Small-molecule therapies for cardiac hypertrophy: moving beneath the cell surface

Timothy A. McKinsey and David A. Kass‡*

Abstract | Pathological stress from cardiovascular disease stimulates hypertrophy of heart cells, which increases the risk of cardiac morbidity and mortality. Recent evidence has indicated that inhibiting such hypertrophy could be beneficial, encouraging drug discovery and development efforts for agents that could achieve this goal. Most existing therapies that have antihypertrophic effects target outside–in signalling in cardiac cells, but their effectiveness seems limited, and so attention has recently turned to the potential of targeting intracellular signalling pathways. Here, we focus on new developments with small-molecule inhibitors of cardiac hypertrophy, summarizing both agents that have been in or are poised for clinical testing, and pathways that offer further promising potential therapeutic targets.

Sarcomere
The basic functional contractile unit of muscle. It is composed largely of the proteins actin and myosin.

Hearts are exposed to physiological stress from the demands of daily living and exercise, and pathological stress from cardiovascular disease. In this latter case, factors such as pressure and volume load, neurohormones and mutations in genes encoding sarcomeric proteins typically stimulate muscle growth — hypertrophy — which increases the risk of cardiac morbidity and mortality[1,2]. Although hypertrophy has been traditionally viewed as a necessary first response to pathological stress that only later exacerbates disease, which might caution against efforts to directly prevent or reverse it, recent data suggest that hypertrophy in response to pathological stress may never be truly adaptive[1,3,4], and clinical studies support benefits from its inhibition[5,6].

Before considering specific inhibitors of cardiac hypertrophy, it is worth summarizing what one is trying to reverse. The heart is composed of multiple cell types, including muscle cells, fibroblasts, immune cells, extracellular matrix and vascular tissue, and all can contribute to greater wall mass. Hypertrophied hearts perform greater mechanical work, consume more energy and have limited reserve capacity, and the muscle is stiffer, which affects both diastolic and systolic function. Reversing these abnormalities is an aim of antihypertrophy treatments. In addition to suppressing growth and reducing fibrosis, such treatments should ideally also improve cardiac function.

From the standpoint of drug development, several questions should be kept in mind. Does an agent prevent hypertrophy development in hearts exposed to increased load? Can it reverse pre-existing pathological hypertrophy? The latter is less often studied, yet is clinically relevant. Does the agent prevent cardiac dilation, improve intrinsic muscle function, reduce fibrosis and improve cardiac vascular supply/demand balance? Last, does the agent enhance cardiac reserve capacity despite blocking the hypertrophic response? This last question is rarely addressed in animal models, but it is also relevant to clinical medicine.

Current small-molecule cardiovascular drugs that have antihypertrophic activity target outside–in signalling; they block neurohormones (catecholamines, angiotensin, aldosterone); or calcium triggers (L-type Ca^{2+}-channel blockers); or target pathological load (vasodilators and diuretics). However, these vary in effectiveness[7], and load-reduction efficacy depends on the pathway that is targeted. Recent efforts to suppress or reverse hypertrophy have turned to signalling inside the cardiac muscle cell, targeting cascades that ultimately alter gene and protein expression, cell enlargement and chamber remodelling (FIG. 1). These pathways have substantial redundancy, as revealed by persistent inducible hypertrophy in models in which one or another of the pathways is genetically inhibited. Although this poses a challenge to efforts to inhibit hypertrophy therapeutically, there are distal strategic nodes where signals converge and intrinsic pathways that serve as multi-effector brakes, and both of these are attractive targets. Small-molecule screens based on cellular phenotype could also lead to

*Gilead Colorado, Inc., 7575 West 103rd Avenue, Westminster, Colorado 80021, USA.
‡Division of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions, Ross Research Building 835, 720 Rutland Avenue, Baltimore, Maryland 21205, USA.
e-mails: timothy.mckinsey@gilead.com; dkass@jhmi.edu
doi:10.1038/nrd2193
Published online 20 July 2007

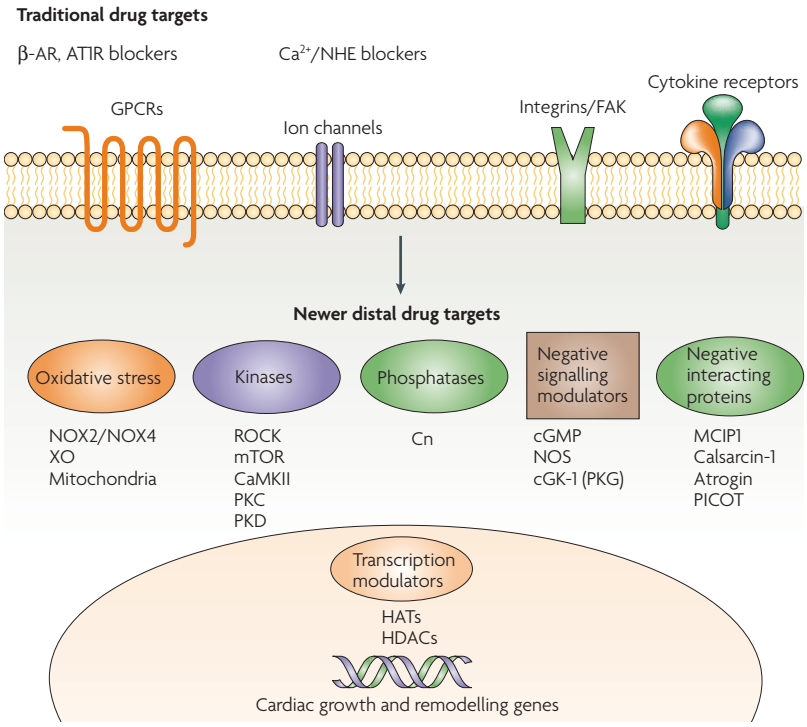


Figure 1 | **Targets for small-molecule drugs that modulate hypertrophic signalling.** Traditional approaches to suppressing hypertrophy have focused on outside–in signalling, targeting G-protein-coupled receptors (GPCRs), ion channels such as L-type Ca^{2+} and Na^+/H^+ exchanger (NHE) blockers, integrins or cytokine receptors. Newer targets include those that influence oxidative stress, regulation of kinases and phosphatases, signalling modulators and interacting proteins. Various examples are shown, many of which are discussed in more detail in the main text. β-AR, β-adrenergic receptor; AT1R, angiotensin II type 1 receptor; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; cGK-1 (PKG) cyclic-GMP dependent kinase -1 (protein kinase G); cGMP, cyclic guanosine monophosphate; Cn, calcineurin; FAK, focal adhesion kinase; HATs, histone acetyltransferases; HDACs, histone deacetylases; MCIP1, modulatory calcineurin-interacting protein 1 (also known as CCP1); mTOR, mammalian target of rapamycin; NOS, nitric oxide synthase; NOX2,4, NADH oxidase 2,4; PICOT, PKC-interacting cousin of thioredoxin; PKC, protein kinase C; PKD, protein kinase D; ROCK, Rho-kinase; XO, xanthine oxidase (also known as XDH).

the identification of agents that affect novel intracellular targets (FIG. 2). In this Review, we focus on recent developments with small-molecule hypertrophy inhibitors. We first present agents that have been in or are poised for clinical testing, and then discuss pathways at earlier development stages.

Rho kinase inhibitors and statins

The Rho family of small GTP-binding proteins act as potent proximal molecular switches. Rho is stimulated by guanine nucleotide exchange factor proteins (GEFs) and is activated by G-protein-coupled receptor (GPCR) agonists, growth-factor receptors, integrins and cytokine receptors[8] (FIG. 3). Once coupled to GTP, activated Rho stimulates downstream effectors, notably Rho-kinase, which has two primary isoforms, ROCK1 (also known as ROCK I, P160ROCK or ROKβ), and ROCK2 (also known as ROCK II or ROKα)[9,10]. ROCK expression is also stimulated by angiotensin and interleukin-1β (IL1β) through a protein kinase C/nuclear factor-κB (PKC/NFκB)-dependent pathway[11]. Knockout of *Rock* isoforms in mice is embryonically lethal[12–14], whereas 50% *Rock1*-knockdown does not blunt pressure-overload hypertrophy, but reduces fibrosis[13].

ROCK has several major targets (FIG. 3), including myosin phosphatase target subunit 1 (MYPT1)[15], which plays an important role in ROCK-mediated vascular smooth-muscle contraction. ROCK-activated MYPT1 inhibits myosin light-chain (MLC) phosphatase, enhancing MLC phosphorylation and augmenting myosin ATPase activity, and thereby the force and velocity of crossbridges[16,17]. Physiological effects include increased vascular tone, cell-stress fibre formation, migration and hypertrophy, and reduced expression of nitric oxide synthase (NOS). Cardiac troponin T is also a ROCK substrate, in which tyrosine phosphorylation reduces tension-Ca^{2+} sensitivity[18]. The phosphatase and tensin homologue (PTEN) is another recently described ROCK substrate[19] that has an important role in the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, and regulates cellular growth, protein synthesis, survival and transcription[20]. PTEN phosphorylation by ROCK reduces PI3K/Akt signalling, whereas ROCK inhibition activates the pathway, perhaps explaining the Akt activation seen in endothelial cells that are treated with ROCK inhibitors[21]. The Na^+/H^+ exchanger, which contributes to hypertrophy in part by increasing intracellular sodium and subsequently calcium via the Na^+/Ca^{2+} exchanger[22], is also activated by ROCK[23,24].

Data supporting the contribution of ROCK to cardiac hypertrophy come largely from pharmacological studies with non-isoform-specific inhibitors[25,26]. Although initially synthesized as calmodulin antagonists, these drugs evolved into protein kinase inhibitors and then into more selective ROCK inhibitors. An early example was HA-1077 (fasudil) (TABLE 1), which was approved in Japan for the treatment of cerebral vasospasm in 1995. Subsequent drug development yielded Y-27632 (REF. 27) (TABLE 1), and together, use of these compounds has provided most of the data that support the antihypertrophic effects of ROCK inhibition. Animal models include angiotensin-II-stimulated hypertrophy in rats[28], apolipoprotein E (*Apoe*)-deficient knockout mice[29], post-infarction models[30] and hypertensive–hypertrophy Dahl salt-sensitive rats[31]. In addition to suppressing hypertrophy[30,31], ROCK inhibitors reduce fibrosis, suppress inflammatory cytokines (transforming growth factor-β2 (TGFβ2) and TGFβ3) and macrophage migration inhibitory factor (MIF)[30], and enhance cardiac function[32]. Antihypertrophic effects occur without significant changes in arterial pressure, supporting primary myocardial effects.

One limitation of existing ROCK inhibitors is their lack of enzymatic specificity. Both fasudil and Y-27632 also inhibit cGMP-dependent protein kinases at an IC_{50} 10–20-times that for ROCK2 (REF. 27). Fasudil also inhibits PKA and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) with an IC_{50} similar to that for PKG, whereas Y-27632 is less active against these kinases[27]. Recent glycyl derivatives of fasudil have yielded more specific ROCK inhibitors that have reduced activity

Myosin
An ATPase that regulates contraction through association with actin.

Crossbridges
A mysosin–actin junction.

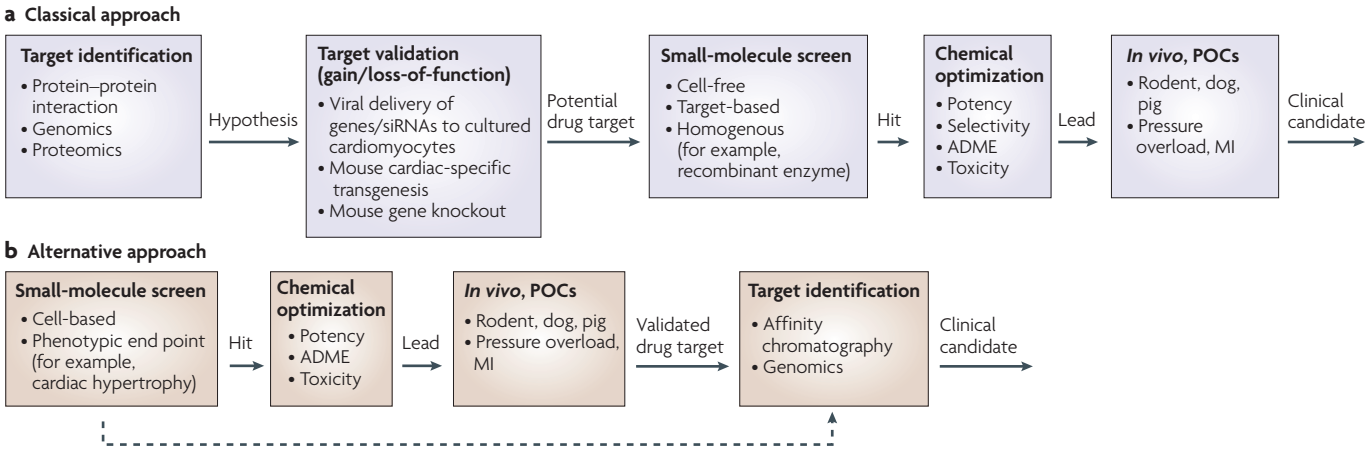


Figure 2 | **Approaches to drug discovery for cardiac hypertrophy. a** | Drug discovery typically involves the identification of potential drug targets and validation of a role for these targets in disease. Validation for cardiac hypertrophy involves gene transduction into cultured cardiomyocytes, production of transgenic and knockout mice, and pharmacological studies. Target discovery and validation often takes many years, thereby delaying the start of small-molecule-screening efforts, chemical optimization of hit compounds and *in vivo* testing to confirm that manipulation of the target will provide therapeutic benefit without unacceptable side effects. **b** | Future drug discovery for cardiac hypertrophy could include an alternative approach, in which the starting point is a 'phenotype-based' high-throughput screen for small-molecule inhibitors of cellular hypertrophy. Screening can be performed in biological systems, such as neonatal rat ventricular myocytes cultured in high-density formats and exposed to stimuli (stretch or hormones) to induce hypertrophy. This unbiased approach will yield classes of compounds that elicit a desired effect (for example, inhibition of pathological cardiac hypertrophy) without prior understanding of the targets of the small molecules. So, small molecules obtained from such screens could be used as tools to identify novel cellular regulators of cardiac hypertrophy that have been recalcitrant to classical discovery efforts. In addition, optimized analogues of hit compounds could be used to validate the target using animal models of pathological cardiac hypertrophy, thereby streamlining efforts to translate preclinical discoveries to clinical testing. It is important to note that chemical optimization is facilitated when the compound target is known, and thus efforts to identify the targets of small molecules will probably be initiated shortly following the demonstration of cellular efficacy and establishment of a structure–activity relationship for given classes of efficacious compounds. ADME, absorption, distribution, metabolism and excretion; MI, myocardial infarction; POC, porcine organ culture.

against PKA and PKC (1×10^3 greater IC_{50}), and $IC_{50} > 1 \times 10^2$ higher for CaMKII and PKG[27]. Additional studies are needed to test their efficacy, and other efforts are aiming to generate isoform-selective inhibitors.

Another way to inhibit Rho/ROCK and upstream Ras signalling is with the widely prescribed HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors, or statins[33–35] (FIG. 3). In addition to blocking cholesterol synthesis, statins impede the activation of Ras by farnesylation (via farnesyl pyrophosphate), and geranylgeranylation of Rho (via geranylgeranyl pyrophosphate)[36,37]. This impairs membrane translocation and activation of both Ras and Rho, leading to inactivity. Simvastatin (TABLE 1) prevents angiotensin-stimulated neonatal myocyte hypertrophy and blocks RhoA and RAC1 GTP binding (RAC1 being linked to oxidative stress)[38]. Its *in vivo* antihypertrophic effect is similar to that of an antioxidant (*N*-acetyl cysteine), suggesting that RAC1 signalling dominates. Transgenic rats overexpressing human renin and angiotensinogen develop hypertrophy, hypertension and fibrosis that can be inhibited by cerivastatin[39] (TABLE 1). Statin benefits have also been reported in the salt-sensitive rat[40], and in rabbits harbouring an R403Q mutation in the myosin heavy chain (MHC), which is a model of genetic hypertrophic cardiomyopathy[41]. The role of Rho, ROCK, and RAC1 in the latter model is unclear, as none was inactivated by the statin. Intriguingly, a retrospective study of patients with heart failure, hypertrophy and normal ejection fraction (diastolic heart failure), found that among common therapies used — beta-blockers, calcium-channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs) and statins — only statin treatment was associated with a survival benefit[42].

mTOR and Akt

Rapamycin (TABLE 1) is a lipophilic macrolide with fungicidal properties that was first isolated from the soil bacterium *Streptomyces hygroscopicus*[43]. Screening for molecules affected by rapamycin led to the purification and cloning of the mammalian target of rapamycin (mTOR) in 1994 (REF. 44). mTOR is a member of the phosphoinositide-kinase-related kinase family, and is essential for cell growth, regulation of mRNA turnover, actin cytoskeletal organization, autophagy, protein stability, transcription and translation[45]. Its regulatory role in gene translation (FIG. 4) is principally effected by phosphorylation of ribosomal S6 kinase (S6K or $p70^{S6K}$) and 4EBP1, a repressor of eukaryotic translation initiation factor 4E (eIF4E)[46,47]. mTOR is activated by growth factors and mechanical and energetic stimuli that converge on the tuberous sclerosis complex (the *TSC1* gene product is hamartin; the *TSC2* product is tuberin) upstream of the small G-protein Rheb[48], which negatively regulates

Actin
A primary thin filament contractile protein in the sarcomere.

