To mark the 60th birthday of Circulation Research (1953–2013), the editors have commissioned Circulation Research Classics, a series of commentaries highlighting seminal articles published in the journal during the past 6 decades that have importantly shaped cardiovascular research. Written by leading experts, Circulation Research Classics are intended to describe the impact of these articles on the field by putting them in a historical perspective. The concept of classic is inextricably linked to time—a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of Circulation Research, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

 Parsing Good Versus Bad Signaling Pathways in the Heart
Role of Calcineurin–Nuclear Factor of Activated T-Cells

Jeffery D. Molkentin

Calcineurin/NFAT Coupling Participates in Pathological, but Not Physiological, Cardiac Hypertrophy
Wilkins et al

Nine years ago, we published an article that suggested a specialized role for calcineurin–nuclear factor of activated T-cells (NFAT) signaling in regulating pathological cardiac hypertrophy preferentially over physiological growth and, in fact, the later response was associated with reduced calcineurin–NFAT activity. Since this time we and others have continued to uncover how this signaling effector pathway functions in the heart in regulating specific aspects of the growth response during disease and with exercise.

Pathological cardiac hypertrophy is associated with neuro-endocrine dysregulation after myocardial infarction injury or in response to long-standing hypertension, as well as in response to a myriad of other disease-causing stimuli, whereas physiological hypertrophy of the heart occurs with intense exercise training.1,2 Although the heart enlarges under both pathological and physiological stimuli, pathological hypertrophy causes negative remodeling of the ventricles and often extreme concentric or eccentric hypertrophy, whereas exercise typically produces a mild 10% to 20% growth of the ventricles in a uniform manner.1,2 The molecular pathways that mediate these 2 responses, as well as the downstream effectors and associated adaptations of the heart, are different. In the broadest sense, physiological cardiac hypertrophy seems to be proximally mediated by insulin-like growth factor-1 signaling through phosphoinositide 3-kinase and Akt and mammalian target of rapamycin, although other minor effectors/pathways can also have specific role in regulating aspects of physiological cardiac hypertrophy. For a more comprehensive review of physiological cardiac hypertrophy and the role of phosphoinositide 3-kinase–Akt and signaling effectors, please see the recent review by Maillet et al.1 In contrast to physiological cardiac hypertrophy, a much larger array of specialized signaling pathways, such as calcineurin–NFAT, seem to underlie pathological cardiac hypertrophy and negative remodeling of the ventricles (Figure).1,2

That divergent signaling pathways can underlie pathological versus physiological cardiac hypertrophy was suggested by Wilkins et al1 when they observed that exercise-induced growth occurred in the absence of induction of the fetal gene program classically associated with the development of pathological hypertrophy. The pathology-induced fetal gene program includes induction of mRNA for atrial natriuretic factor, b-type natriuretic peptide, skeletal α-actin, and β-myosin heavy chain.1,2 Wilkins et al1 showed that these pathological marker genes were induced by transverse aortic constriction (TAC) in mice, but not after swimming exercise or growth hormone/insulin-like growth factor-1 injections. Indeed, Konhilas et al4 also showed that exercise-induced physiological remodeling of the heart was associated with downregulation of the pathological fetal gene program and suppression of NFAT activity. These very simple observations suggested that physiological growth of the myocardium uses specialized regulatory pathways and signaling circuits compared with many of the disease-causing pathways that are associated with induction of the fetal gene program. For example, signaling through calcineurin–NFAT leads to induction of b-type natriuretic peptide and β-myosin heavy chain expression in cardiac myocytes, which may be a direct effect because NFAT has been shown to bind the promoters of these 2 genes.5,6

The Ca2+-dependent serine–threonine protein phosphatase calcineurin was identified as a central prohypertrophic signaling effector in the myocardium by us ≈15 years ago.5 Calcineurin is the target of 2 widely used immunosuppressant drugs, cyclosporin A and FK506, which exert their effect by
agents for treating hypertrophic heart disease given that much higher dosing is required that can lead to severe side effects.7 More definitively, we showed that cardiac-specific expression of the noncompetitive calcineurin inhibitory domain from A-kinase anchoring protein 79 or Cabin-1 reduced myocardial hypertrophy after pathological agonist infusion or TAC stimulation.15 Similarly, transgene-mediated overexpression of regulator of calcineurin 1 (previously known as MCIP1) also inhibited cardiac hypertrophy to diverse pathological stimuli.12,13 Analogous to regulator of calcineurin 1 overexpression, transgene-mediated expression of a dominant negative mutant of calcineurin in the heart reduced pathological cardiac hypertrophy in mice.14 Finally, CnAβ (Ppp3cb) gene–targeted mice, which had a 70% reduction in total cardiac calcineurin activity, were generated by us and shown to have blunted hypertrophy after 2 weeks of angiotensin II or isoproterenol infusion, as well as after TAC stimulation.15 With respect to gain-of-function studies, mice expressing an activated calcineurin mutant protein in the heart developed massive cardiac hypertrophy with negative ventricular remodeling,7 whereas mice lacking the calcineurin docking and inhibitory protein calcsarcin-1 showed greater endogenous calcineurin activity and were sensitized to negative cardiac remodeling and greater disease with pressure overload stimulation.16 Collectively, these genetic approaches have established the role of calcineurin–NFAT signaling as an obligate regulator of the pathological hypertrophic response.

