetic deletion of components of the signaling pathway, thereby avoiding complications from drug toxicity.

**Current Clinical Treatments for Hypertrophy and Heart Failure**

Countless studies have focused on elucidating the intracellular pathways that mediate cardiac hypertrophy with the goal of identifying drugs to potentially alter these pathways. Cardiac hypertrophy associated with essential hypertension is currently treated with pharmacological antagonists for various cardiac signaling pathways. Angiotensin-converting enzyme (ACE) inhibitors; β-blockers, which block β-adrenergic receptor stimulation; and Ca$_{2+}$ channel blockers, which lower [Ca$_{2+}$]i inotropy, are the most commonly prescribed therapies. Echocardiographic analysis of hypertensive patients treated with ACE inhibitors, β-blockers, or Ca$_{2+}$ channel blockers, showed a 5% to 15% decrease in left ventricular hypertrophy.$^{97,102}$ Although these regimens are successful in prophylactic treatment of heart disease in hypertensive patients, they are not as successful in treating advanced heart failure.$^{103}$

Of the estimated 4 to 5 million individuals in the United States afflicted with heart disease each year,$^{1,2}$ a few hundred thousand are estimated to have advanced heart failure, characterized by a 2-year mortality rate of >90%.$^{103}$ Recent studies demonstrated a 4-fold increase in calcineurin activation in human heart failure samples compared with controls,$^{103a}$ consistent with the possibility that calcineurin may play an important role in heart failure. Current treatment protocols for heart failure include a combination of ACE inhibitors, diuretics, β-blockers, and digoxin.$^{104}$ However, these therapies have shown only limited success.$^{104}$ Inhibiting calcineurin activity in heart failure patients may represent a novel approach toward uncoupling progressive increases in [Ca$_{2+}$i], from maladaptive hypertrophic signaling.

CsA and FK-506 are currently used to prevent allograft tissue rejection after organ transplantation. Because these agents have been used in patients for many years, it is anticipated that positive or negative correlations would have been made with cardiac hypertrophy. However, the clinical data correlating CsA treatment with cardiac function are inconclusive.$^{105}$ It is certainly apparent that heart transplant patients live longer on CsA because of its immunosuppressive actions.$^{106}$ However, only a handful of studies have analyzed ventricular wall thickness or cardiac function in patients receiving calcineurin inhibitors. Two separate studies report left ventricular hypertrophy in pediatric transplant patients receiving FK-506. In one study, 2 of 5 patients receiving FK-506 developed cardiomyopathy, which resolved after switching to CsA.$^{107}$ In another study, 2 liver transplant patients on long-term FK-506 treatment demonstrated cardiac hypertrophy.$^{108}$ In contrast to these case reports, a longitudinal study of 107 heart transplant patients showed a dramatic benefit with CsA treatment.$^{109}$ Patients who received CsA had higher left ventricular ejection fractions and fewer ischemic episodes.

Studies of the potential beneficial effects of CsA on cardiac function in humans are also confounded by renal toxicity associated with chronic treatment with the drug and resulting hypertension leading to cardiac hypertrophy.$^{106}$ Moreover, the immunosuppressive effects of CsA and FK-506 preclude their use as routine inhibitors of hypertrophy. Despite these side effects, an investigation into the usefulness of CsA and FK-506 in treating heart failure should be considered. Ideally, it might be possible to target CsA or FK-505 to the heart or to develop new agents that selectively inhibit cardiac calcineurin activity and thereby bypass adverse effects on the immune system and kidneys. Approaches for cell type–specific drug targeting have recently been developed.$^{110}$ In addition, because these immunosuppressants act by forming complexes with immunophilins in target cells, it is conceivable that cardiac-specific immunophilins might exist that show ligand-binding properties distinct from those that function in the immune system and thereby provide a potential means of selectively targeting calcineurin inhibitors to the heart.

**Potential Control Points in the Calcineurin-NFAT Pathway**

In addition to inhibiting calcineurin activity, the hypertrophic signaling pathway shown in Figure 3 suggests several potential control points that might be targeted pharmacologically. For example, stimulation of NFAT3 phosphorylation represents a potential means of inhibiting the calcineurin signaling pathway, assuming NFAT3 activation is an essential downstream step in the pathway. Once in the nucleus, NFAT proteins can be
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rephosphorylated, resulting in their translocation back to the cytoplasm and termination of the transcriptional signal. Several kinases have been implicated in NFAT phosphorylation and thereby function as negative regulators of calcineurin signaling. Protein kinase A can phosphorylate NFAT proteins in the cytoplasm, preventing their nuclear translocation, whereas glycogen synthase-3 kinase phosphorylates NFAT proteins in the nucleus, resulting in nuclear exit. JNK also phosphorylates the amino terminus of NFAT4, thereby opposing calcineurin signaling. Of note, recent studies showed that JNK blocked activation of ANF transcription in response to hypertrophic signaling, whether this inhibition is mediated by NFAT phosphorylation has not been determined. Casein kinase Iα and the MAPK family member MEKK1 (mitogen-activated protein/ERK kinase-1) also disrupt calcineurin signaling by masking the nuclear localization signal of NFAT4. It might also be possible to design peptide decoys that would block interaction of GATA4 and NFAT3. The attractiveness of this approach is that it could provide cardiac specificity to drug inhibition and potentially avoid complications of immune suppression.

Conclusions

In summary, we view cardiac hypertrophy as a fundamental problem of gene regulation in which prolonged and aberrant activation of Ca2+-dependent intracellular signaling systems results in reprogramming of cardiomyocyte gene expression. The hypertrophic signaling system we have outlined provides a framework for further delineating the detailed molecular mechanisms underlying cardiac hypertrophy and heart failure and suggests several novel drug targets. However, it is clear that other signaling systems can also induce hypertrophy. Defining the cytoplasmic and nuclear components of these alternate pathways and distinguishing which pathways control the various forms of hypertrophy represent important problems for the future. It should now be possible to determine those forms of cardiac disease in which calcineurin plays a role, by assessing sensitivity to calcineurin inhibitors. Given the staggering number of patients suffering from heart failure, if calcineurin inhibitors are effective in only a fraction of these patients, such inhibitors either alone or in conjunction with other agents could have a significant impact on this disease. Resolutions to these issues promise to be fascinating and rapidly forthcoming.

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Prevention of Cardiac Hypertrophy by Calcineurin Inhibition: Hope or Hype?

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