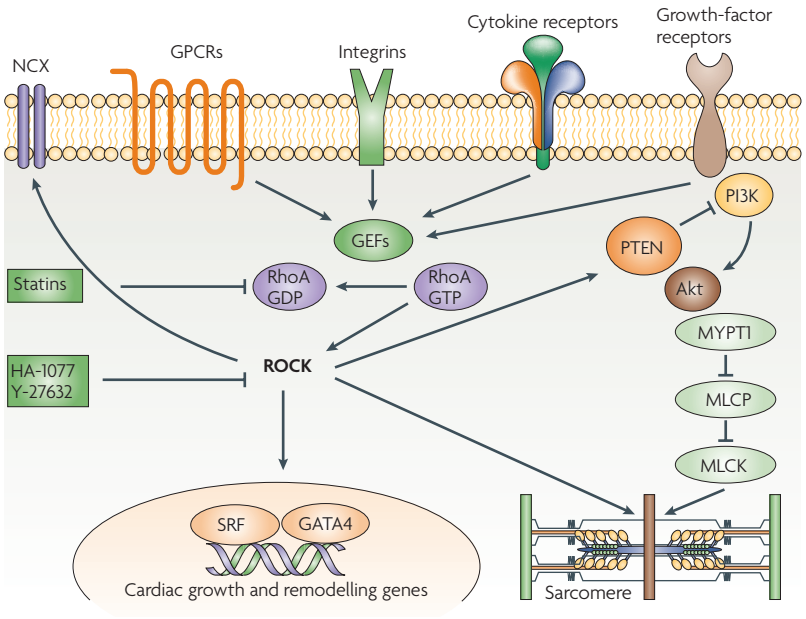


Figure 3 | **Rho/Rho kinase (ROCK) signalling and its potential role in hypertrophy.** ROCK, the principal effector kinase for Rho signalling, can affect membrane ion channels, block protective signalling cascades coupled to Akt, alter sarcomeric proteins and induce gene transcriptional changes. Statins inhibit this pathway by interfering with RhoA activation, whereas inhibitors such as Y-27632 and HA-1077 act more directly on ROCK. GATA4, GATA-binding protein 4; GEF, guanine nucleotide exchange factor; MLCK, myosin light-chain kinase; MLCP, myosin light-chain phosphatase; MYPT1, myosin phosphatases target subunit 1; NCX, Na^+/Ca^{2+} exchanger; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue; SRF, serum response factor.

growth by inhibiting mTOR[46]. Disinhibition occurs when the serine/threonine kinase Akt phosphorylates TSC2, a mechanism for hypertrophic signalling through the PI3K/Akt pathway (for reviews see REFS. 46,47).

mTOR and Akt hypertrophic signalling are thought to be important for physiological hypertrophy that is stimulated by exercise[20,49]. However, pathological hormonal stimulation by phenylephrine or endothelin 1 also activates TSC2 and de-represses mTOR through the small GTP-binding protein Ras and subsequent activation of mitogen-activated protein kinase/ERK kinase (MEK) and extracellular response kinase (ERK)[47]. mTOR is energetically regulated by 5′AMP-activated protein kinase (AMPK), which phosphorylates and activates TSC2. Reduction in AMPK occurs in animals lacking the lipocytokine adiponectin[50], which exacerbates pathological hypertrophy from pressure overload[51].

Rapamycin binds to the 12-kDa immunophilin FK506-binding protein, and the combined rapamycin–FKBP12 complex in turn inhibits mTOR and S6K activation (FIG. 4). Rapamycin reduces hypertrophy in neonatal myocytes[52,53] and in hearts with modest hypertrophy stimulated by pressure overload[54,55]. In both instances, inactivation of $p70^{S6K1}$ seems to be central to the response, which is intriguing as $p70^{S6K1}$ activity peaks early (4 hours of banding), returning to baseline by 24 hours. Another interesting feature is that although hypertrophy is reduced, expression of fetal genes, such as those encoding natriuretic peptides and β-MHC, remains stimulated despite rapamycin treatment[54,55]. Rapamycin was also shown to regress pre-existing cardiac hypertrophy induced by pressure overload[56]. Rapamycin is currently used as an immunosuppressant for transplant rejection, reflecting its broader impact on other cell systems. Improving cardiac targeting and/or modifying cell sensitivity will probably be important to its future use for cardiac hypertrophy.

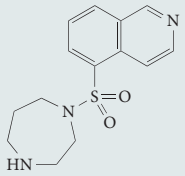
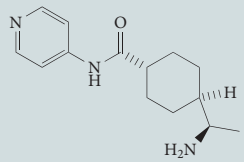
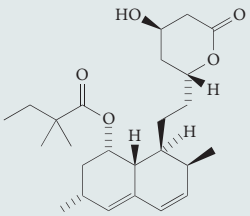
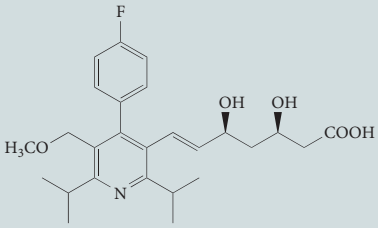
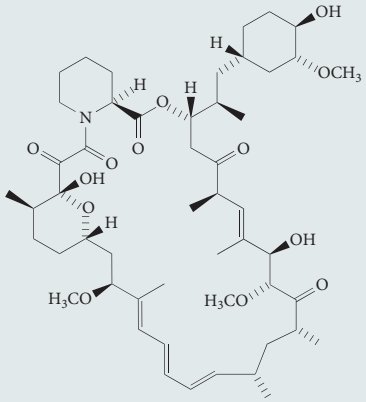
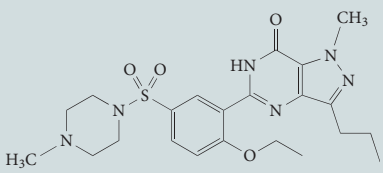
The PI3K/Akt pathway modulates hypertrophy through other pathways besides mTOR. Hearts that lack Class I_A PI3K are reduced in size and show blunted hypertrophic response to physiological stimuli such as swimming[49], whereas mice that lack PTEN, an inhibitor of PI3K-stimulated signalling, have greater basal and stimulated hypertrophy[57]. PI3K activates Akt, which in turn phosphorylates glycogen synthase kinase 3β (GSK3β) (FIG. 4), another suppressor of hypertrophy that is negatively regulated by Akt (reviewed in REF. 58). GSK3β phosphorylates the transcriptional regulator myocardin[59], an important mechanism of its hypertrophy suppression. Although Akt is thought to regulate physiological hypertrophy[60], sustained or marked activation alters expression profiling[61] to that typical of pathological hypertrophy and failure[62,63]. Akt intracellular localization may also determine its impact, with nuclear translocation providing anti-apoptotic[64] and antihypertrophic effects[65], while improving myocyte function[66]. Enhancing the right type of Akt stimulation or preventing the wrong type remain intriguing approaches, although small-molecule approaches may be difficult owing to the pleiomorphic effects and ubiquitous expression of Akt.

Oxidative stress

Myocardial oxidative stress is generated by various enzymes, including NADPH oxidases (NOXs), xanthine oxidase (XO), mitochondrial electron transport chain complexes, and NOS[67] (FIG. 5). Oxidase-generated radicals are normally maintained at physiological levels by enzymatic antioxidants, including superoxide dismutatase (SOD), which converts O_2^- to H_2O_2; catalase and glutathione peroxidases, which convert reduced glutathione (GSH) and H_2O_2 to H_2O and oxidized glutathione (GSSG); and glutathione reductase (GR), which reduces GSSG back to GSH. Antioxidant systems centrally involve thiol-disulphide oxidoreductase systems such as the cytosolic proteins thioredoxin (TRX) and glutaredoxin (GRX), each containing a signature -Cys-X-X-Cys- motif at their active sites. TRX operates with NADPH and thioredoxin reductase (TRXR) to catalyse the reduction of intramolecular or intermolecular protein disulphides[68,69], whereas GRX catalyses the reduction of protein-mixed disulphides in a system coupled with GSH and GR.

Growing evidence supports a role for pathological superoxide generation in cardiac hypertrophy[38,70–74] and chamber dilation and dysfunction[75,76]. Reactive oxygen species (ROS) modulate transcriptional regulation[77] and modify proteins to influence signal transduction (FIG. 5). For example, mechanical strain of myocytes stimulates ROS activation through the post-translational activation

Table 1 | **Compounds that have antihypertrophic activity *in vivo****

Target	Compound	Structure	Model	Inhibition of hypertrophy	Inhibition of fibrosis	Improved LV function	Refs
ROCK	HA-1077 (Fasudil)		Ang II infusion (4 weeks)	Yes	n/a	n/a	28
			Ang II infusion in *Apoe*-knockout mice (4 weeks)	Yes	Yes	Yes	29
			Mouse MI (4 weeks)	Yes	Yes	Yes	30
ROCK	Y-27632		Dahl S rat (7 weeks)	Yes	No	Yes	31
			Dahl S rat (7 weeks)	Yes	Yes	Yes	32
HMG-CoA reductase/ Rho/Rac	Simvastatin		Rabbit Tg β-MHC (Q403) (12 Weeks)	Yes	Yes	Yes	41
HMG-CoA reductase/ Rho/Rac	Cerivastatin		Rat Tg Renin/Ang (3 weeks)	Yes	Yes	n/a	39
			Dahl S rat (11 weeks)	Yes	Yes	Yes	40
mTOR	Rapamycin		Mouse TAB (1 week)	Yes	n/a	No‡	54
			Mouse TAB (2 weeks; compound added at 1 week)	Yes	n/a	Yes	56
			Rat TAB (3 days)	Yes	n/a	n/a	55
PDE5	Sildenafil		Mouse TAB (3 and 9 weeks)	Yes	Yes	Yes	148

*Continued in TABLE 2. ‡Indicates no improved LV function or no blockade of fibrosis and so on. Ang, angiotensin; Apoe, apolipoprotein E; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LV, left-ventricular; MI, myocardial infarction; β-MHC, β-myosin heavy chain; mTOR, mammalian target of rapamycin; n/a, data not available; PDE5, phosphodiesterase-5; ROCK, Rho kinase; TAB, thoracic aortic banding; Tg, transgenic.

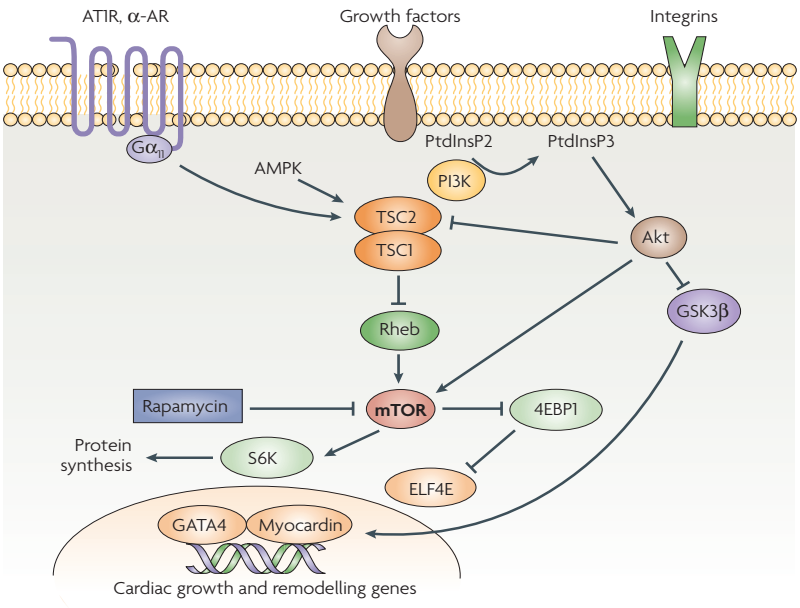


Figure 4 | **mTOR signalling and its role in cardiac hypertrophy.** Proximal activators include G-protein-coupled receptors (GPCRs), growth factors and integrin signalling, as well as metabolic stimulation (AMP kinase). Rapamycin acts distally in this cascade by inhibiting mammalian target of rapamycin (mTOR). α-AR, α-adrenergic receptor; AMPK, 5′-AMP activated protein kinase; 4EBP1, eIF4E-binding protein 1; ELF4E, eukaryotic translation initiation factor 4E; GATA4, GATA-binding protein 4; GSK3β, glycogen synthase kinase 3β; PI3K, phosphatidylinositol 3-kinase; PtdInsP2, phosphatidylinositol bisphosphate; PtdInsP3, phosphatidylinositol-3,4,5-trisphosphate; Rheb, Ras homologue enriched in brain; S6K, ribosomal S6 kinase; TSC1,2, tuberous sclerosis complex 1,2.

of the GTP-binding protein RAS, activating the RAF/MEK/ERK pathway[78]. Genetic inhibition of antioxidant proteins such as TRX increases hypertrophy and the hypertrophic response to pressure load[79]. Oxidant stress activates metalloproteinases, which in turn contribute to matrix remodelling in various forms of cardiomyopathy[67]. Enhancing antioxidant proteins *in vitro* is antihypertrophic[73,80,81], although *in vivo* demonstration of this remains scant. Oxidative stress can also be blunted by inhibiting oxidase-generating species, enhancing intrinsic antioxidants or with broad reducing agents.

The primary enzyme involved with uric acid formation is XO, and selective XO inhibitors such as allopurinol (active metabolite being oxypurinol) are clinically used to treat gout, a hyperuricaemic syndrome affecting the joints and kidney. XO may have an important role in cardiac failure[82–85], although its involvement with hypertrophy remains unclear. Allopurinol alters the redox balance in failing and post-infarct ventricles[82,85] and can improve cardiac function while limiting chamber remodelling[84–87]. Small unblinded studies have found positive[88] or negligible[83] effects on heart function, whereas larger placebo-controlled trials have reported effects on lowering plasma uric acid, but no benefit on clinical end-points of symptoms or exercise capacity[89].

NOXs have roles in normal oxidant cell signalling[90], but are stimulated in pressure-overload hypertrophy and heart failure[91–95]. Genetic models that have deleted isoforms of NOX such as gp91^{phox} (*NOX2*) found little role for the enzyme in hypertrophy in response to pressure load[95,96], but some influence on myocardial function and fibrosis. Mice lacking NOX2 have blunted angiotensin-stimulated hypertrophy[95,97]. At present, pharmacologically viable small-molecule NADPH inhibitors are lacking.

NOS is a homodimeric oxidoreductase with a flavin-containing reductase domain and a haem-containing oxygenase domain connected by a regulatory calmodulin-binding domain. Binding of Ca^{2+}/calmodulin orientates the other domains to allow NADPH-derived electrons generated in the reductase domain to flow to the oxygenase domain[98] to convert L-arginine to nitric oxide (NO) and L-citrulline. This occurs if tetrahydrobiopterin (BH_4) is bound in the dimer interface[99,100], which then interacts with residues from both monomers to stabilize NOS dimerization and enhance NO generation. Functional NOS uncoupling occurs if the zinc core or BH_4 become oxidized, or levels of BH_4 or arginine substrate decline, which is observed in cardiac hypertrophy[101]. The likely result of this is the malrotation of the oxidase domains leading to the molecular uncoupling[101,102] and functional uncoupling of catalytic activity enhancing O_2^- or H_2O_2 formation. ROS generation by uncoupled NOS may have a role in hypertension[103], heart failure[104] and cardiac hypertrophic remodelling and dysfunction[101]. Diabetic vasculopathy can be prevented by upregulating the primary synthetic enzyme for BH_4, GTP-cyclohydrolase[102,105]. BH_4 is being examined as a potential treatment for hypertension and vascular disease[105–107], and may have a role in inhibiting and/or reversing cardiac hypertrophy. New synthetic molecules may provide cost-effective methods to use BH_4 as a drug, and clinical studies are currently underway.