More recently, we also generated mice with an inducible loss of all calcineurin activity in the heart by cardiac-specific deletion of the CnB1 (Ppp3r1) gene.17 Although we were not able to assess hypertrophy in these mice after pathological pressure overload stimulation because of acute lethality, the baseline phenotype actually suggested that calcineurin can have some adaptive and protective signaling functions in the heart. Specifically, cardiac-specific CnB1-loxp–deleted mice showed reduced myocyte proliferation rates with fewer cells.17 These results are also consistent with greater myocyte apoptosis rates in the absence of CnB1 in the heart with acute pressure overload,17 as well as greater cell death and injury after ischemia-reperfusion injury in CnAβ-deleted mice.18 Moreover, transgenic mice expressing an activated calcineurin mutant were protected from cell death after ischemia-reperfusion injury, despite the underlying pathological hypertrophic phenotype.19 Thus, although calcineurin–NFAT signaling contributes to and is necessary for pathological cardiac hypertrophy, this pathway also has some degree of physiological importance in protecting from cell death and permitting myocyte proliferation.

A number of equally supportive studies have emerged with respect to NFAT and its role in the heart. Interestingly Nfatc1 gene–deleted mice die during embryogenesis from a defect in heart valve maturation.20,21 With respect to cardiac hypertrophy induced by pathological stimuli, mice lacking either Nfatc2 or Nfatc3, but not Nfatc4, showed a significant reduction in the response.22,23 Moreover, mice with inhibited c-Jun N-terminal kinase or p38α mitogen–activated protein kinase signaling in the heart, which directly resulted in greater NFAT activity (they are both important NFAT kinases that inhibit its activity), showed greater cardiac hypertrophy after pathological hypertrophy partici-
stimulation. Finally, mice lacking Mkk4, which lies upstream of p38 and c-Jun N-terminal kinase signaling, showed increased NFAT activity and greater pathological cardiac hypertrophy, but swimming-induced cardiac hypertrophy was unaffected.

The Wilkins et al article was the first to suggest that calcineurin–NFAT signaling was more highly specialized in the adult heart for inducing cardiac hypertrophy and negative ventricular remodeling in response to pathological insults. At that time, it was also one the first articles describing a genetic tool allowing the direct in vivo assessment of the activity of a transcription factor. The transgenic line we generated contained an NFAT-dependent luciferase transcriptional reporter consisting of 9 multimerized NFAT-binding sites upstream of a minimal promoter, which we showed specifically responded to calcineurin–NFAT signaling in vivo. For example, when this reporter transgenic line was crossed with mice containing the activated calcineurin transgene, NFAT activity was increased >15-fold in a cyclosporin A-inhibitable manner. The NFAT reporter transgene was also significantly induced in the adult heart at essentially all stages of TAC stimulation, both early (2 days) and late when the hearts transitioned to failure (56 days). The reporter was also activated after MI injury to the heart, both the early compensatory phase and later when the hearts began to fail. However, this reporter was not stimulated in the adult heart after either swimming or voluntary wheel running-induced physiological hypertrophy and, in fact, it was significantly repressed. This later result suggested that reductions in calcineurin–NFAT signaling were part of a more adaptive profile of physiological hypertrophy of the myocardium to exercise stimulation. Consistent with these data, we also showed that insulin-like growth factor-1 or an activated Akt mutant, which are known regulators of adaptive or physiological hypertrophy, did not induce the NFAT-luciferase reporter in cultured myocytes, but in fact likely repressed it. Finally, we also showed that Cnβ−/− mice, which are largely refractory to hypertrophy after pathological stimulation as discussed earlier, hypertrophied normally after insulin-like growth factor 1–growth hormone injections during 2 weeks time, further indicating that calcineurin–NFAT signaling does not underlie adaptive or physiological growth of the heart in vivo.

Since our report in 2004, others have used the NFAT-luciferase reporter transgenic mice and confirmed our results. For example, the reporter is significantly activated within the heart with pathological pressure overload stimulation, but in fact, it was significantly repressed. This later result suggested that reductions in calcineurin–NFAT signaling were part of a more adaptive profile of physiological hypertrophy of the myocardium to exercise stimulation. Consistent with these data, we also showed that insulin-like growth factor-1 or an activated Akt mutant, which are known regulators of adaptive or physiological hypertrophy, did not induce the NFAT-luciferase reporter in cultured myocytes, but in fact likely repressed it. Finally, we also showed that Cnβ−/− mice, which are largely refractory to hypertrophy after pathological stimulation as discussed earlier, hypertrophied normally after insulin-like growth factor 1–growth hormone injections during 2 weeks time, further indicating that calcineurin–NFAT signaling does not underlie adaptive or physiological growth of the heart in vivo.

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