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CAMTA in Cardiac Hypertrophy

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In this issue of *Cell*, the Calmodulin binding transcription activator 2 (CAMTA2), is shown by Song et al. (2006) to be an indispensable transcription coactivator for cardiac hypertrophy. CAMTA2 is activated by the dissociation of class II histone deacetylase 5 and promotes transcription of genes involved in cardiac hypertrophy through its interaction with Nkx2-5.

For adult cardiac muscle—the single most common target of disease in the elderly—the characteristic response to injury is myocyte hypertrophy (Olson and Schneider, 2003). However, myocyte hypertrophy is much more than a global growth response and includes other stereotypic features such as intrinsic myocyte dysfunction that is due in part to transcriptional reprogramming. This reprogramming leads to the reactivation of cardiac-specific genes associated with the fetal myocardium and the downregulation of several essential genes whose normal expression is highest in adult hearts. Depending on the severity, duration, and type of instigating signal, a second hallmark of hypertrophy can be progression to a "heart failure" phenotype, with ventricular wall thinning, chamber dilation, and, typically, ongoing myocyte death through apoptosis. In short, in heart disease, the myocytes are both depressed and suicidal.

Over the past two decades, a principal objective in cardiac biology has been to comprehend in molecular detail how pathological signals such as high blood pressure, or the signals that compensate for the ischemic loss of myocytes in heart attacks, lead to and orchestrate the observed ensemble of genomic responses. Many signaling modules have been implicated as sufficient or, more rarely, as necessary for hypertrophy. Many of these, in turn, share three properties. The first is extensive crosstalk and interconnection, posing challenges that are common to studies of signal transduction in any context. The second, more uniquely, is that many hypertrophic signaling cascades ultimately engage the cellular circuits that control growth and gene expression in the embryonic heart. A third, established in large part by Eric Olson and colleagues, is the role of intracellular calcium, involving the calcium binding protein calmodulin, calcium-dependent export of particular corepressors (such as class II histone deacetylases), and other calcium-dependent events that have eluded definition.

In this issue of *Cell*, Olson and colleagues report their discovery of a new regulator of cardiac gene expression and growth (Song et al., 2006). Using a eukaryotic expression screen for cDNAs that activate the *atrial natriuretic factor (ANF)* promoter, a cardiac-specific marker of hypertrophy and pathological remodeling, the authors hit upon a potent inducer, called calmodulin binding transcription activator 2 (CAMTA2), one of a family of previously described but little-studied proteins that are conserved from plants to humans. They were first discovered in plants as stress-responsive regulators of gene transcription that respond to calcium/calmodulin (Yang and Poovaiah, 2002; Bouche et al., 2002). The existence of two *CAMTA* genes was known for mice and humans, but their functions were utterly unknown. Both are most highly expressed in heart and brain, with relative expression in heart highest for CAMTA2.

Interestingly, the physical recruitment of CAMTAs to the *ANF* promoter occurred indirectly, at least in part, through association with an essential cardiac homeodomain protein, Nkx2-5, and CAMTA2 hence acted as a coactivator for Nkx2-5. The Nkx2-5 binding site in the *ANF* promoter was required for maximal activation by CAMTA2. A single copy of the Nkx2-5 binding site was sufficient to confer CAMTA responsiveness to a basal promoter, and mutations blocking the protein-protein interaction abolished the ability of CAMTA2 to support Nkx2-5-dependent ANF induction.

Nkx2-5 is the mouse ortholog of *Tinman*, a homeobox gene that is obligatory for the formation of the rudimentary heart tube in flies. Although it is dispensable in mice for cardiac myogenesis, perhaps because of family members with overlapping expression, Nkx2-5 is nevertheless essential in mice for normal cardiac morphogenesis to transpire, and mutations in Nkx2-5 cause a spectrum of cardiac abnormalities in humans (reviewed in Prall et al., 2002). However, evidence implicating Nkx2-5 in the control of cardiac growth is problematic in a developmental context because altered cardiac muscle mass might reflect altered cell fate decisions or be a secondary response to dysmorphogenesis and the resultant abnormal hemodynamics. Hence, it is intriguing that Olson's study suggests that the induction of cardiac hypertrophy by CAMTA2 was medi-