Small-molecule activators of intrinsic antioxidants include Tempol, a membrane-permeant SOD mimetic. However, in models such as hyperthyroid-induced or catecholamine-induced hypertrophy, in which ROS activation is clearly present in the heart, Tempol did not blunt hypertrophy[108,109]. Ebselen is a glutathione peroxidase mimetic that improves ischaemia reperfusion injury[110], although antihypertrophic effects have not yet been reported. Antioxidants such as vitamin E suppress neonatal myocyte hypertrophy[71], but *in vivo* data demonstrating effectiveness remain lacking. More potent antioxidants derived from red grapes and natural polyphenols such as resveratrol and its analogues (isorhapontigenin[111]) blunt cellular and chamber hypertrophy in conjunction with reducing mitogen-activated protein kinase (MAPK), Akt/GSK3β, and S6K activation. However, it should be noted that broad antioxidant treatment trials (vitamins C, E and so on) in cardiovascular disease have been disappointing[112]. Nevertheless, the pursuit of more potent and perhaps better targeted treatments holds considerable promise.

cGMP and PKG: exploiting an intrinsic brake

Cyclic guanosine monophosphate (cGMP) and its primary effector kinase, PKG (or cGK), have important roles in acute and chronic cardiac regulation[113–115]. cGMP has been thought to exert a yin/yang-like balancing of cAMP-stimulated responses[116–118] (FIG. 6), although

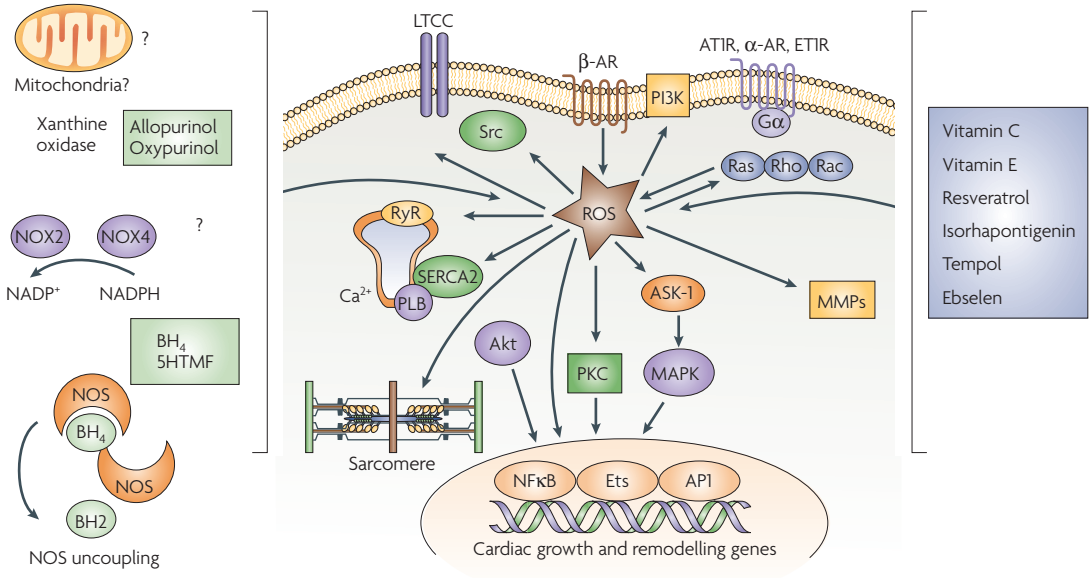


Figure 5 | **Reactive oxygen species (ROS) signalling in hypertrophy.** ROS-generating systems are shown on the left and include xanthine oxidase, NADPH oxidases (NOX2, NOX4), nitric oxide synthase (NOS) and mitochondrial complexes. ROS activation has protean effects on calcium handling, myofilament function, matrix activation, kinase and phosphatase stimulation, and transcriptional regulation. Small-molecule interference can target specific ROS generating systems (left), or more broadly affect ROS once generated by acting as scavengers (shown on right). 5HTMF, 5-hydrotetramethylfolate; α/β-AR, α/β-adrenergic receptor; ASK1, apoptosis signal-regulating kinase 1 (similar to MEKK5/MAP3K5); AT1R, angiotensin II type 1 receptor; BH_4, tetrahydrobiopterin; ET1R, endothelin 1 receptor (also known as EDNRA); Ets, E26 transformation-specific sequence transcription factor; LTCC, L-type calcium channel; MAPK, mitogen-activated protein kinase; MMPs, metalloproteinases; NFκB, nuclear factor-κB; PKC, protein kinase C; PI3K, phosphatidylinositol 3 kinase; PLB, phospholamban; RyR, ryanodine receptor; SERCA2, sarcoplasmic reticular ATPase 2.

cAMP-independent signal modification also occurs. Importantly, clinical agents exist that enhance the genesis (for example, NO donors, natriuretic peptides (NPs) and nitrates) or block hydrolysis (phosphodiesterase 5 (PDE5) inhibitors) of cGMP. Recent studies highlight the complex regulation of the heart by cGMP/PKG interacting at multiple nodes in the hypertrophy cascade[113,115]; generally showing little influence on rest function, but a greater impact when hearts are acutely or chronically stressed, much like an automotive brake.

Myocyte cGMP is generated by membrane-receptor-coupled guanylate cyclase (GCA, also rGC) or by soluble NO-stimulated guanylate cyclase (sGC). Binding of NP (atrial NP (ANP) or brain (BNP)) to the extracellular domain of the NP receptor (NPRA) de-represses the intracellular kinase homology domain to activate GCA[119,120]. Cardiac myocytes have both NPRA and NPRB receptors, the latter being stimulated by ANP and C-type NP (CNP)[121]. sGC activation principally results from NO binding to its reduced haem moiety, enhancing the conversion of GTP to cGMP. Once formed, cGMP can affect function by feedback on cAMP and stimulation of PKG.

PKG phosphorylates proteins regulating ion channels, excitation–contraction coupling (troponin I[122], phospholamban[123,126]) and growth and survival signalling (for example, RhoA[125,126]) (FIG. 6). In vascular muscle, PKG stimulates MLC phosphatase (MLCP), leading to reduced myosin force generation and thus vasorelaxation. MLCP exists in myocytes, although its role in hypertrophy is unclear. PKG also interacts with proteins such as regulator of G-coupled signalling (RGS2) through a leucine-zipper motif[127]. The combined protein complex, with activated RGS2, migrates to the membrane, where it can induce the re-assembly of the $G\alpha_q$ complex, suppressing receptor-coupled signalling via this pathway. This may have an important role in arterial proliferation and hypertension[127], and recent studies suggest a similar role in myocyte hypertrophy[128,129]. PKG also signals to mitochondria, resulting in cytoprotection to ischaemia/reperfusion injury through a PKC-dependent pathway[130,131] and mitochondrial biogenesis[132]. This may blunt oxidant stress and stimulate energy production to meet stress demands.

Most studies showing antihypertrophic effects of cGMP/PKG *in vivo* have focused on NP/NPRA-coupled stimulation pathways. Mice that lack GCA in cardiac myocytes develop greater left-ventricular mass at rest and enhanced hypertrophy in response to stress despite a slightly lower arterial pressure[133]. Mice that lack BNP develop fibrosis, but little left-ventricular hypertrophy[134]. NP, NO and cGMP inhibit cardiomyocyte hypertrophy *in vitro*[114,135]. NP has been clinically available since 2001, when nesiritide (Natrecor; Scios) was approved for treating decompensated heart failure. However, this synthetic peptide requires intravenous infusion, and effects on central vascular pressures and arterial pressure are sufficiently large that targeting hypertrophic growth is difficult. Recent studies have raised concerns of renal dysfunction with this therapy, which may relate

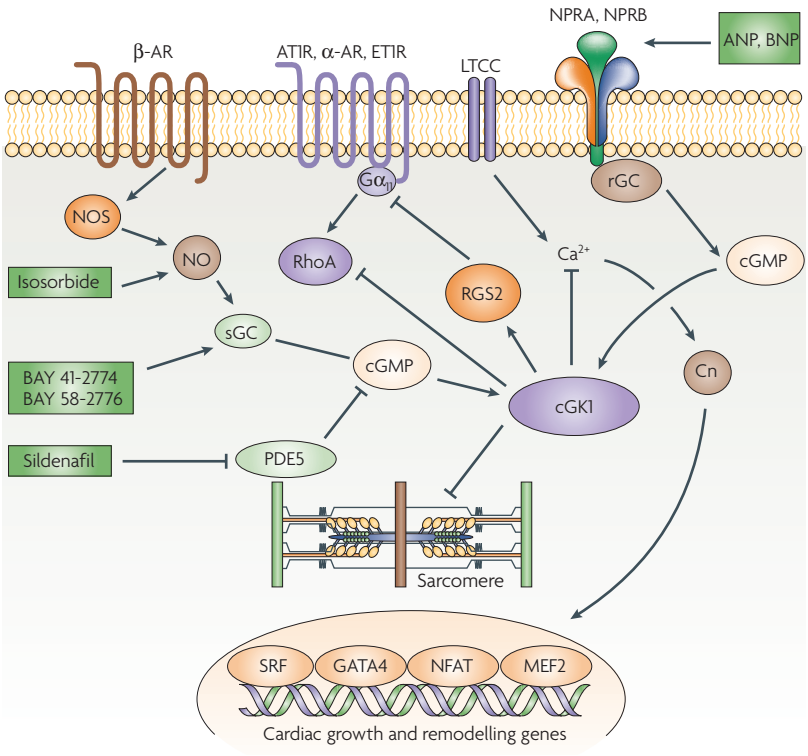


Figure 6 | **Small-molecule regulators of the cGMP/cGK1 (PKG1) pathway.** The cGMP/cGK1 system acts like an internal brake in the cardiac myocyte to suppress stress-stimulated signalling. Much of this action is thought to be mediated by cGMP-targeted kinase (cGK1) also known as protein kinase G (PKG1), which can suppress $G\alpha_q$ stimulation by interaction with regulator of G-protein-coupled signalling 2 (RGS2), inhibit calcium stimulation of calcineurin and RhoA, modify sarcomeric function, and influence mitochondrial function and gene transcription. cGMP synthesis is regulated by nitric oxide synthase (NOS) and by natriuretic peptides (ANP, BNP) working through two different isoforms of soluble guanylate cyclase (rGC and sGC). Available small-molecule stimulators of this process are forms of the NPs, NO-generating compounds such as isosorbide dinitrate, and direct activators of sGC (BAY 58-2776 or BAY 41-2774). cGMP can also be increased by blocking selective phosphodiesterases (PDEs) such as PDE5a (for example, with sildenafil). α/β-AR, α/β-adrenergic receptor; AT1R, angiotensin II type 1 receptor; Cn, calcineurin; ET1R, endothelin 1 receptor (also known as EDNRA); GATA4, GATA-protein binding 4; LTCC, L-type calcium channel; MEF2, myocyte enhancer factor 2; NFAT, nuclear factor of activated T cells; NPRA/B, natriuretic peptide receptor type A/B; SRF, serum response factor.

to vasodilative effects[136]. Oral NP has been developed by alkylPEGylation, in which short monodispersed amphiphilic oligomers are covalently attached to specific sites to alter BNP hydrophilicity and hydrophobicity, protecting it from stomach digestion[137]. cGMP can also be increased by providing NO donors such as isosorbide dinitrate (FIG. 6), and more recently by directly stimulating sGC (for example, BAY 58-2776, and 41-2772)[138,139]. Novel agents for sGC activation have been developed that work in the presence of an oxidized central haem, making them more effective in pathological but not physiological environments[138]. They are, therefore, more potent as antihypertensives in disease models[139], but their effect on hypertrophy remains to be determined.

Last, cGMP/PKG signalling can be enhanced by inhibiting cGMP hydrolysis with targeted PDE inhibitors[140]. In human myocytes, PDE1, 2, 3, 5 and 9 are expressed, with PDE5 and PDE9 being specific for cGMP[141]. PDE1 and PDE2 are dual-substrate enzymes. The role of PDE1 in myocytes remains largely unknown, whereas PDE2 seems to regulate NP-generated cGMP in myocytes[142], and can also hydrolyse cAMP when stimulated by cGMP to blunt contractility[118]. The best known cGMP-esterase is PDE5, the inhibition of which by drugs such as sildenafil (Viagra; Pfizer) (TABLE 1) is a main treatment of erectile dysfunction and more recently pulmonary hypertension[143,144]. PDE5 seems to be localized to z bands in cardiac myocytes, where it may regulate a localized compartment with particular activity on acute and chronic cardiac stress response[145,146]. Acute PDE5 inhibition inhibits β-adrenergic-stimulated contractility in mice[146], dogs[145] and humans[147], and is cardioprotective against ischaemia-reperfusion injury and apoptosis[131]. Chronic PDE5 inhibition prevents and can reverse cardiac hypertrophy, fibrosis and contractile dysfunction induced by pressure overload[148]. These data have led to the initiation of a recent clinical trial to determine the efficacy of PDE5 inhibition — using the PDE5 inhibitor tadalafil (Cialis; Eli Lilly) — on hypertensive cardiac hypertrophy.

Calcineurin

Calcineurin is a ubiquitously expressed serine/threonine phosphatase that exists as a heterodimer containing a catalytic (A) and regulatory (B) subunit. In response to abnormally increased intracellular Ca^{2+}, Ca^{2+}/calmodulin complexes bind to the B subunit, inducing a conformational change that frees the enzyme to dephosphorylate downstream substrates[149] — notably members of the nuclear factor of activated T cells (NFAT) transcription factor family[150]. On dephosphorylation, NFAT translocates to the nucleus, where it binds DNA and activates transcription by intrinsic transactivation domains and combinatorial interactions with other transcription factors, including GATA-binding protein 4 (GATA4) and myocyte enhancer factor 2 (MEF2). Calcineurin was first reported to regulate pathological cardiac hypertrophy by Molkentin and colleagues in 1998 (REF. 151), and subsequent genetic manipulations of the calcineurin/NFAT axis have confirmed this role[152–157].

Pharmacological inhibition of calcineurin effects can be achieved by suppressing calcineurin directly, or by targeting its modulating proteins or downstream effectors. Calcineurin inhibition by cyclosporine A and FK506 — both immunosuppressants that are highly specific for calcineurin when complexed with their endogenous cytosolic receptors (cyclophilin and FKBP, respectively) — was first tested for antihypertrophic properties *in vivo* in rodents. However, these compounds had equivocal effects, probably due to the pleiomorphic functions of calcineurin and the complex actions of the inhibitors[158,159].

Rather than directly targeting calcineurin, efforts have turned to stimulating the expression and/or activity of endogenous calcineurin inhibitors, among which three are enriched in muscle: modulatory calcineurin-interacting protein (MCIP1; also known as DSCR1), calsarcin (also known as myozenin) and atrogin, a muscle atrophy F-box protein (FIG. 7). MCIP1 blocks calcineurin by its high-affinity binding to the catalytic site[160].

Z bands
Delimit the sarcomere.

Transgenic overexpression of MCIP1 in the mouse heart inhibits pathological remodelling from pressure overload[154] and infarction[155], without promoting cardiac decompensation.

These results suggest that small molecules that increase cardiac MCIP1 levels will block pathological hypertrophy. A cell-based, high-throughput screen monitoring a luciferase reporter gene under the control of the *MCIP1* gene promoter identified a novel series of 4-aminopyridine-containing compounds that resemble serotonin (5-hydroxytryptamine, 5-HT). The compounds activate MCIP1 expression by binding to and agonism of the 5-HT_{2B} receptor[161]. However, this compound series, referred to as pyridine activator of myocyte hypertrophy (PAMH) (FIG. 7), promoted *MCIP1* gene expression through the activation of calcineurin/NFAT signalling, stimulating rather than inhibiting hypertrophy. A modified screen for compounds that post-translationally enhance MCIP1-protein expression yielded a novel class of benzothiophenone-containing molecules that stabilize a calcineurin-independent form of MCIP1 (REF. 162) (FIG. 7). These compounds block agonist-dependent hypertrophy of cardiac myocytes in a dose-dependent manner, suggesting promise for this approach.

The net impact of MCIP1, however, may depend on its phosphorylation status. For example, phosphorylation of MCIP by GSK3β in yeast promotes calcineurin signalling[163], which is paradoxical given the ability of this kinase to inhibit calcineurin-mediated cardiac hypertrophy in mice[164]. MEKK3, an MAPK, stimulates calcineurin in response to angiotensin II by triggering MCIP1 phosphorylation[165], resulting in the release of MCIP1 from the calcineurin complex.

Calsarcins are muscle-enriched calcineurin inhibitory proteins. Expression of calsarcin 1 is specific for heart and skeletal muscle, whereas calsarcin 2 and calsarcin 3 are only expressed in skeletal muscle[166,167]. In cardiomyocytes, calsarcin 1 localizes to sarcomere z-disks through the physical association with α-actinin, tethering a pool of calcineurin to the contractile apparatus. Genetic deletion of calsarcin 1 exaggerates calcineurin signalling and exacerbates pressure-overload hypertrophy[168], but has no effect on physiological hypertrophy caused by exercise. Compounds that stabilize calsarcin–calcineurin interactions may prove useful for inhibition of pathological cardiac hypertrophy.

A third muscle-enriched calcineurin inhibitor is atrogin[169], which functions as an adaptor that couples calcineurin to the SCF ubiquitin ligase complex, thereby targeting the phosphatase for degradation by the proteasome. Ectopic overexpression of atrogin in the mouse heart blunts calcineurin signalling and associated cardiac hypertrophy in response to pressure overload[170]. The *atrogin* gene promoter is stimulated by the FOXO transcription factor[171], and adenoviral gene transfer of FOXO to cultured cardiac myocytes[172,173] or adult mouse hearts[173] results in atrogin-dependent blockade of calcineurin signalling and cardiac hypertrophy. A high-throughput screen identified small molecules that enhance FOXO activity by blocking its nuclear export[174]; these compounds warrant testing in models of cardiac hypertrophy.

MCIP1, calsarcin and atrogin are non-classical drug targets for which the desired pharmacological effect is stimulatory rather than inhibitory. Nonetheless, they remain intriguing because of their enriched expression in the heart, and they suggest possibilities for small-molecule treatment of cardiac hypertrophy without side effects associated with calcineurin inhibition in other tissues (for example, immunosuppression and nephrotoxicity).

Last, calcineurin signalling may be blocked by targeting the interaction of the phosphatase with specific downstream substrates. A cell-permeable peptide inhibitor of calcineurin–NFAT interaction was recently shown to block hypertrophy in response to pressure overload in rats[175]. Such protein–protein interactions are historically difficult to target with small molecules, yet recent high-throughput screens have uncovered compounds that block the association of calcineurin with NFAT[176]. These newly discovered small molecules — INCAs (inhibitors of NFAT–calcineurin association) — bind covalently and non-covalently to a cysteine residue adjacent to the calcineurin docking site on NFAT[176,177] (FIG. 7). Future studies will test if these allosteric inhibitors solely modify NFAT or also alter cysteines in other proteins.

TRPC channels

Canonical transient receptor potential (TRPC) channels are a family of membrane-spanning, non-selective cation channels that mediate non-voltage-gated influx of Ca^{2+} in response to GPCR signalling, receptor tyrosine kinase signalling and depletion of internal Ca^{2+} stores[178,179]. Cardiac TRPC expression rises in rodent models of pathological cardiac hypertrophy and in humans with heart failure[180]. Intriguingly, downregulation of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump expression, a hallmark of pathological hypertrophy that impairs Ca^{2+} reuptake into the sarcoplasmic reticulum, is associated with the upregulation of TRPC-channel expression and calcineurin activation[181].

Pathological stimuli cause phospholipase (PLC) activation and Ca^{2+} store depletion, potentiating TRPC activity and producing a Ca^{2+} signal that activates calcineurin/NFAT signalling. Overexpression of the TRPC3 isoform in cultured cardiomyocytes promotes pathological cardiac hypertrophy by the superactivation of calcineurin signalling[180], whereas RNAi-mediated knockdown of endogenous TRPC3 or TRPC6 blunts agonist-dependent growth of these cells[182]. Transgenic overexpression of TRPC3 (REF. 183) or TRPC6 (REF. 184) in the adult mouse heart promotes pathological cardiac hypertrophy in association with exacerbated calcineurin signalling. As TRPC channels are mechanosensitive[185], abnormal mechanical stress in hypertrophied or failing hearts might activate them directly. Among the genes modulated by the calcineurin/NFAT pathway are TRPCs themselves[180,184,186], completing a positive feedback circuit that stabilizes a state of hypertrophic gene expression.

It remains to be determined whether existing TRPC inhibitors or future derivatives that have greater selectivity for TRPC channels will block pathological cardiac hypertrophy *in vivo*. TRPC inhibition has historically relied on the use of 2-aminoethoxydiphenyborane (2-APB)[187],

which also targets TRPV and TRPM channels. Recent high-throughput screens for compounds that suppress the activation of the NFAT-responsive *IL2* promoter identified a new series of 3,5-bistrifluoromethyl pyrazole (BTP) inhibitors[188,189] (FIG. 7), which were subsequently shown to function as TRPC antagonists[190,191]. An alternative high-throughput screen for compounds that block NFAT nuclear translocation also yielded TRPC inhibitors[192], suggesting an intriguing future for this approach.

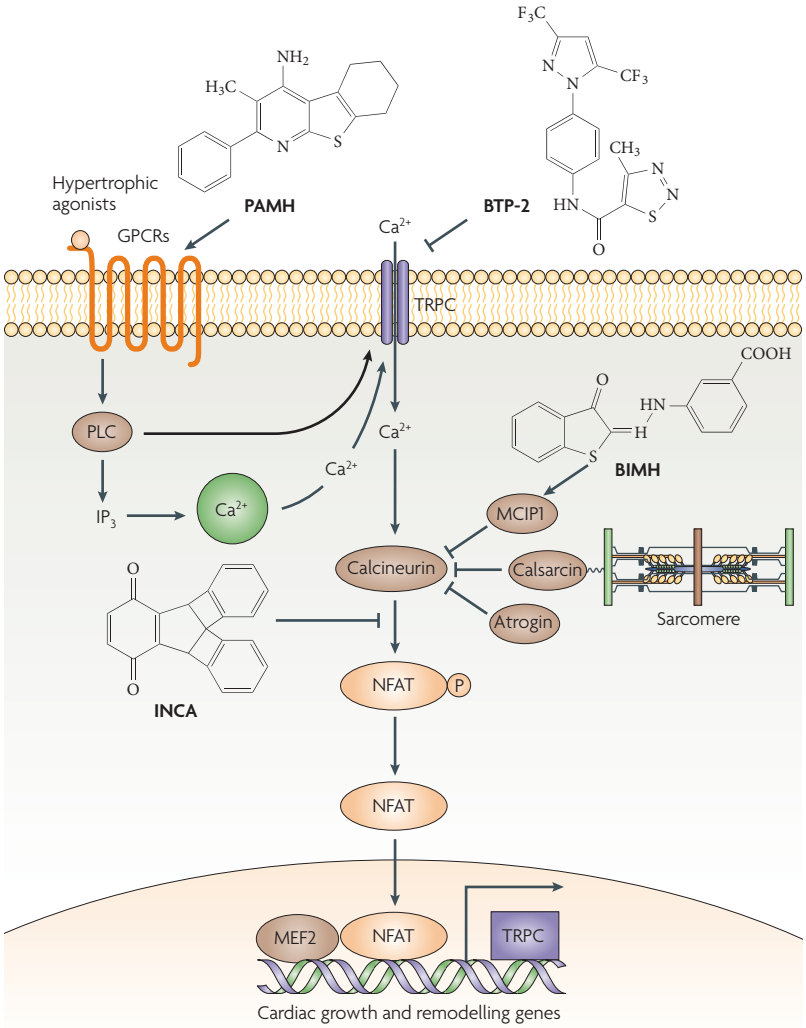


Figure 7 | **Small-molecule regulators of the cardiac calcineurin axis.** On dephosphorylation by Ca^{2+}-dependent calcineurin, the nuclear factor of activated T cells (NFAT) transcription factor translocates to the nucleus, where it regulates pathological cardiac gene expression through interactions with other transcription factors, including myocyte enhancer factor 2 (MEF2) and GATA-binding protein 4 (GATA4). The pool of Ca^{2+} derived from TRPC (canonical transient receptor potential) ion channels contributes to calcineurin activation. Stress signals, such as those emanating from G-protein-coupled receptors (GPCRs), trigger phospholipase C (PLC) activation and subsequent formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 promotes the release of Ca^{2+} from internal stores, which potentiates TRPC channel activity. PLC is capable of activating TRPC channels by direct binding to the channel and indirectly via DAG, which also associates with TRPC channels. Endogenous inhibitors of calcineurin include modulatory calcineurin-interacting protein 1 (MCIP1; also known as DSCR1); calsarcin, which is tethered to the sarcomere; and atrogin. Small-molecule regulators of the calcineurin–NFAT pathway include pyridine activator of myocyte hypertrophy (PAMH), which stimulates calcineurin through 5-hydroxytryptamine (serotonin) 2B ($5\text{-}HT_{2B}$) receptors; 3,5-bistrifluoromethyl pyrazole 2 (BTP-2), which inhibits TRPC channels; benzothiophenone inhibitor of myocyte hypertrophy (BIMH), which blocks calcineurin signalling by stimulating expression of MCIP; and inhibitor of NFAT-calcineurin association (INCA), which binds directly to NFAT. P, phosphate.

Ca^{2+}/calmodulin-dependent protein kinases

Another Ca^{2+}-dependent signalling mechanism that promotes pathological cardiac hypertrophy involves the CaMKs. Six distinct genes encode members of the CaMK family: *CaMKI*, *CaMKIIα*, *β*, *γ*, *δ* and *CaMKIV*, among which CaMKIIγ and CAMKIIδ are expressed at significant levels in the heart[193]. CaMKII forms homo- and heteromeric complexes consisting of 6–12 subunits that assemble into a structure resembling a bicycle wheel with spokes. CaMKII exists in an inactive state by virtue of a 16-amino-acid autoinhibitory domain, which binds to the active site of the enzyme and sterically precludes its association with ATP and protein substrates. Ca^{2+}/calmodulin complexes bind to and dislodge the autoinhibitory domain, permitting subsequent autophosphorylation of this domain, which locks the enzyme in an active, Ca^{2+}-independent state[194,195].

In the late 1990s and early 2000s, studies with transgenic mice and chemical inhibitors of CaMKs started to suggest a role of CaMKs in the control of cardiac hypertrophy[196–200]. CaMK activity was also found to be elevated in animal models of pathological hypertrophy[199,201–205], and in human end-stage failing hearts[206]. CaMKIIδ is the most abundant isoform in the heart, and its mRNA transcript is alternatively spliced to produce distinct isoforms, including δ_B and δ_C, which localize to the nucleus and cytoplasm, respectively. Cardiac-specific overexpression of $CaMKII\delta_B$ (REF. 207) or $CAMKII\delta_C$ (REF. 208) is sufficient to trigger pathological hypertrophy and cardiac failure. Cardiac-specific, pan-CaMKII inhibition was achieved in mice through the overexpression of the autoinhibitory domain, and is well tolerated[209]. Such mice are resistant to hypertrophy from β-AR stimulation and have reduced heart dilation and dysfunction following myocardial infarction (MI). CaMKII inhibition also blocked cardiac myocyte death in these models[210,211].

Downstream effectors of CaMKII include those controlling cardiac Ca^{2+} handling[212]. For example, CaMKII phosphorylates L-type Ca^{2+} channels[213], increasing Ca^{2+} entry into myocytes; the ryanodine receptor (RyR)[214], promoting Ca^{2+} release from the sarcoplasmic reticulum[215,216]; and phospholamban (PLB)[217], causing derepression of the SERCA pump and enhanced Ca^{2+} uptake by the sarcoplamsic reticulum. The net effect is increased calcium release and uptake, often with a net rise in Ca^{2+} that impairs contractility[218] and is arrhythmogenic[219–221]. CaMKII signalling also promotes the phosphorylation of transcriptional regulators that govern expression of pro-growth genes[222,223].

Pharmacological inhibition of CaMKs has relied on KN62 and KN93 (TABLE 2), both isoquinolone-sulphonamides that bind to the calmodulin docking site on CaMK, thereby preventing the activation of CaMKs by Ca^{2+}/calmodulin. KN93 was shown to block post-MI cardiac

remodelling in mice[209]. However, whether inhibitors that suppress the CaMKII activation step are efficacious for the treatment of chronic hypertrophy and failure, in which drugs need to block autophosphorylated Ca^{2+}-independent CaMKII to reverse disease, remains unclear. An alternative approach is the development of an ATP-competitive small-molecule inhibitor of the CaMKII catalytic domain[224]. Small molecules that achieve this have been identified, but are related to the general serine/threonine kinase inhibitor staurosporine and are thus promiscuous kinase inhibitors[225]. Development of selective, ATP-competitive inhibitors of CaMK remains in question, but may be helped by computational chemistry studies that are based on the CaMKII crystal structure[226].

Protein kinase C

Members of the PKC family of serine/threonine kinases have key roles in cardiac hypertrophy and have been recently reviewed in detail[60]. Classical and conventional PKCs (cPKCs) such as α, βI, βII and γ are activated by Ca^{2+} and the lipid-derived second messenger diacylglycerol (DAG). Novel PKCs (nPKCs) δ, ε, η and θ are also activated by DAG but are Ca^{2+}-independent, whereas atypical PKCs (aPKCs) λ and ζ require neither Ca^{2+} or DAG for activation. PKCα, β, δ and ε appear to be most abundant in adult hearts and have been most scrutinized with regard to regulation of pathological hypertrophy.

Analogous to MCIP1 for calcineurin, PKC-interacting cousin of thioredoxin (PICOT) is an endogenous inhibitor of PKC signalling[227] that is upregulated during cardiac hypertrophy[228]. Transgenic overexpression of PICOT in the mouse heart blocks pressure-overload-induced hypertrophy and improves cardiac function, suggesting that PKC inhibitors may be therapeutically useful for pathological hypertrophy[228]. However, the precise PKC isoform(s) targeted by PICOT to control hypertrophy remain unknown, as PICOT can inhibit multiple PKC family members.

Attempts to target PKC will probably need to selectively inhibit some but not other isoforms. Mochley-Rosen and colleagues showed that the activation of either PKCδ or PKCε was sufficient to induce hypertrophy, but this was more physiological in character[229]. Conversely, peptide-mediated inhibition of PKCε (REFS 230,231) and PKCδ (REF. 232) exaggerated pathological hypertrophy and promoted dilation and heart failure, supporting a role for these PKC isoforms in adaptive rather than pathological hypertrophy. *Pkcε*-knockout mice develop hypertrophy with increased collagen deposition and diastolic dysfunction[233]. So, PKC-directed strategies for cardiac hypertrophy should attempt to spare PKCδ and PKCε from inhibition.

Ruboxistaurin (LY333531) (TABLE 1b) is a bisindolylmaleimide PKC inhibitor that is in clinical testing for patients with retinopathy or nephropathy secondary to diabetes[234,235]. This compound, which seems to exhibit modest selectivity for the PKCβ isoform, was recently tested for its ability to block pathological cardiac remodelling in rats subjected to MI[236]. Ruboxistaurin treatment was well tolerated for up to four weeks post-MI, and was associated with a marked inhibition of cardiac hypertrophy and fibrosis in the non-infarct zone of the myocardium. Although both control rats and compound-treated rats developed ventricular dilation in response to MI, cardiac performance was maintained in animals receiving the PKC inhibitor versus those treated with vehicle alone. A key question for the future is whether the beneficial effects of ruboxistaurin in the rat MI model result from selective PKCβ inhibition or targeting of other PKC isoforms and/or unrelated kinases, as related compounds can inhibit members of several kinase families *in vitro*[237]. It will be important to test if ruboxistaurin inhibits hypertrophy from alternative stresses such as pressure overload, as knockout studies suggest that PKCβ is dispensable for cardiac growth[238].

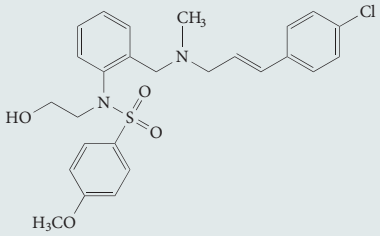
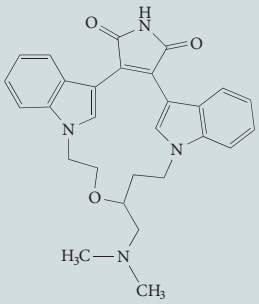
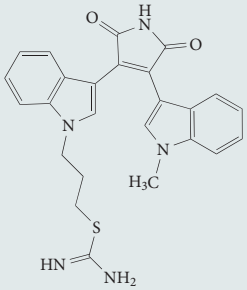
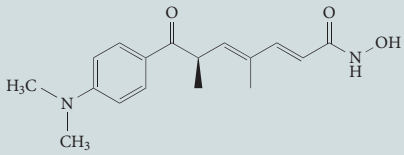
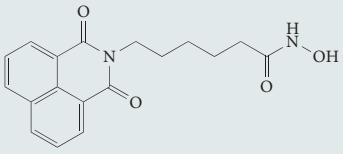
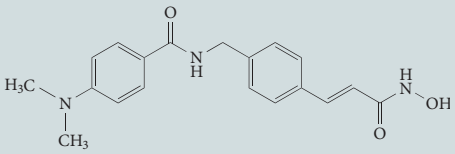
A PKC isoform that is probably involved in pathological cardiac remodelling is PKCα. The impact of PKCα seems to be more directed towards contractility rather than hypertrophy, as PKCα activation by a peptide inducer[239] or transgenic overexpression[240] does not promote overt cardiac hypertrophy, and *Pkcα*-knockout mice develop similar hypertrophy to controls in response to pressure overload[240]. PKCα was shown to inhibit PLB phosphorylation by activating protein phosphatase 1, resulting in the reduced Ca^{2+} uptake by the sarcoplasmic reticulum. PLB becomes hyperphosphorylated in *Pkcα*-knockout mice, leading to de-repression of SERCA and hypercontractility. As PKCα protein levels and activity rise with heart failure[241], and *PKCα* gene deletion helps rescue heart failure in $Mlp^{-/-}$ mice[242], this is a promising target.

Two small-molecule bisindolylmaleimide inhibitors of PKC, Ro-32-0432 and Ro-31-8220 (TABLE 2), have been recently explored in the heart[242]. Both compounds acutely and chronically increased cardiac contractility in normal mice and in mice with pathological cardiac hypertrophy induced by MI, *Mlp* deletion or transgene-mediated overexpression of the $G\alpha_q$ protein in the heart. Although contractility improved in $Mlp^{-/-}$ mice receiving Ro-31-8220 for up to 6 weeks, hearts continued to progress to failure, in contrast to protective effects of *PKCα* deletion in this model. This discrepancy may reflect the inadequate inhibition of cardiac PKCα or negative, counterbalancing effects of Ro-31-8220 on other kinases that are cardioprotective, as it has the capacity to inhibit multiple kinases[237]. Nevertheless, small-molecule targeting of PKCα may provide therapeutic benefit by normalizing impaired cardiac contractility.

Class IIa HDACs: targeting the nucleus

The most distal approach to targeting pathological cardiac hypertrophy is by modulating nuclear gene transcription[243]. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) function as central regulators of gene transcription through their effects on chromatin structure. HATs stimulate gene transcription by acetylating nucleosomal histones, which relaxes the chromatin structure and allows DNA-binding transcription factors access to gene regulatory elements. Conversely, deacetylation of nucleosomal histones by HDACs results in transcriptional repression.

Table 2 | **Compounds that have antihypertrophic activity *in vivo****

Target	Compound	Structure	Model	Inhibition of hypertrophy	Inhibition of fibrosis	Improved LV function	Refs
CaMKII	KN93		Mouse MI (3 weeks)	n/a	n/a	Yes	209
PKCβ	Ruboxistaurin		Rat MI (4 weeks)	Yes	Yes	Yes	236
PKC	Ro-31-8220		*Mlp*-null mouse (6 weeks)	No	n/a	Yes	242
HDAC	Trichostatin A		Mouse ISO infusion (2 weeks)	Yes	n/a	n/a	266
			Mouse Ang II infusion (2 weeks)	Yes	Yes	n/a	264
			Mouse and rat TAB (2 weeks)	Yes	Yes	n/a	264
			Mouse TAB (3 and 9 weeks)	Yes	Yes	Yes	265
HDAC	Scriptaid		Mouse TAB (3 weeks)	Yes	n/a	n/a	265
HDAC	SK-7041		Mouse TAB (2 weeks)	Yes	Yes	n/a	264

*Continued from TABLE 1. Ang, angiotensin; CaMKII, Ca^{2+} calmodulin-dependent protein kinase II; HDAC, histone deacetylase; ISO, isoproterenol; LV, left-ventricular; MI, myocardial infarction; MLP, muscle LIM protein; n/a, data not available; PKC, protein kinase C; TAB, thoracic aortic banding.