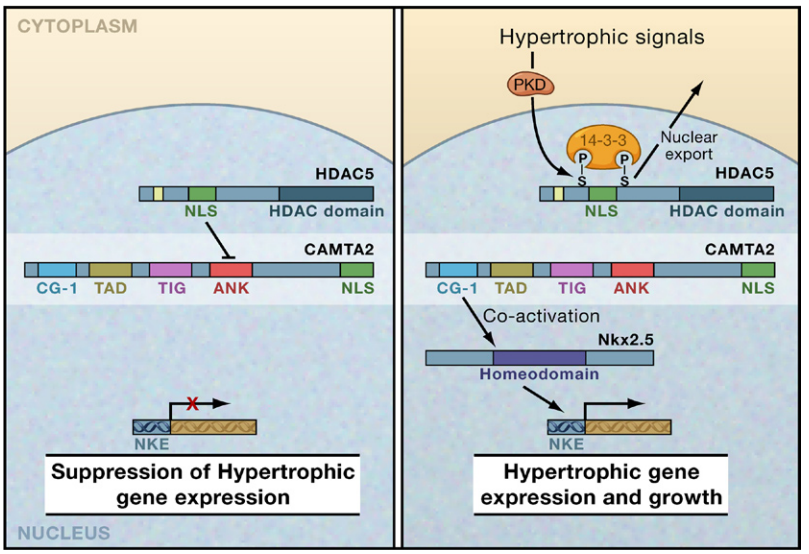

Figure 1. CAMTA2 in Hypertrophic Signaling in the Heart

(Left panel) Based on Song et al. (2006), CAMTA2 is repressed by an association with class II histone deacetylases (HDACs). (Right panel) Activation of protein kinase D (PKD) signaling leads to phosphorylation of class II HDACs, which create docking sites for 14-3-3 proteins and their nuclear export, thus releasing CAMTA2 from repression and promoting cardiac growth. The interaction between the CG-1 domain of CAMTA2 and the homeodomain of Nkx2-5 activates downstream cardiac hypertophic gene targets via binding to the Nkx2-5-response element (NKE). CAMTA2 domains defined by mutagenesis and functional analysis are as follows: CG-1 is involved in Nkx2-5 binding and nuclear export, TAD is required for transcriptional activation, TIG stabilizes the interaction with Nkx2-5, ANK is an ankyrin repression domain that binds HDAC5, and the nuclear localization signal (NLS) functions in nuclear import.

ated, at least in part, through its association with Nkx2-5. The association of transcription factors with coactivators (and corepressors) allows for signal-dependent regulation of gene expression and expands the regulatory potential of *cis*-acting DNA sequences as a consequence of combinatorial protein-protein interactions. The present findings do not exclude other possibilities, including the potential for CAMTA to bind the DNA sequence, CGCG (Yang and Poovaiah, 2002), which might stabilize its interactions with Nkx2-5 and other cofactors. Notably, because Nkx2-5 associates with many cardiogenic transcription factors, including SRF, GATA4, and Tbx5 (Chen and Schwartz, 1996; Durocher et al., 1997; Hiroi et al., 2001), it will be important to determine if CAMTA can activate Nkx2-5-dependent transcription in those contrasting settings where Nkx2-5 is tethered to DNA indirectly via these other factors. In addition, as CAMTA and many other factors physically interact with the homeodomain of Nkx2-5, it will be intriguing to learn which interactions can coexist and which, if any, are mutually exclusive. CAMTA2 did not show appreciable expression in the heart until after birth, whereas CAMTA1 was strongly expressed in the embryonic heart (Song et al., 2006); given their high degree of sequence conservation, it is surmised (but not yet proven) that CAMTA1 will modulate the developmental functions of Nkx2-5.

Multiple functional domains were found in CAMTA2 that are evolutionarily conserved (Figure 1). These include the CG-1 domain (required for association of CAMTA2 with Nkx2-5 and activation of Nkx2-5-dependent promoters), the TIG domain (which may stabilize the interaction with Nkx2-5), the IQ motifs (which bind calmodulin, in the case of plant CAMTA proteins, yet are dispensable for trans-activation here; Bouche et al., 2002; Yang and Poovaiah, 2002), and an ankyrin repeat. The ankyrin repeat of CAMTA2 associates with class II HDACs and negatively modulates the function of the trans-activation domain, much as in other ankyrin-repeat-containing transcriptional activators (McKinsey et al., 2006).