HDACs fall into three classes[244]: I (HDACs 1, 2, 3, 8 and 11), II (IIa: HDACs 4, 5, 7, 9; IIb: HDACs 6 and 10) and III (sirtuins), which generally seem to have pro-hypertrophic, antihypertrophic and anti-apoptotic functions in cardiomyocytes, respectively. Overexpression of class IIa HDAC5 or HDAC9 in cultured cardiomyocytes prevents hypertrophy in response to agonists, whereas disruption of the gene encoding either HDAC results in exaggerated hypertrophy in response to pressure overload and spontaneous hypertrophy with age[245,246]. Class IIa HDACs (FIG. 8) suppress cardiac hypertrophy through association with the MEF2 transcription factor, forming repressor complexes on regulatory elements for pro-growth genes[247]. Class IIa HDACs also inhibit cardiac hypertrophy by MEF2-independent mechanisms, for example, repressing NFAT-driven gene expression by interacting with an NFAT chaperone protein, mammalian relative of DnaJ (Mrj)[248]; by blunting serum response factor (SRF)-mediated transcription by interacting with the myocardin co-activator[249]; or through association with the YY1 transcription factor[250]. In addition, class IIa HDACs block cardiac hypertrophy through a novel association with calmodulin binding transcription activator (CAMTA), a co-activator for the NKX2–5 transcription factor[251].

Cardiac hypertrophy is contingent on the nuclear export of class IIa HDACs into the cytoplasm, which de-represses pro-growth genes. This shuttling relies on the phosphorylation of two serine-containing motifs conserved in all class IIa HDACs, which induces association with a chaperone protein, 14-3-3, and unmasking of a CRM1 (also known as XPO1)-dependent nuclear export sequence[252–256]. Suppression of class IIa HDAC nuclear export by disruption of the phospho-acceptor sites[161,223,246] or with the CRM1 antagonist leptomycin B[257] inhibits cardiomyocyte hypertrophy. PKC can induce the nuclear export of class IIa HDACs, but it does so indirectly by activating a downstream effector kinase, PKD[258]. PKD directly phosphorylates class IIa HDACs and thereby de-represses downstream target genes (FIG. 8). Knockdown of PKD expression with small-interfering RNA (siRNA) blunts hypertrophy, and transgenic mice expressing constitutively active PKD in the heart develop dilated cardiomyopathy[259].

Class IIa HDACs can also be regulated by CaMKII (FIG. 8). Although PKD phosphorylates all class IIa HDACs, CaMKII only targets HDAC4 by virtue of a unique docking site not conserved in other HDACs[223]. Interestingly, inositol 1,4,5-trisphosphate (IP_3) receptors in the nuclear envelope have been shown to transduce Ca^{2+} to a nuclear pool of CaMKII that triggers the export of HDAC5 to the cytoplasm of adult rabbit cardiac myocytes[222]. CaMKII-mediated nuclear export of HDAC5 may occur indirectly via another HDAC kinase (for example, PKD) or through an HDAC5-associated factor.

These data suggest that small-molecule inhibitors of a HDAC kinase(s) will block cardiac hypertrophy by retaining class IIa HDACs in the nucleus. However, as described above for CaMKII, specific PKD inhibitors have yet to be identified. One heterocyclic derivative of staurosporine, termed GO-6976, is a potent PKD inhibitor[260], but also is active towards several other kinases[237]. Related compounds such as UCN-01, CEP-1347 and PKC412 are in clinical development for cancer, neurodegeneration and diabetic retinopathy[261]. PKD is expressed in many cell types beyond cardiomyocytes, and is implicated in processes as diverse as Golgi transport, proliferation and apoptosis[262]. Therefore, PKD inhibitors may have pleiotropic effects in tissues other than heart. Whether suppression of class IIa HDAC nuclear export will prevent cardiac disease without causing unwanted side effects awaits *in vivo* proof-of-concept testing with selective kinase inhibitors.

HDAC inhibitors

Small-molecule inhibitors of HDAC catalytic activity, such as the hydroxamic acid trichostatin A (TSA) (TABLE 2), are potent repressors of cardiac hypertrophy. In cultured cardiac myocytes, HDAC inhibitors block cell growth and induction of pro-hypertrophic genes in response to multiple agonists[263]. Studies with mice demonstrated that HDAC inhibitors (Scriptaid, TSA and SK-7041; TABLE 2) block cardiac hypertrophy driven by pressure overload[264,265], by stimulation of β-ARs[266] or by angiotensin II receptors[264]. HDAC-inhibitor treatment blocked fibrosis, preserved systolic performance and improved animal survival. Importantly, HDAC inhibitors were able to reverse pre-established cardiac hypertrophy[264]. *In vivo* delivery of HDAC inhibitors also stimulates expression of α-MHC in the heart[267,268], which is predicted to enhance cardiac contractility[269].

The antihypertrophic activity of HDAC inhibitors seems paradoxical given the fact that class IIa HDACs repress cardiac hypertrophy. However, class IIa HDACs are largely devoid of catalytic activity[270], and instead appear to block gene expression by functioning as adaptors that couple co-repressors such as C-terminal-binding protein (CTBP) and heterochromatin protein 1 (HP1) to transcription factors[271,272], and prohibit the binding of HAT co-activators to these same transcription factors[273,274]. TSA does not inhibit class III HDACs, which are involved in cardiomyocyte survival[275]. Disruption of the gene encoding *Hdac2* in mice was recently shown to blunt cardiac hypertrophy in response to β-AR stimulation and pressure overload, suggesting that suppression of this class I HDAC may contribute to the antihypertrophic action of HDAC inhibitors[276]. Blockade of cardiac hypertrophy in *Hdac2*-targeted mice appears to involve the upregulation of the gene encoding a phosphoinositide phosphatase[276,277], which results in inactivation of AKT, a pro-hypertrophic kinase that normally neutralizes the action of antihypertrophic GSK3β (REF. 276).

It is not known whether HDACs stimulate cardiomyocyte growth entirely through deacetylation of nucleosomal histones with resultant effects on gene expression, or through deacetylation of non-histone targets. A growing body of evidence suggests the existence of diverse non-histone proteins that are regulated by acetylation/deacetylation[278], and thus it is likely that HDACs also play non-genomic roles in the control of cardiac growth.

Other pathways

This Review has focused on a fraction of the vast array of intracellular pathways implicated in the control of pathological cardiac hypertrophy[279]. Other potential targets for treatment of cardiac hypertrophy include p38 MAP kinases — as small-molecule inhibitors of p38 have been shown to block cardiac hypertrophy and adverse remodelling in hypertensive stroke-prone rats[280], cardiomyopathic hamsters[281], in mice subjected to MI[282] and in mice expressing dominant-negative 14-3-3 chaperone protein in the heart[283]. Inhibitors of janus kinase 2 (JAK2)[284] and the pro-apoptotic factor, poly ADP-ribose polymerase (PARP)[285], also block cardiac hypertrophy in response to pressure overload and chronic β-AR stimulation, respectively. Furthermore, in addition to the discovery of novel mediators of cardiac hypertrophy (for example, CAMTA[251]), biochemical and genetic studies continue to uncover new roles for known signalling factors in the control of cardiac hypertrophy. And in some cases, such as for cyclin-dependent kinase 9 (REFS. 286,287), checkpoint kinase 2 (REFS. 288,289), Raf-1 kinase[290,291], and the p300 HAT[292,293], existing small-molecule inhibitors may be suitable for *in vivo* proof-of-concept testing.

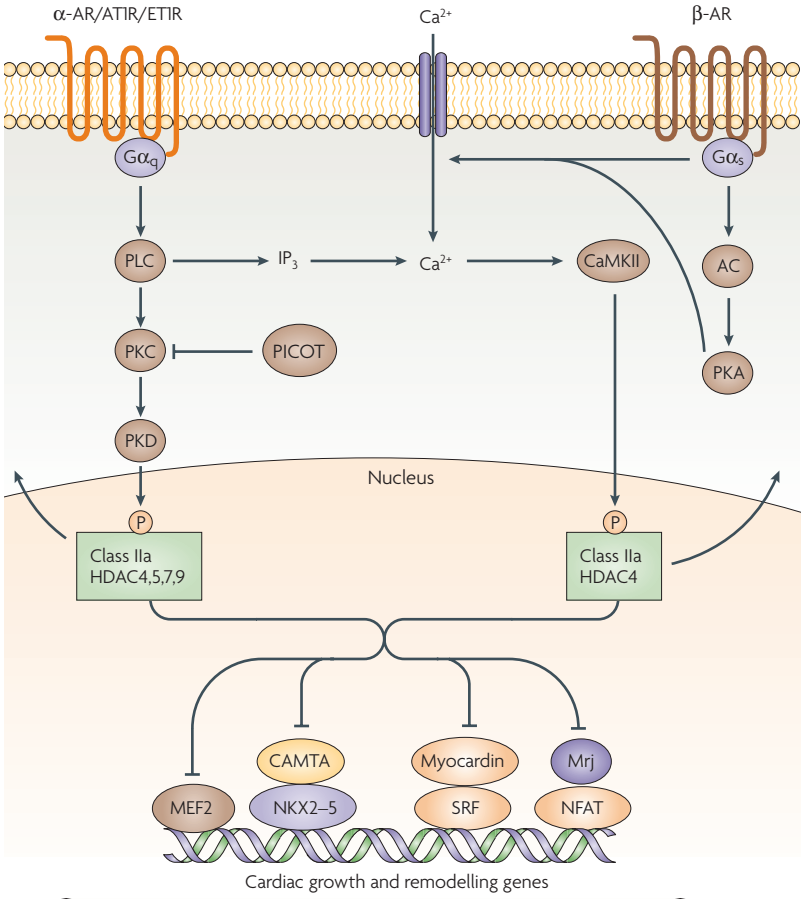


Figure 8 | **Repression of pathological cardiac genes by class IIa histone deacetylases.** Pathological cardiac gene expression is activated by sequence-specific DNA-binding transcription factors, including myocyte enhancer factor 2 (MEF2), NK2 transcription factor related, locus 5 (NKX2–5), serum response factor (SRF) and nuclear factor of activated T cells (NFAT). Class IIa histone deacetylases (HDACs) bind directly to MEF2 and suppress expression of downstream target genes. Class IIa HDACs indirectly associate with NKX2–5, SRF and NFAT via bridging cofactors, which are calmodulin binding transcription activator (CAMTA), myocardin and Mrj (mammalian relative of DnaJ), respectively. Genes under the control of class IIa HDACs are de-repressed by signals that trigger phosphorylation-dependent nuclear export of the HDACs. $G\alpha_q$-coupled receptors trigger class IIa HDAC phosphorylation by activating protein kinase D (PKD). Phospholipase C (PLC) promotes PKD activation via diacyl glycerol (DAG), which stimulates protein kinase C (PKC) to phosphorylate the catalytic domain of PKD. Inositol 1,4,5-trisphosphate (IP_3) produced via PLC can also trigger release of intracellular Ca^{2+}, with subsequent activation of calcium/calmodulin-dependent protein kinase II (CaMKII), which selectively phosphorylates HDAC4. The pathway for CaMKII activation also involves $G\alpha_s$-coupled β-adrenergic receptors (β-ARs), which cause rises in intracellular Ca^{2+} through multiple mechanisms, including activation of voltage-gated Ca^{2+} channels by cyclic AMP-dependent protein kinase (PKA) and direct binding of the channels by $G\alpha_s$. α-AR, α-adrenergic receptor; AT1R, angiotensin II type 1 receptor; AC, adenylate cyclase; ET1R, endothelin 1 receptor (also known as EDNRA); P, phosphate; PICOT, PKC-interacting cousin of thioredoxin.

Translating antihypertrophic therapy to patients

The preceding discussion has sought to systematically highlight novel ways in which we may be able to suppress pathological cardiac hypertrophy. Before concluding, it is important to consider how such treatments might be clinically used. Heart failure is certainly a logical target, especially for patients with preserved ejection fraction (also known as diastolic heart failure). The prevalence of this syndrome is increasing more than is systolic heart failure[294], and many of these patients have marked cardiac hypertrophy, which is thought to contribute significantly to symptoms[295]. This is also an important patient group, as there are virtually no evidence-based proven treatments. Patients with systolic heart failure also develop hypertrophy, and its suppression may prove to be an important clinical target. In both groups, the signalling cascades discussed here have a broader impact on the myocardium beyond left-ventricular mass reduction, and these could prove beneficial. Treatment would probably be added to existing therapy, although it could ultimately replace some, and probably continue for the life of the patient as these syndromes are complex, and it is unlikely that the targeted pathway would turn out to be the primary 'fire' which when 'put out' would result in a permanent cure. The high prevalence of systemic hypertension with cardiac hypertrophy serving as a potent risk factor[1,5–7] for subsequent heart disease suggests that these agents might also be valuable as primary prevention therapies. Proof of efficacy for this indication is undeniably a major undertaking, but the potential could be profound. We do not know if all forms of pathological hypertrophy should be prevented, and certainly efforts to remove the inciting stimulus remain central to any treatment. Last, we do not yet know if hypertrophic disease from genetic mutations can be effectively targeted by the strategies we have described. Few of the approaches have as yet been tested in models of this disease, with statins being one exception[41], and efforts to suppress calcineurin in another genetic model actually triggered more hypertrophy[296].

Conclusions

The recent major advances in the understanding of molecular signalling in hearts exposed to pathological stress has paved the way for different approaches to treating heart disease, in particular to modulate distal

targets and intrinsic regulators on the intracellular side of the cell membrane. Although much more work is needed to develop safe and effective small molecules that interact with some of these pathways, there are several molecules that are ready for clinical testing, and others looking promising in the near future. The potential patient populations for which such therapy could be appropriate have substantial morbidity and mortality, reflect a major target of health-care resources and are growing in size. If the promise of approaches described in this article is realised, therapeutic interventions that improve the health of heart muscle and help it better confront pathological stimuli that otherwise trigger progressive disease could become widespread.

1. Frey, N., Katus, H. A., Olson, E. N. & Hill, J. A. Hypertrophy of the heart: a new therapeutic target? *Circulation* **109**, 1580–1589 (2004).
2. Koren, M. J., Devereux, R. B., Casale, P. N., Savage, D. D. & Laragh, J. H. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann. Intern. Med.* **114**, 345–352 (1991).
3. Esposito, G. *et al.* Genetic alterations that inhibit *in vivo* pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. *Circulation* **105**, 85–92 (2002).
4. Nienaber, J. J. *et al.* Inhibition of receptor-localized PI3K preserves cardiac β-adrenergic receptor function and ameliorates pressure overload heart failure. *J. Clin. Invest* **112**, 1067–1079 (2003).
5. Devereux, R. B. *et al.* Prognostic significance of left ventricular mass change during treatment of hypertension. *JAMA* **292**, 2350–2356 (2004).
6. Gardin, J. M. & Lauer, M. S. Left ventricular hypertrophy: the next treatable, silent killer? *JAMA* **292**, 2396–2398 (2004).
7. Devereux, R. B. *et al.* Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the losartan intervention for endpoint reduction in hypertension (LIFE) trial. *Circulation* **110**, 1456–1462 (2004).
8. Brown, J. H., Del Re, D. P. & Sussman, M. A. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ. Res.* **98**, 730–742 (2006).
9. Nakagawa, O. *et al.* ROCK-I and ROCK-II, two isoforms of Rho-associated coiled–coil forming protein serine/threonine kinase in mice. *FEBS Lett.* **392**, 189–193 (1996).
10. Riento, K. & Ridley, A. J. Rocks: multifunctional kinases in cell behaviour. *Nature Rev. Mol. Cell Biol.* **4**, 446–456 (2003).
11. Hiroki, J. *et al.* Inflammatory stimuli upregulate Rho-kinase in human coronary vascular smooth muscle cells. *J. Mol. Cell. Cardiol.* **37**, 537–546 (2004).
12. Thumkeo, D. *et al.* Targeted disruption of the mouse rho-associated kinase 2 gene results in intrauterine growth retardation and fetal death. *Mol. Cell. Biol.* **23**, 5043–5055 (2003).
13. Rikitake, Y. *et al.* Decreased perivascular fibrosis but not cardiac hypertrophy in *Rock1*$^{+/-}$ haploinsufficient mice. *Circulation* **112**, 2959–2965 (2005).
14. Shimizu, Y. *et al.* ROCK-I regulates closure of the eyelids and ventral body wall by inducing assembly of actomyosin bundles. *J. Cell Biol.* **168**, 941–953 (2005).
15. Kimura, K. *et al.* Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248 (1996).
16. Somlyo, A. P. & Somlyo, A. V. Signal transduction and regulation in smooth muscle. *Nature* **372**, 231–236 (1994).
17. Somlyo, A. P. & Somlyo, A. V. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. *J. Physiol.* **522**, 177–185 (2000).
18. Vahebi, S., Kobayashi, T., Warren, C. M., de Tombe, P. P. & Solaro, R. J. Functional effects of rho-kinase-dependent phosphorylation of specific sites on cardiac troponin. *Circ. Res.* **96**, 740–747 (2005).
19. Li, Z. *et al.* Regulation of PTEN by Rho small GTPases. *Nature Cell Biol.* **7**, 399–404 (2005).
20. Oudit, G. Y. *et al.* The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J. Mol. Cell. Cardiol.* **37**, 449–471 (2004).
21. Wolfrum, S. *et al.* Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1842–1847 (2004).
22. Ennis, I. L. *et al.* Regression of isoproterenol-induced cardiac hypertrophy by Na^+/H^+ exchanger inhibition. *Hypertension* **41**, 1324–1329 (2003).
23. Denker, S. P., Yan, W. & Barber, D. L. Effect of Rho GTPases on Na–H exchanger in mammalian cells. *Methods Enzymol.* **325**, 334–348 (2000).
24. Tominaga, T. & Barber, D. L. Na–H exchange acts downstream of RhoA to regulate integrin-induced cell adhesion and spreading. *Mol. Biol. Cell* **9**, 2287–2303 (1998).
25. Lai, A. & Frishman, W. H. Rho-kinase inhibition in the therapy of cardiovascular disease. *Cardiol. Rev.* **13**, 285–292 (2005).
26. Shimokawa, H. & Takeshita, A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1767–1775 (2005).
27. Tamura, M. *et al.* Development of specific Rho-kinase inhibitors and their clinical application. *Biochim. Biophys. Acta* **1754**, 245–252 (2005).
28. Higashi, M. *et al.* Long-term inhibition of Rho-kinase suppresses angiotensin II-induced cardiovascular hypertrophy in rats *in vivo*: effect on endothelial NAD(P)H oxidase system. *Circ. Res.* **93**, 767–775 (2003).
29. Wang, Y. X. *et al.* Inhibition of Rho-kinase by fasudil attenuated angiotensin II-induced cardiac hypertrophy in apolipoprotein E deficient mice. *Eur. J. Pharmacol.* **512**, 215–222 (2005).
30. Hattori, T. *et al.* Long-term inhibition of Rho-kinase suppresses left ventricular remodeling after myocardial infarction in mice. *Circulation* **109**, 2234–2239 (2004).
31. Satoh, S. *et al.* Chronic inhibition of Rho kinase blunts the process of left ventricular hypertrophy leading to cardiac contractile dysfunction in hypertension-induced heart failure. *J. Mol. Cell. Cardiol.* **35**, 59–70 (2003).
This demonstrates the effectiveness of a Rho-kinase inhibitor to blunt progressive hypertrophy and heart failure in the Dahl sensitive rat.
32. Kobayashi, N. *et al.* Critical role of Rho-kinase pathway for cardiac performance and remodeling in failing rat hearts. *Cardiovasc. Res.* **55**, 757–767 (2002).
33. Martin G, *et al.* Statin-induced inhibition of the Rho-signaling pathway activates PPARα and induces HDL apoA-I. *J. Clin. Invest.* **107**, 1423–1432 (2001).
34. Liao, J. K. & Laufs, U. Pleiotropic effects of statins. *Annu. Rev. Pharmacol. Toxicol.* **45**, 89–118 (2005).
35. Nakagami, H. & Liao, J. K. Statins and myocardial hypertrophy. *Coron. Artery Dis.* **15**, 247–250 (2004).
36. Laufs, U. & Liao, J. K. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. *J. Biol. Chem.* **273**, 24266–24271 (1998).
37. Liao, J. K. Statin therapy for cardiac hypertrophy and heart failure. *J. Investig. Med.* **52**, 248–253 (2004).
38. Takemoto, M. *et al.* Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy. *J. Clin. Invest.* **108**, 1429–1437 (2001).
This shows that statin therapy suppresses hypertrophy coupled to the inhibition of GTP binding to RhoA and RAC1.
39. Dechend, R. *et al.* Amelioration of angiotensin II-induced cardiac injury by a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor. *Circulation* **104**, 576–581 (2001).
40. Hasegawa, H. *et al.* 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors prevent the development of cardiac hypertrophy and heart failure in rats. *J. Mol. Cell. Cardiol.* **35**, 953–960 (2003).
41. Patel, R. *et al.* Simvastatin induces regression of cardiac hypertrophy and fibrosis and improves cardiac function in a transgenic rabbit model of human hypertrophic cardiomyopathy. *Circulation* **104**, 317–324 (2001).
42. Fukuta, H., Sane, D. C., Brucks, S. & Little, W. C. Statin therapy may be associated with lower mortality in patients with diastolic heart failure: a preliminary report. *Circulation* **112**, 357–363 (2005).
43. Vezina, C., Kudelski, A. & Sehgal, S. N. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot. (Tokyo)* **28**, 721–726 (1975).
44. Brown, E. J. *et al.* A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* **369**, 756–758 (1994).
45. Jacinto, E. & Hall, M. N. Tor signalling in bugs, brain and brawn. *Nature Rev. Mol. Cell Biol.* **4**, 117–126 (2003).
46. Inoki, K., Corradetti, M. N. & Guan, K. L. Dysregulation of the TSC-mTOR pathway in human disease. *Nature Genet.* **37**, 19–24 (2005).
47. Proud, C. G. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. *Cardiovasc. Res.* **63**, 403–413 (2004).
48. Manning, B. D. & Cantley, L. C. Rheb fills a GAP between TSC and TOR. *Trends Biochem. Sci.* **28**, 573–576 (2003).
49. Luo, J. *et al.* Class IA phosphoinositide 3-kinase regulates heart size and physiological cardiac hypertrophy. *Mol. Cell Biol.* **25**, 9491–9502 (2005).
50. Shibata, R. *et al.* Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nature Med* **10**, 1384–1389 (2004).
51. Chan, A. Y., Soltys, C. L., Young, M. E., Proud, C. G. & Dyck, J. R. Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. *J. Biol. Chem.* **279**, 32771–32779 (2004).
52. Sadoshima, J. & Izumo, S. Rapamycin selectively inhibits angiotensin II-induced increase in protein synthesis in cardiac myocytes *in vitro*. Potential role of 70-kD S6 kinase in angiotensin II-induced cardiac hypertrophy. *Circ. Res.* **77**, 1040–1052 (1995).
53. Boluyt, M. O. *et al.* Rapamycin inhibits α1-adrenergic receptor-stimulated cardiac myocyte hypertrophy but not activation of hypertrophy-associated genes. Evidence for involvement of p70 S6 kinase. *Circ. Res.* **81**, 176–186 (1997).
54. Shioi, T. *et al.* Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation* **107**, 1664–1670 (2003).
The first study to demonstrate the benefit of rapamycin in treating pre-existing load-induced cardiac hypertrophy.
55. Boluyt, M. O. *et al.* The mTOR/p70S6K signal transduction pathway plays a role in cardiac hypertrophy and influences expression of myosin heavy chain genes *in vivo*. *Cardiovasc. Drugs Ther.* **18**, 257–267 (2004).
56. McMullen, J. R. *et al.* Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* **109**, 3050–3055 (2004).
57. Crackower, M. A. *et al.* Regulation of myocardial contractility and cell size by distinct PI3K–PTEN signaling pathways. *Cell* **110**, 737–749 (2002).
58. Hardt, S. E. & Sadoshima, J. Glycogen synthase kinase-3β: a novel regulator of cardiac hypertrophy and development. *Circ. Res.* **90**, 1055–1063 (2002).
59. Badorff, C., Seeger, F. H., Zeiher, A. M. & Dimmeler, S. Glycogen synthase kinase 3β inhibits myocardin-dependent transcription and hypertrophy induction through site-specific phosphorylation. *Circ. Res.* **97**, 645–654 (2005).
60. Dorn, G. W. & Force, T. Protein kinase cascades in the regulation of cardiac hypertrophy. *J. Clin. Invest.* **115**, 527–537 (2005).
61. Schiekofer, S. *et al.* Microarray analysis of Akt1 activation in transgenic mouse hearts reveal transcript expression profiles associated with compensatory hypertrophy and failure. *Physiol. Genomics* **27**, 156–170 (2006).
62. Matsui, T. *et al.* Phenotypic spectrum caused by transgenic overexpression of activated Akt in the heart. *J. Biol. Chem.* **277**, 22896–22901 (2002).

63. Shiojima, I. *et al.* Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J. Clin. Invest.* **115**, 2108–2118 (2005).
64. Kato, T. *et al.* Atrial natriuretic peptide promotes cardiomyocyte survival by cGMP-dependent nuclear accumulation of zyxin and Akt. *J. Clin. Invest.* **115**, 2716–2730 (2005).
65. Tsujita, Y. *et al.* Nuclear targeting of Akt antagonizes aspects of cardiomyocyte hypertrophy. *Proc. Natl Acad. Sci. USA* **103**, 11946–11951 (2006).
66. Rota, M. *et al.* Nuclear targeting of Akt enhances ventricular function and myocyte contractility. *Circ. Res.* **97**, 1332–1341 (2005).
67. Giordano F. J. Oxygen, oxidative stress, hypoxia, and heart failure. *J. Clin. Invest*. **115**, 500–508 (2005)
68. Casagrande, S. *et al.* Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. *Proc. Natl Acad. Sci. USA* **99**, 9745–9749 (2002).
69. Yamawaki, H. & Berk, B. C. Thioredoxin: a multi-functional antioxidant enzyme in kidney, heart and vessels. *Curr. Opin. Nephrol. Hypertens.* **14**, 149–153 (2005).
70. Xie, Z. *et al.* Intracellular reactive oxygen species mediate the linkage of Na^+/K^+-ATPase to hypertrophy and its marker genes in cardiac myocytes. *J. Biol. Chem.* **274**, 19323–19328 (1999).
71. Nakamura, K. *et al.* Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. *Circulation* **98**, 794–799 (1998).
72. Amin, J. K. *et al.* Reactive oxygen species mediate α-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes. *J. Mol. Cell. Cardiol.* **33**, 131–139 (2001).
73. Date, M. O. *et al.* The antioxidant *N*-2-mercaptopropionyl glycine attenuates left ventricular hypertrophy in *in vivo* murine pressure-overload model. *J. Am. Coll. Cardiol.* **39**, 907–912 (2002).
74. Siwik, D. A. *et al.* Inhibition of copper-zinc superoxide dismutase induces cell growth, hypertrophic phenotype, and apoptosis in neonatal rat cardiac myocytes *in vitro*. *Circ. Res.* **85**, 147–153 (1999).
75. Ide, T. *et al.* Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. *Circ. Res.* **86**, 152–157 (2000).
76. Kinugawa, S. *et al.* Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. *Circ. Res.* **87**, 392–398 (2000).
77. Liu, H., Colavitti, R., Rovira, I. I. & Finkel, T. Redox-dependent transcriptional regulation. *Circ. Res.* **97**, 967–974 (2005).
78. Pimentel, D. R. *et al.* Strain-stimulated hypertrophy in cardiac myocytes is mediated by reactive oxygen species-dependent Ras *S*-glutathiolation. *J. Mol. Cell. Cardiol.* **41**, 613–622 (2006).
79. Yamamoto, M. *et al.* Inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy. *J. Clin. Invest* **112**, 1395–1406 (2003).
80. Matsushima, S. *et al.* Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* **113**, 1779–1786 (2006).
81. Zhang, X. *et al.* Cardiac-specific overexpression of catalase rescues ventricular myocytes from ethanol-induced cardiac contractile defect. *J. Mol. Cell. Cardiol.* **35**, 645–652 (2003).
82. Berry, C. E. & Hare, J. M. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J. Physiol.* **555**, 589–606 (2004).
83. Cappola, T. P. *et al.* Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation* **104**, 2407–2411 (2001).
84. Engberding, N. *et al.* Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? *Circulation* **110**, 2175–2179 (2004).
85. Saavedra, W. F. *et al.* Imbalance between xanthine oxidase and nitric oxide synthase signaling pathways underlies mechanoenergetic uncoupling in the failing heart. *Circ. Res.* **90**, 297–304 (2002).
86. Minhas, K. M. *et al.* Xanthine oxidoreductase inhibition causes reverse remodeling in rats with dilated cardiomyopathy. *Circ. Res.* **98**, 271–279 (2006).
87. Amado, L. C. *et al.* Xanthine oxidase inhibition ameliorates cardiovascular dysfunction in dogs with pacing-induced heart failure. *J. Mol. Cell. Cardiol.* **39**, 531–536 (2005).
88. Baldus, S. *et al.* Inhibition of xanthine oxidase improves myocardial contractility in patients with ischemic cardiomyopathy. *Free Radic. Biol. Med.* **41**, 1282–1288 (2006).
89. Reyes, A. J. & Leary, W. P. Allopurinol or oxypurinol in heart failure therapy — a promising new development or end of story? *Cardiovasc. Drugs Ther.* **19**, 311–313 (2005).
90. Byrne, J. A., Grieve, D. J., Cave, A. C. & Shah, A. M. Oxidative stress and heart failure. *Arch. Mal Coeur Vaiss.* **96**, 214–221 (2003).
91. Li, J. M., Gall, N. P., Grieve, D. J., Chen, M. & Shah, A. M. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension* **40**, 477–484 (2002).
92. Murdoch, C. E., Grieve, D. J., Cave, A. C., Looi, Y. H. & Shah, A. M. NADPH oxidase and heart failure. *Curr. Opin. Pharmacol.* **6**, 148–153 (2006).
93. Lambeth, J. D. NOX enzymes and the biology of reactive oxygen. *Nature Rev. Immunol.* **4**, 181–189 (2004).
94. Nakagami, H., Takemoto, M. & Liao, J. K. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced cardiac hypertrophy. *J. Mol. Cell. Cardiol.* **35**, 851–859 (2003).
95. Byrne, J. A. *et al.* Contrasting roles of NADPH oxidase isoforms in pressure-overload versus angiotensin II-induced cardiac hypertrophy. *Circ. Res.* **93**, 802–805 (2003).
96. Maytin, M. *et al.* Pressure overload-induced myocardial hypertrophy in mice does not require gp91phox. *Circulation* **109**, 1168–1171 (2004).
97. Bendall, J. K., Cave, A. C., Heymes, C., Gall, N. & Shah, A. M. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation* **105**, 293–296 (2002).
98. bu-Soud, H. M. & Stuehr, D. J. Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. *Proc. Natl Acad. Sci. USA* **90**, 10769–10772 (1993).
99. Schmidt, H. H. *et al.* No •NO from NO synthase. *Proc. Natl Acad. Sci. USA* **93**, 14492–14497 (1996).
100. Wever, R. M., van, D. T., van Rijn, H. J., de, G. F. & Rabelink, T. J. Tetrahydrobiopterin regulates superoxide and nitric oxide generation by recombinant endothelial nitric oxide synthase. *Biochem. Biophys. Res. Commun.* **237**, 340–344 (1997).
101. Takimoto, E. *et al.* Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. *J. Clin. Invest.* **115**, 1221–1231 (2005).
This demonstrated that oxidant stress from NOS uncoupling is important in hypertrophy and dilation of hearts subjected to pressure overload, and that its prevention by exogenous BH_4 also blunts pathological remodelling.
102. Alp, N. J. *et al.* Tetrahydrobiopterin-dependent preservation of nitric oxide-mediated endothelial function in diabetes by targeted transgenic GTP-cyclohydrolase I overexpression. *J Clin. Invest.* **112**, 725–735 (2003).
103. Landmesser, U. *et al.* Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J. Clin. Invest.* **111**, 1201–1209 (2003).
104. Dixon, L. J. *et al.* Functional consequences of endothelial nitric oxide synthase uncoupling in congestive cardiac failure. *Circulation* **107**, 1725–1728 (2003).
105. Alp, N. J. & Channon, K. M. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **24**, 413–420 (2004).
106. Madeddu, P. Correction of endothelial dysfunction by tetrahydrobiopterin: new hope for the treatment of arterial hypertension? *J. Hypertens.* **23**, 1335–1336 (2005).
107. Kase, H., Hashikabe, Y., Uchida, K., Nakanishi, N. & Hattori, Y. Supplementation with tetrahydrobiopterin prevents the cardiovascular effects of angiotensin II-induced oxidative and nitrosative stress. *J. Hypertens.* **23**, 1375–1382 (2005).
108. Moreno, J. M. *et al.* Cardiac and renal antioxidant enzymes and effects of tempol in hyperthyroid rats. *Am. J. Physiol. Endocrinol. Metab.* **289**, E776–E783 (2005).
109. Zhang, G. X. *et al.* Cardiac oxidative stress in acute and chronic isoproterenol-infused rats. *Cardiovasc. Res.* **65**, 230–238 (2005).
110. Hoshida, S. *et al.* Ebselen protects against ischemia-reperfusion injury in a canine model of myocardial infarction. *Am. J. Physiol.* **267**, H2342–H2347 (1994).
111. Li, H. L. *et al.* Isorhapontigenin, a new resveratrol analog, attenuates cardiac hypertrophy via blocking signaling transduction pathways. *Free Radic. Biol. Med.* **38**, 243–257 (2005).
112. Clarke, R. & Armitage, J. Antioxidant vitamins and risk of cardiovascular disease. Review of large-scale randomised trials. *Cardiovasc. Drugs Ther.* **16**, 411–415 (2002).
113. Hoffmann, F., Feil, R., Kleppisch, T. & Scholssman, J. Function of cGMP-dependent protein kinases as revealed by gene deletion. *Physiol. Rev.* **86**, 1–23 (2006).
114. Calderone, A., Thaik, C. M., Takahashi, N., Chang, D. F. & Colucci, W. S. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. *J. Clin. Invest.* **101**, 812–818 (1998).
115. Pilz, R. B. & Casteel, D. E. Regulation of gene expression by cyclic GMP. *Circ. Res.* **93**, 1034–1046 (2003).
116. Balligand, J. L. Regulation of cardiac β-adrenergic response by nitric oxide. *Cardiovasc. Res.* **43**, 607–620 (1999).
117. Maurice, D. H. Cyclic nucleotide phosphodiesterase-mediated integration of cGMP and cAMP signaling in cells of the cardiovascular system. *Front. Biosci.* **10**, 1221–1228 (2005).
118. Mongillo, M. *et al.* Compartmentalized phosphodiesterase-2 activity blunts β-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circ. Res.* **98**, 226–234 (2006).
119. Silberbach, M. *et al.* Extracellular signal-regulated protein kinase activation is required for the anti-hypertrophic effect of atrial natriuretic factor in neonatal rat ventricular myocytes. *J. Biol. Chem.* **274**, 24858–24864 (1999).
120. Silberbach, M. & Roberts, C. T. Jr. Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. *Cell. Signal.* **13**, 221–231 (2001).
121. Koller, K. J. *et al.* Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* **252**, 120–123 (1991).
122. Layland, J., Li, J. M. & Shah, A. M. Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *J. Physiol.* **540**, 457–467 (2002).
123. Wollert, K. C. *et al.* Increased effects of C-type natriuretic peptide on contractility and calcium regulation in murine hearts overexpressing cyclic GMP-dependent protein kinase I. *Br. J. Pharmacol.* **140**, 1227–1236 (2003).
124. MacLennan, D. H. & Kranias, E. G. Phospholamban: a crucial regulator of cardiac contractility. *Nature Rev. Mol. Cell Biol.* **4**, 566–577 (2003).
125. Murthy, K. S., Zhou, H., Grider, J. R. & Makhlouf, G. M. Inhibition of sustained smooth muscle contraction by PKA and PKG preferentially mediated by phosphorylation of RhoA. *Am. J. Physiol. Gastrointest. Liver Physiol.* **284**, G1006–G1016 (2003).
126. Sauzeau, V. *et al.* Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca^{2+} sensitization of contraction in vascular smooth muscle. *J. Biol. Chem.* **275**, 21722–21729 (2000).
127. Tang, K. M. *et al.* Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. *Nature Med* **9**, 1506–1512 (2003).
128. Zou, M. X. *et al.* RGS2 is upregulated by and attenuates the hypertrophic effect of α(1)-adrenergic activation in cultured ventricular myocytes. *Cell. Signal.* **18**, 1655–1663 (2006).
129. Zhang, W. *et al.* Selective loss of fine tuning of Gq/11 signaling by RGS2 protein exacerbates cardiomyocyte hypertrophy. *J. Biol. Chem.* **281**, 5811–5820 (2006).
130. Costa, A. D. *et al.* Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. *Circ. Res.* **97**, 329–336 (2005).
131. Das, A., Xi, L. & Kukreja, R. C. Phosphodiesterase-5 inhibitor sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis. Essential role of nitric oxide signaling. *J. Biol. Chem.* **280**, 12944–12955 (2005).
132. Nisoli, E. *et al.* Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* **299**, 896–899 (2003).