Through gain- and loss-of-function approaches in vivo and in vitro, Olson and colleagues show persuasively that class II HDACs restrain trans-activation by CAMTA proteins. Nuclear export of class II HDACs in response to signaling by protein kinase C (PKC) and PKD releases CAMTAs from HDAC-dependent repression and consequently induces the genes for cardiac hypertrophy. By constrast, a mutant of HDAC that is "signal resistant" because it lacks the phosphorylation sites for nuclear export blocks CAMTA activity even in the face of PKC signaling. Elevating CAMTA2 expression by adenoviral gene transfer caused cardiomyocytes in culture to become both larger and more numerous. Elevating CAMTA2 in transgenic mice, using the heart-specific α-myosin heavy chain (MHC) promoter, caused cardiac hypertrophy as assessed by wall thickness, cell size, and molecular markers, and all transgenic animals died by 12 weeks of age, with dilated cardiomyopathy and heart failure. Despite equal or greater expression of the transgene-encoded protein, mice expressing the Δ206 CAMTA2 mutant, which cannot associate with Nkx2-5, did not develop hypertrophy. Conversely, hypertrophy induced by expression of CAMTA2 by the αMHC promoter was potentiated in mice lacking HDAC5, whereas mice lacking CAMTA2 showed diminished hypertrophy in response to constriction of the aorta (which causes increased workload) and the infusion of the hypertropic agonists, angiotensin II or isoproterenol.

Thus, CAMTA2 was found to be an essential signal-responsive transcriptional coactivator for cardiac hypertrophy that becomes activated by the dissociation of class II HDACs. Based on the authors' prior work on calcium-dependent export of HDACs from the nucleus, it is implied, quite convincingly, that CAMTA proteins

likely function in the pathway for "excitation-transcription coupling" in the heart. Inositol 1,4,5-trisphosphate receptors in the nuclear envelope mediate spatially restricted calcium release, which is instrumental to this process (Wu et al., 2006).

The present report in *Cell* poses as many exciting questions as it answers—a very fair and rewarding trade. How does CAMTA2 engage a growth program—is Nkx2-5 or some other DNA bound factor the critical intermediate for growth? Given the many growth factors that are induced or activated in hypertrophic cells, are the trophic effects of CAMTA exclusively cell autonomous? Does CAMTA2 participate in those aspects of hypertrophy that were not yet studied (the predisposition to cell death, the dysregulation of nuclear genes for mitochondrial proteins, the loss of αMHC and calcium-handling proteins) or in those modes of hypertrophy referred to as more "physiological" because they are triggered by exercise or the IGF-PI3K-Akt pathway? Even if CAMTA2 and CAMTA1 are functionally indistinguishable as ascertained in vitro, the differential expression of CAMTA1 (in the early stages of heart development) versus CAMTA2 (at later ages) prompts the speculation that CAMTA1 might have the more obvious place in both cardiac development and in human congenital heart disease.

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Forcing the Third Dimension

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One goal of biomedical research is to reliably construct surrogate tissues for replacement therapy and to promote tissue regeneration. In this issue of *Cell*, Chun et al. (2006) provide insight into the molecular basis of tissue-specific differentiation. The authors show that remodeling of the extracellular matrix by the matrix metalloproteinase MT1-MMP contributes to the three-dimensional development of white adipose tissue in mice.

Tissue development proceeds within the context of an organized extracellular matrix (ECM) microenvironment and is temporally controlled by transcriptional programs initiated by soluble and insoluble cues. Tissues are three dimensional (3D) and exert—and are subject to—forces that profoundly alter their behavior. Dynamic extrinsic and intrinsic forces modify cell fate by influencing membrane-receptor and ion-channel activity, by altering cytoskeletal organization and nuclear shape, and by modulating tissue organization and gene expression (for review, see Orr et al., 2006). As development proceeds, increasingly elaborate and specialized 3D tissue structures are assembled, with the cytoarchitecture and tissue-specific forces in each organ reflecting the specialized function of the tissue (for review, see Nelson et al., 2005). As each specialized tissue develops, it produces a unique biochemical and organizational ECM in which it becomes enmeshed. The ECM in turn influences the ability of the tissue to sense the magnitude and duration of extrinsic forces. This force then impacts signaling cascades that direct expression of genes important for differentiation. Accordingly, the biochemical