133. Holtwick, R. *et al.* Pressure-independent cardiac hypertrophy in mice with cardiomyocyte-restricted inactivation of the atrial natriuretic peptide receptor guanylyl cyclase-A. *J. Clin. Invest.* **111**, 1399–1407 (2003).
134. Ogawa, Y. *et al*. Brain natriuretic peptide appears to act locally as an antifibrotic factor in the heart. *Can. J. Physiol. Pharmacol.* **79**, 723–729 (2001).
135. Ritchie, R. H., Schiebinger, R. J., LaPointe, M. C. & Marsh, J. D. Angiotensin II-induced hypertrophy of adult rat cardiomyocytes is blocked by nitric oxide. *Am. J. Physiol.* **275**, H1370–H1374 (1998).
136. Sackner-Bernstein, J. D., Kowalski, M., Fox, M. & Aaronson, K. Short-term risk of death after treatment with nesiritide for decompensated heart failure: a pooled analysis of randomized controlled trials. *JAMA* **293**, 1900–1905 (2005).
137. Cataliotti, A. & Burnett, J. C. Jr. Natriuretic peptides: novel therapeutic targets in heart failure. *J. Investig. Med.* **53**, 378–384 (2005).
138. Evgenov, O. V. *et al*. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nature Rev. Drug Discov*. **5**, 755–768 (2006).
139. Stasch, J. P. *et al*. Targeting the heme-oxidized nitric oxide receptor for selective vasodilatation of diseased blood vessels. *J. Clin. Invest.* **116**, 2552–2561 (2006).
140. Lugnier, C. Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. *Pharmacol. Ther*. **109**, 366–398 (2006).
141. Bender, A. T. & Beavo, J. A. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol. Rev*. **58**, 488–520 (2006).
142. Castro, L. R., Verde, I., Cooper, D. M. & Fischmeister, R. Cyclic guanosine monophosphate compartmentation in rat cardiac myocytes. *Circulation* **113**, 2221–2228 (2006).
143. Galie, N. *et al.* Sildenafil citrate therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* **353**, 2148–2157 (2005).
144. Goldstein, I. *et al.* Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. *N. Engl. J. Med.* **338**, 1397–1404 (1998).
145. Senzaki, H. *et al.* Cardiac phosphodiesterase 5 (cGMP-specific) modulates β-adrenergic signaling *in vivo* and is down-regulated in heart failure. *FASEB J.* **15**, 1718–1726 (2001).
146. Takimoto, E. *et al.* cGMP catabolism by phosphodiesterase 5A regulates cardiac adrenergic stimulation by NOS3-dependent mechanism. *Circ. Res.* **96**, 100–109 (2005).
147. Borlaug, B. A., Melenovsky, V., Marhin, T., Fitzgerald, P. & Kass, D. A. Sildenafil inhibits β-adrenergic-stimulated cardiac contractility in humans. *Circulation* **112**, 2642–2649 (2005).
148. Takimoto, E. *et al.* Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nature Med.* **11**, 214–222 (2005). **This showed that the chronic inhibition of PDE5a with sildenafil blunted progression of hypertrophy, while enhancing heart function, and reversed pre-existing early stage hypertrophy in pressure-overloaded hearts.**
149. Aramburu, J., Rao, A. & Klee, C. B. Calcineurin: from structure to function. *Curr. Top. Cell. Regul.* **36**, 237–295 (2000).
150. Crabtree, G. R. & Olson, E. N. NFAT signaling: choreographing the social lives of cells. *Cell* **109** (Suppl 1), 67–79 (2002).
151. Molkentin, J. D. *et al.* A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* **93**, 215–228 (1998).
152. Bueno, O. F. *et al.* Impaired cardiac hypertrophic response in calcineurin Aβ-deficient mice. *Proc. Natl Acad. Sci. USA* **99**, 4586–4591 (2002).
153. De Windt, L. J. *et al.* Targeted inhibition of calcineurin attenuates cardiac hypertrophy *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 3322–3327 (2001).
154. Rothermel, B. A. *et al.* Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy *in vivo*. *Proc. Natl Acad. Sci. USA* **98**, 3328–3333 (2001).
155. van Rooij, E. *et al.* MCIP1 overexpression suppresses left ventricular remodeling and sustains cardiac function after myocardial infarction. *Circ. Res.* **94**, e18–e26 (2004).
156. Wilkins, B. J. *et al.* Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth. *Mol. Cell. Biol.* **22**, 7603–7613 (2002).
157. Zou, Y. *et al.* Calcineurin plays a critical role in the development of pressure overload-induced cardiac hypertrophy. *Circulation* **104**, 97–101 (2001).
158. Frey, N. & Olson, E. N. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu. Rev. Physiol.* **65**, 45–79 (2003).
159. Wilkins, B. J. & Molkentin, J. D. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem. Biophys. Res. Commun.* **322**, 1178–1191 (2004).
160. Chan, B., Greenan, G., McKeon, F. & Ellenberger, T. Identification of a peptide fragment of DSCR1 that competitively inhibits calcineurin activity *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **102**, 13075–13080 (2005).
161. Bush, E. *et al.* A small molecular activator of cardiac hypertrophy uncovered in a chemical screen for modifiers of the calcineurin signaling pathway. *Proc. Natl Acad. Sci. USA* **101**, 2870–2875 (2004).
162. Bush, E. & Hood, D. Use of modulators of a novel form of muscle selective calcineurin interacting protein (MCIP-1-38) as a treatment for cardiovascular diseases, and modulator screening methods. Patent WO200533461 (2005).
163. Hilioti, Z. *et al.* GSK-3 kinases enhance calcineurin signaling by phosphorylation of RCNs. *Genes Dev.* **18**, 35–47 (2004).
164. Antos, C. L. *et al.* Activated glycogen synthase kinase-3β suppresses cardiac hypertrophy *in vivo*. *Proc. Natl Acad. Sci. USA* **99**, 907–912 (2002).
165. Abbasi, S. *et al.* Protein kinase-mediated regulation of calcineurin through the phosphorylation of modulatory calcineurin-interacting protein 1. *J. Biol. Chem.* **281**, 7717–7726 (2006).
166. Frey, N., Richardson, J. A. & Olson, E. N. Calsarcins, a novel family of sarcomeric calcineurin-binding proteins. *Proc. Natl Acad. Sci. USA* **97**, 14632–14637 (2000).
167. Frey, N. & Olson, E. N. Calsarcin-3, a novel skeletal muscle-specific member of the calsarcin family, interacts with multiple Z-disc proteins. *J. Biol. Chem.* **277**, 13998–14004 (2002).
168. Frey, N. *et al.* Mice lacking calsarcin-1 are sensitized to calcineurin signaling and show accelerated cardiomyopathy in response to pathological biomechanical stress. *Nature Med.* **10**, 1336–1343 (2004).
169. Gomes, M. D., Lecker, S. H., Jagoe, R. T., Navon, A. & Goldberg, A. L. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc. Natl Acad. Sci. USA* **98**, 14440–14445 (2001).
170. Li, H. H. *et al.* Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J. Clin. Invest.* **114**, 1058–1071 (2004).
171. Sandri, M. *et al.* Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117**, 399–412 (2004).
172. Ni, Y. G. *et al.* Foxo transcription factors blunt cardiac hypertrophy by inhibiting calcineurin signaling. *Circulation* **114**, 1159–1168 (2006).
173. Skurk, C. *et al.* The FOXO3 a transcription factor regulates cardiac myocyte size downstream of AKT signaling. *J. Biol. Chem.* **280**, 20814–20823 (2005).
174. Kau, T. R. *et al.* A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells. *Cancer Cell* **4**, 463–476 (2003).
175. Kuriyama, M. *et al.* A cell-permeable NFAT inhibitor peptide prevents pressure-overload cardiac hypertrophy. *Chem. Biol. Drug Des.* **67**, 238–243 (2006).
176. Roehrl, M. H. *et al.* Selective inhibition of calcineurin-NFAT signaling by blocking protein–protein interaction with small organic molecules. *Proc. Natl Acad. Sci. USA* **101**, 7554–7559 (2004).
177. Kang, S., Li, H., Rao, A. & Hogan, P. G. Inhibition of the calcineurin-NFAT interaction by small organic molecules reflects binding at an allosteric site. *J. Biol. Chem.* **280**, 37698–37706 (2005).
178. Ramsey, I. S., Delling, M. & Clapham, D. E. An introduction to TRP channels. *Annu. Rev. Physiol.* **68**, 619–647 (2006).
179. Vazquez, G., Wedel, B. J., Aziz, O., Trebak, M. & Putney, J. W. Jr. The mammalian TRPC cation channels. *Biochim. Biophys. Acta* **1742**, 21–36 (2004).
180. Bush, E. W. *et al.* Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling. *J. Biol. Chem.* **281**, 33487–33496 (2006).
181. Seth, M. *et al.* Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) gene silencing and remodeling of the Ca^{2+} signaling mechanism in cardiac myocytes. *Proc. Natl Acad. Sci. USA* **101**, 16683–16688 (2004).
182. Onohara, N. *et al*. TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *EMBO J.* **25**, 5305–5316 (2006).
183. Nakayama, H., Wilkin, B. J., Bodi, I. & Molkentin, J. D. Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. *FASEB J.* **20**, 1660–1670 (2006).
184. Kuwahara, K. *et al.* TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J. Clin. Invest.* **116**, 3114–3126 (2006).
185. Maroto, R. *et al.* TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nature Cell Biol.* **7**, 179–185 (2005).
186. Rosenberg, P. *et al.* TRPC3 channels confer cellular memory of recent neuromuscular activity. *Proc. Natl Acad. Sci. USA* **101**, 9387–9392 (2004).
187. Lievremont, J. P., Bird, G. S. & Putney, J. W. Jr. Mechanism of inhibition of TRPC cation channels by 2-aminoethoxydiphenylborane. *Mol. Pharmacol.* **68**, 758–762 (2005).
188. Djuric, S. W. *et al*. **3**, 5-Bis(trifluoromethyl)pyrazole s: a novel class of NFAT transcription factor regulator. *J. Med. Chem.* **43**, 2975–2981 (2000).
189. Trevillyan, J. M. *et al.* Potent inhibition of NFAT activation and T cell cytokine production by novel low molecular weight pyrazole compounds. *J. Biol. Chem.* **276**, 48118–48126 (2001).
190. He, L. P., Hewavitharana, T., Soboloff, J., Spassova, M. A. & Gill, D. L. A functional link between store-operated and TRPC channels revealed by the 3, 5-bis (trifluoromethyl)pyrazole derivative, BTP2. *J. Biol. Chem.* **280**, 10997–11006 (2005).
191. Zitt, C. *et al.* Potent inhibition of Ca^{2+} release-activated Ca^{2+} channels and T-lymphocyte activation by the pyrazole derivative BTP2. *J. Biol. Chem.* **279**, 12427–12437 (2004).
192. Venkatesh, N. *et al.* Chemical genetics to identify NFAT inhibitors: potential of targeting calcium mobilization in immunosuppression. *Proc. Natl Acad. Sci. USA* **101**, 8969–8974 (2004).
193. Tobimatsu, T. & Fujisawa, H. Tissue-specific expression of four types of rat calmodulin-dependent protein kinase II mRNAs. *J. Biol. Chem.* **264**, 17907–17912 (1989).
194. Means, A. R. Regulatory cascades involving calmodulin-dependent protein kinases. *Mol. Endocrinol.* **14**, 4–13 (2000).
195. Schulman, H. The multifunctional Ca^{2+}/calmodulin-dependent protein kinases. *Curr. Opin. Cell Biol.* **5**, 247–253 (1993).
196. Kato, T. *et al.* Calmodulin kinases II and IV and calcineurin are involved in leukemia inhibitory factor-induced cardiac hypertrophy in rats. *Circ. Res.* **87**, 937–945 (2000).
197. Passier, R. *et al.* CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor *in vivo*. *J. Clin. Invest.* **105**, 1395–1406 (2000).
198. Ramirez, M. T., Zhao, X. L., Schulman, H. & Brown, J. H. The nuclear δB isoform of Ca^{2+}/calmodulin-dependent protein kinase II regulates atrial natriuretic factor gene expression in ventricular myocytes. *J. Biol. Chem.* **272**, 31203–31208 (1997).
199. Zhang, T., Miyamoto, S. & Brown, J. H. Cardiomyocyte calcium and calcium/calmodulin-dependent protein kinase II: friends or foes? *Recent Prog. Horm. Res.* **59**, 141–168 (2004).
200. Zhu, W. *et al.* Ca^{2+}/calmodulin-dependent kinase II and calcineurin play critical roles in endothelin-1-induced cardiomyocyte hypertrophy. *J. Biol. Chem.* **275**, 15239–15245 (2000).
201. Boknik, P. *et al.* Enhanced protein phosphorylation in hypertensive hypertrophy. *Cardiovasc. Res.* **51**, 717–728 (2001).
202. Colomer, J. M., Mao, L., Rockman, H. A. & Means, A. R. Pressure overload selectively up-regulates Ca^{2+}/calmodulin-dependent protein kinase II *in vivo*. *Mol. Endocrinol.* **17**, 183–192 (2003).
203. Currie, S. & Smith, G. L. Calcium/calmodulin-dependent protein kinase II activity is increased in sarcoplasmic reticulum from coronary artery ligated rabbit hearts. *FEBS Lett.* **459**, 244–248 (1999).
204. Hagemann, D., Bohlender, J., Hoch, B., Krause, E. G. & Karczewski, P. Expression of Ca^{2+}/calmodulin-dependent protein kinase II δ-subunit isoforms in rats with hypertensive cardiac hypertrophy. *Mol. Cell. Biochem.* **220**, 69–76 (2001).

205. Zhang, T. *et al.* The δC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. *Circ. Res.* **92**, 912–919 (2003).
206. Kirchhefer, U., Schmitz, W., Scholz, H. & Neumann, J. Activity of cAMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase in failing and nonfailing human hearts. *Cardiovasc. Res.* **42**, 254–261 (1999).
207. Zhang, T. *et al.* The cardiac-specific nuclear δB isoform of Ca^{2+}/calmodulin-dependent protein kinase II induces hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. *J. Biol. Chem.* **277**, 1261–1267 (2002).
208. Zhang T. *et al.* The δC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. *Circ. Res.* **92**, 912–919 (2003).
209. Zhang, R. *et al.* Calmodulin kinase II inhibition protects against structural heart disease. *Nature Med.* **11**, 409–417 (2005).
This demonstrated that CaM kinase inhibition in the heart, using either a peptide or a small molecule, is well tolerated and suppresses adverse cardiac remodelling in animal models.
210. Yang, Y. *et al.* Calmodulin kinase II inhibition protects against myocardial cell apoptosis *in vivo*. *Am. J. Physiol. Heart Circ. Physiol.* **291**, H3065–H3075 (2006).
211. Zhu, W. Z. *et al.* Linkage of β1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca^{2+}/calmodulin kinase II. *J. Clin. Invest.* **111**, 617–625 (2003).
212. Bers, D. M. & Guo, T. Calcium signaling in cardiac ventricular myocytes. *Ann. N. Y. Acad. Sci.* **1047**, 86–98 (2005).
213. Dzhura, I., Wu, Y., Colbran, R. J., Balser, J. R. & Anderson, M. E. Calmodulin kinase determines calcium-dependent facilitation of L-type calcium channels. *Nature Cell Biol.* **2**, 173–177 (2000).
214. Witcher, D. R., Kovacs, R. J., Schulman, H., Cefali, D. C. & Jones, L. R. Unique phosphorylation site on the cardiac ryanodine receptor regulates calcium channel activity. *J. Biol. Chem.* **266**, 11144–11152 (1991).
215. Kohlhaas, M. *et al.* Increased sarcoplasmic reticulum calcium leak but unaltered contractility by acute CaMKII overexpression in isolated rabbit cardiac myocytes. *Circ. Res.* **98**, 235–244 (2006).
216. Maier, L. S. *et al.* Transgenic CaMKIIδC overexpression uniquely alters cardiac myocyte Ca^{2+} handling: reduced SR Ca^{2+} load and activated SR Ca^{2+} release. *Circ. Res.* **92**, 904–911 (2003).
217. Simmerman, H. K., Collins, J. H., Theibert, J. L., Wegener, A. D. & Jones, L. R. Sequence analysis of phospholamban. Identification of phosphorylation sites and two major structural domains. *J. Biol. Chem.* **261**, 13333–13341 (1986).
218. Wagner, S. & Maier, L. S. Modulation of cardiac Na(+) and Ca(2+) currents by CaM and CaMKII. *J. Cardiovasc. Electrophysiol.* **17** (Suppl. 1), 26–33 (2006).
219. Ai, X., Curran, J. W., Shannon, T. R., Bers, D. M. & Pogwizd, S. M. Ca^{2+}/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. *Circ. Res.* **97**, 1314–1322 (2005).
220. Anderson, M. E. The fire from within: the biggest Ca^{2+} channel erupts and dribbles. *Circ. Res.* **97**, 1213–1215 (2005).
221. Wu, Y. *et al.* Calmodulin kinase II and arrhythmias in a mouse model of cardiac hypertrophy. *Circulation* **106**, 1288–1293 (2002).
222. Wu, X. *et al.* Local InsP3-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. *J. Clin. Invest.* **116**, 675–682 (2006).
223. Backs, J., Song, K., Bezprozvannaya, S., Chang, S. & Olson, E. N. CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J. Clin. Invest.* **116**, 1853–1864 (2006).
224. Anderson, M. E., Higgins, L. S. & Schulman, H. Disease mechanisms and emerging therapies: protein kinases and their inhibitors in myocardial disease. *Nature Clin. Pract. Cardiovasc. Med.* **3**, 437–445 (2006).
225. Hashimoto, Y. *et al.* Potent and preferential inhibition of Ca^{2+}/calmodulin-dependent protein kinase II by K252a and its derivative, KT5926. *Biochem. Biophys. Res. Commun.* **181**, 423–429 (1991).
226. Rosenberg, O. S., Deindl, S., Sung, R. J., Nairn, A. C. & Kuriyan, J. Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. *Cell* **123**, 849–860 (2005).
227. Witte, S. *et al.* Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-κB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *J. Biol. Chem.* **275**, 1902–1909 (2000).
228. Jeong, D. *et al.* PICOT inhibits cardiac hypertrophy and enhances ventricular function and cardiomyocyte contractility. *Circ. Res.* **99**, 307–314 (2006).
229. Chen, L. *et al.* Opposing cardioprotective actions and parallel hypertrophic effects of δPKC and εPKC. *Proc. Natl Acad. Sci. USA* **98**, 11114–11119 (2001).
230. Mochly-Rosen, D. *et al.* Cardiotrophic effects of protein kinase C ε: analysis by *in vivo* modulation of PKCε translocation. *Circ. Res.* **86**, 1173–1179 (2000).
231. Wu, G., Toyokawa, T., Hahn, H. & Dorn, G. W. Epsilon protein kinase C in pathological myocardial hypertrophy. Analysis by combined transgenic expression of translocation modifiers and $G\alpha^9$. *J. Biol. Chem.* **275**, 29927–29930 (2000).
232. Hahn, H. S. *et al.* Ischemic protection and myofibrillar cardiomyopathy: dose-dependent effects of *in vivo* δPKC inhibition. *Circ. Res.* **91**, 741–748 (2002).
233. Klein, G. *et al.* Increased collagen deposition and diastolic dysfunction but preserved myocardial hypertrophy after pressure overload in mice lacking PKCε. *Circ. Res.* **96**, 748–755 (2005).
234. Tuttle, K. R. *et al.* The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* **28**, 2686–2690 (2005).
235. The PKC-DRS Study Group. The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: initial results of the protein kinase C β inhibitor diabetic retinopathy study (PKC-DRS) multicenter randomized clinical trial. *Diabetes* **54**, 2188–2197 (2005).
236. Boyle, A. J. *et al.* Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction. *J. Mol. Cell. Cardiol.* **39**, 213–221 (2005).
This demonstrated a beneficial effect of a small-molecule inhibitor of PKC in the prevention of post-MI remodelling in rats.
237. Davies, S. P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**, 95–105 (2000).
238. Roman, B. B., Geenen, D. L., Leitges, M. & Buttrick, P. M. PKC-β is not necessary for cardiac hypertrophy. *Am. J. Physiol Heart Circ. Physiol.* **280**, H2264–H2270 (2001).
239. Hahn, H. S. *et al.* Protein kinase Cα negatively regulates systolic and diastolic function in pathological hypertrophy. *Circ. Res.* **93**, 1111–1119 (2003).
240. Braz, J. C. *et al.* PKC-α regulates cardiac contractility and propensity toward heart failure. *Nature Med.* **10**, 248–254 (2004).
241. Bowling, N. *et al.* Increased protein kinase C activity and expression of Ca^{2+}-sensitive isoforms in the failing human heart. *Circulation* **99**, 384–391 (1999).
242. Hambleton, M. *et al.* Pharmacological- and gene therapy-based inhibition of protein kinase Cα/β enhances cardiac contractility and attenuates heart failure. *Circulation* **114**, 574–582 (2006).
This demonstrated that small-molecule inhibitors of PKC enhance cardiac contractility in animal models.
243. McKinsey, T. A. & Olson, E. N. Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. *J. Clin. Invest.* **115**, 538–546 (2005).
244. Verdin, E., Dequiedt, F. & Kasler, H. G. Class II histone deacetylases: versatile regulators. *Trends Genet.* **19**, 286–293 (2003).
245. Chang, S. *et al.* Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. *Mol. Cell. Biol.* **24**, 8467–8476 (2004).
246. Zhang, C. L. *et al.* Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* **110**, 479–488 (2002).
247. Han, A., He, J., Wu, Y., Liu, J. O. & Chen, L. Mechanism of recruitment of class II histone deacetylases by myocyte enhancer factor-2. *J. Mol. Biol.* **345**, 91–102 (2005).
248. Dai, Y. S., Xu, J. & Molkentin, J. D. The DnaJ-related factor Mrj interacts with nuclear factor of activated T cells c3 and mediates transcriptional repression through class II histone deacetylase recruitment. *Mol. Cell Biol.* **25**, 9936–9948 (2005).
249. Xing, W. *et al.* Myocardin induces cardiomyocyte hypertrophy. *Circ. Res.* **98**, 1089–1097 (2006).
250. Sucharov, C. C., Langer, S. J., Bristow, M. R. & Leinwand, L. A. Shuttling of HDAC5 in H9C2 cells regulates YY1 function through CaMKIV/PKD and PP2A. *Am. J. Physiol. Cell. Physiol.* **291**, C1029–C1037 (2006).
251. Song, K. *et al.* The transcriptional coactivator CAMTA2 stimulates cardiac growth by opposing class II histone deacetylases. *Cell* **125**, 453–466 (2006).
252. Grozinger, C. M. & Schreiber, S. L. Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. *Proc. Natl Acad. Sci. USA* **97**, 7835–7840 (2000).
253. McKinsey, T. A., Zhang, C. L. & Olson, E. N. Activation of the myocyte enhancer factor-2 transcription factor by calcium/calmodulin-dependent protein kinase-stimulated binding of 14-3-3 to histone deacetylase 5. *Proc. Natl Acad. Sci. USA* **97**, 14400–14405 (2000).
254. McKinsey, T. A., Zhang, C. L., Lu, J. & Olson, E. N. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* **408**, 106–111 (2000).
255. McKinsey, T. A., Zhang, C. L. & Olson, E. N. Identification of a signal-responsive nuclear export sequence in class II histone deacetylases. *Mol. Cell. Biol.* **21**, 6312–6321 (2001).
256. Wang, A. H. *et al.* Regulation of histone deacetylase 4 by binding of 14-3-3 proteins. *Mol. Cell. Biol.* **20**, 6904–6912 (2000).
257. Harrison, B. C. *et al.* The CRM1 nuclear export receptor controls pathological cardiac gene expression. *Mol. Cell. Biol.* **24**, 10636–10649 (2004).
258. Vega, R. B. *et al.* Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5. *Mol. Cell. Biol.* **24**, 8374–8385 (2004).
259. Harrison, B. C. *et al.* Regulation of cardiac stress signaling by protein kinase D1. *Mol. Cell. Biol.* **26**, 3875–3888 (2006).
260. Gschwendt, M. *et al.* Inhibition of protein kinase C μ by various inhibitors. Differentiation from protein kinase c isoenzymes. *FEBS Lett.* **392**, 77–80 (1996).
261. Cohen, P. Protein kinases — the major drug targets of the twenty-first century? *Nature Rev. Drug Discov.* **1**, 309–315 (2002).
262. Rozengurt, E., Rey, O. & Waldron, R. T. Protein kinase D signaling. *J. Biol. Chem.* **280**, 13205–13208 (2005).
263. Antos, C. L. *et al.* Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J. Biol. Chem.* **278**, 28930–28937 (2003).
264. Kee, H. J. *et al.* Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* **113**, 51–59 (2006).
Together with reference 265, demonstrated that small-molecule inhibitors of HDACs are efficacious in the prevention and reversal of pathological cardiac hypertrophy in animal models.
265. Kong, Y. *et al.* Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* **113**, 2579–2588 (2006).
266. Kook, H. *et al.* Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. *J. Clin. Invest* **112**, 863–871 (2003).
267. Danzi, S., Dubon, P. & Klein, I. Effect of serum triiodothyronine on regulation of cardiac gene expression: role of histone acetylation. *Am. J. Physiol Heart Circ. Physiol.* **289**, H1506–H1511 (2005).
268. Davis, F. J., Pillai, J. B., Gupta, M. & Gupta, M. P. Concurrent opposite effects of trichostatin A, an inhibitor of histone deacetylases, on expression of α-MHC and cardiac tubulins: implication for gain in cardiac muscle contractility. *Am. J. Physiol Heart Circ. Physiol.* **288**, H1477–H1490 (2005).
269. Braunwald, E. & Bristow, M. R. Congestive heart failure: fifty years of progress. *Circulation* **102**, IV14-IV23 (2000).
270. Fischle, W. *et al.* Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol. Cell* **9**, 45–57 (2002).
271. Zhang, C. L., McKinsey, T. A., Lu, J. R. & Olson, E. N. Association of COOH-terminal-binding protein (CtBP) and MEF2-interacting transcription repressor (MITR) contributes to transcriptional repression of the MEF2 transcription factor. *J. Biol. Chem.* **276**, 35–39 (2001).

272. Zhang, C. L., McKinsey, T. A. & Olson, E. N. Association of class II histone deacetylases with heterochromatin protein 1: potential role for histone methylation in control of muscle differentiation. *Mol. Cell. Biol.* **22**, 7302–7312 (2002).
273. Han, A. *et al.* Sequence-specific recruitment of transcriptional co-repressor Cabin1 by myocyte enhancer factor-2. *Nature* **422**, 730–734 (2003).
274. Youn, H. D., Grozinger, C. M. & Liu, J. O. Calcium regulates transcriptional repression of myocyte enhancer factor 2 by histone deacetylase 4. *J. Biol. Chem.* **275**, 22563–22567 (2000).
275. Alcendor, R. R., Kirshenbaum, L. A., Imai, S., Vatner, S. F. & Sadoshima, J. Silent information regulator 2α, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. *Circ. Res.* **95**, 971–980 (2004).
276. Trivedi, C. M. *et al.* HDAC2 regulates the cardiac hypertrophic response by modulating GSK3β activity. *Nature Med.* **13**, 324–331 (2007).
277. Minagawa, T., Ijuin, T., Mochizuki, Y. & Takenawa, T. Identification and characterization of a sac domain-containing phosphoinositide 5-phosphatase. *J. Biol. Chem.* **276**, 22011–22015 (2001).
278. Glozak, M. A., Sengupta, N., Zhang, X. & Seto, E. Acetylation and deacetylation of non-histone proteins. *Gene* **363**, 15–23 (2005).
279. Heineke, J. & Molkentin, J. D. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nature Rev. Mol. Cell Biol.* **7**, 589–600 (2006).
280. Behr, T. M. *et al.* Hypertensive end-organ damage and premature mortality are p38 mitogen-activated protein kinase-dependent in a rat model of cardiac hypertrophy and dysfunction. *Circulation* **104**, 1292–1298 (2001).
281. Kyoi, S. *et al.* Opposing effect of p38 MAP kinase and JNK inhibitors on the development of heart failure in the cardiomyopathic hamster. *Cardiovasc. Res.* **69**, 888–898 (2006).
282. Liu, Y. H. *et al.* Inhibition of p38 mitogen-activated protein kinase protects the heart against cardiac remodeling in mice with heart failure resulting from myocardial infarction. *J. Card. Fail.* **11**, 74–81 (2005).
283. Zhang, S. *et al.* Role of 14-3-3-mediated p38 mitogen-activated protein kinase inhibition in cardiac myocyte survival. *Circ. Res.* **93**, 1026–1028 (2003).
284. Beckles, D. L., Mascareno, E. & Siddiqui, M. A. Inhibition of Jak2 phosphorylation attenuates pressure overload cardiac hypertrophy. *Vascul. Pharmacol.* **45**, 350–357 (2006).
285. Palfi, A. *et al.* PARP inhibition prevents postinfarction myocardial remodeling and heart failure via the protein kinase C/glycogen synthase kinase-3β pathway. *J. Mol. Cell. Cardiol.* **41**, 149–159 (2006).
286. Sano, M. *et al.* Activation and function of cyclin T-Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. *Nature Med.* **8**, 1310–1317 (2002).
287. Sausville, E. A. Cyclin-dependent kinase modulators studied at the NCI: pre-clinical and clinical studies. *Curr. Med. Chem. Anticancer Agents* **3**, 47–56 (2003).
288. Oh, H. *et al.* Telomere attrition and CHK2 activation in human heart failure. *Proc. Natl Acad. Sci. USA* **100**, 5378–5383 (2003).
289. Arienti, K. L. *et al.* Checkpoint kinase inhibitors: SAR and radioprotective properties of a series of 2-arylbenzimidazoles. *J. Med. Chem.* **48**, 1873–1885 (2005).
290. Harris, I. S. *et al.* Raf-1 kinase is required for cardiac hypertrophy and cardiomyocyte survival in response to pressure overload. *Circulation* **110**, 718–723 (2004).
291. Schreck, R. & Rapp, U. R. Raf kinases: oncogenesis and drug discovery. *Int. J. Cancer* **119**, 2261–2271 (2006).
292. Davidson, S. M. *et al.* The transcriptional coactivator p300 plays a critical role in the hypertrophic and protective pathways induced by phenylephrine in cardiac cells but is specific to the hypertrophic effect of urocortin. *Chembiochem.* **6**, 162–170 (2005).
293. Lau, O. D. *et al.* HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. *Mol. Cell* **5**, 589–595 (2000).
294. Owan, T. E. *et al*. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N. Engl. J. Med.* **355**, 251–259 (2006).
295. Klapholz, M. *et al*. Hospitalization for heart failure in the presence of a normal left ventricular ejection fraction: results of the New York Heart Failure Registry. *J. Am. Coll. Cardiol.* **43**, 1432–1438 (2004).
296. Fatkin, D. *et al*. An abnormal Ca(2+) response in mutant sarcomere protein-mediated familial hypertrophic cardiomyopathy. *J. Clin. Invest*. **106**, 1351–1359 (2000).

Acknowledgements
T.A.M. thanks E. Bush for helpful discussions and L. Castonguay for assistance with the compound structures. D.A.K. is supported by NIH-PO1-HL-077180 and PO1-HL-59408 grants, and the Peter Belfer Laboratory Research Fund.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
The following terms in this article are linked online to:
David A. Kass's homepage:
http://www.hopkinshospital.org/Faculty/Heart/Kass.html
Gilead: http://www.gilead.com
Access to this interactive links box is free online.