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Gene Expression in Fibroblasts and Fibrosis: Involvement in Cardiac Hypertrophy
Roles of Cardiac Transcription Factors in Cardiac Hypertrophy
Ras, Akt, and Mechanotransduction in the Cardiac Myocyte

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Ryozo Nagai, Guest Editor

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An RNAPII Kinase at the Nexus of Cardiac Growth and Death Cascades

Motoaki Sano, Michael D. Schneider

Abstract—Over the past decade and a half, the paradigm has emerged of cardiac hypertrophy and ensuing heart failure as fundamentally a problem in signal transduction, impinging on the altered expression or function of gene-specific transcription factors and their partners, which then execute the hypertrophic phenotype. Strikingly, RNA polymerase II (RNAPII) is itself a substrate for two protein kinases—the cyclin-dependent kinases Cdk7 and Cdk9—that are activated by hypertrophic cues. Phosphorylation of RNAPII in the carboxyl terminal domain (CTD) of its largest subunit controls a number of critical steps subsequent to transcription initiation, among them enabling RNAPII to overcome its stalling in the promoter–proximal region and to engage in efficient transcription elongation. Here, we summarize our current understanding of the RNAPII-directed protein kinases in cardiac hypertrophy. Cdk9 activation is essential in tissue culture for myocyte enlargement and sufficient in transgenic mice for hypertrophy to occur and yet is unrelated to the "fetal" gene program that is typical of pathophysiological heart growth. Although this trophic effect of Cdk9 appears benign superficially, pathophysiological levels of Cdk9 activity render myocardium remarkably susceptible to apoptotic stress. Cdk9 interacts adversely with Gq-dependent pathways for hypertrophy, impairing the expression of numerous genes for mitochondrial proteins, and, in particular, suppressing master regulators of mitochondrial biogenesis and function, perioxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), and nuclear respiratory factor-1 (NRF-1). Given the dual transcriptional roles of Cdk9 in hypertrophic growth and mitochondrial dysfunction, we suggest the potential usefulness of Cdk9 as a target in heart failure drug discovery. **(*Circ Res*. 2004;95:867-876.)**

Key Words: apoptosis ■ cyclin-dependent kinases ■ hypertrophy ■ mitochondria ■ perioxisome proliferator-activated receptor-γ coactivator-1

The two fundamental problems of transcriptional regulation in eukaryotes are, first, how genes are transcribed in cell type-specific and developmentally regulated patterns during normal biological circumstances (development, maturation, aging) and, second, how cells modulate their ensemble of expressed genes in response to pathobiological cues. Among the paramount and best-studied examples of a pathobiological gene program is "hypertrophic phenotype" in cardiac muscle, induced by mechanical stress, by hypertrophic agonists like angiotensin II and endothelin, and by intracellular signaling proteins for these and related or interconnected cascades.[1,2] Evolving knowledge of this phenotype, in reductionist molecular terms, has encompassed identifying genes whose induction or suppression is characteristic

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of hypertrophy, mapping the promoter regions of those genes to find the responsible transcription factor binding sites, and proving which transcription factors there bind DNA directly. More complexly, activity of the cognate transcription factors is subjected to multiple modes of regulation. To be sure, levels of a transcription factor can be affected by hypertrophic signals. Yet, perhaps more importantly, the many functional properties of a transcription factor are potential targets of hypertrophic signaling cascades, involving modulation by diverse post-translational modifications (phosphorylation, acetylation, methylation, and ubiquitination among these) whereby DNA- and protein-binding can be controlled. Gene activation or repression by DNA-binding factors also can depend on specific partners (coactivators or corepressors, including chromatin remodeling proteins) that are tethered to DNA indirectly, and the trafficking between the nuclear and cytoplasmic compartments of the cell likewise determines transcription factor activity. Collectively, the many transcription factors, coactivators, and corepressors that are complexed to a gene and the many processes that modify them serve but one elementary purpose: to permit transcription initiation through the physical recruitment of a DNA-directed RNA polymerase.

The RNAPII Phosphorylation–Dephosphorylation Cycle

In eukaryotes, transcription of protein-coding genes is catalyzed by RNAPII. Gene-specific DNA-binding factors interact with RNAPII and with general transcription factors via a 20- to 30-protein molecular bridge known as Mediator, together forming the so-called pre-initiation complex.[3] However, the onset of transcription (initiation) is just one step in the process of transcription. Production of more than merely a nascent transcript requires that RNAPII escape from the promoter (promoter clearance) and move 5′ to 3′ along the genomic sequence (transcription elongation). Capping of the 5′ end and splicing are coupled cotranscriptionally to elongation of the transcript, polyadenylation of the 3′ end is coupled to transcription termination, and RNAPII is then recycled, allowing its reuse in a fresh round of the transcription cycle.[4–10] These postinitiation steps are also critical targets in transcriptional control.

Growing evidence indicates that a central role in this orchestration of promoter escape, transcription elongation, and pre-mRNA processing is played by the phosphorylation state of the largest subunit of RNAPII (Rpb1), within its repetitive serine-rich C-terminal domain (CTD).[4,5,11,12] This C-terminal region is unique to RNAPII, which otherwise shares high homology with RNA polymerases I and III. The CTD consists of a highly multimerized tandem heptad repeat (Tyr^1-Ser^2-Pro^3-Thr^4-Ser^5-Pro^6-Ser^7) that has been conserved remarkably across eukaryotic taxa, including all animals, green plants, and fungi for which the sequence is known, and even Microsporidia, the simplest eukaryote.[13] Although the number of repeats varies widely (26 in yeast, 52 in mammals), deletions within the repeat led to loss of viability in both yeast and mammalian cells,[14–17] and deletion of 13 repeats in mice caused growth retardation with neonatal demise.[18] The predominant phosphorylated residues in this seven-amino acid motif are serine 2 and serine 5, both conforming to the S/T*-P minimal consensus site for Cdc28, the prototypical cyclin-dependent kinase in *Saccharomyces cerevisiae*. Substitution of alanine at either position throughout the repeat is lethal in yeast, as is the inversion to Pro^2Ser^3 (ref.[16]). In the C-terminal part of the CTD, the sequence of the heptad repeat departs from the perfect consensus, suggesting that the N-terminal and C-terminal portions might serve distinguishable functions.[19–21]

CTD phosphorylation is required to coordinate the sequential recruitment of transcription elongation factors, mRNA processing machinery, and chromatin remodeling factors.[6,8,19,22–25] By chromatin immunoprecipitation, it has been possible to map the serial changes in CTD phosphorylation that are associated with the discrete stages of mRNA synthesis described.[10,11,23,26,27] Unphosphorylated RNAPII (IIa) is recruited to promoters and forms the pre-initiation complex with the assistance with Mediator and general transcription factors. Phosphorylation of serine 5 is concentrated near the promoter, whereas serine 2 phosphorylation predominates in the coding region. Three atypical RNAPII-directed cyclin-dependent protein kinases—Cdk7, Cdk8, and Cdk9—are responsible for CTD phosphorylation, two of which are key constituents of multicomponent transcription factors (Figure 1). Soon after initiation, serine 5 is phosphorylated by Cdk7/cyclin H/MAT-1, the kinase subunit of TFIIH,[28,29] whereupon RNAPII becomes arrested in the promoter–proximal region by its physical association with negative elongation factors.[30,31] Phosphorylated serine 5 likewise mediates the recruitment of mRNA capping enzymes.[6,26,32–36] After capping, serine 5 is dephosphorylated by an unknown means and serine 2 becomes extensively phosphorylated by Cdk9/cyclin T (positive transcription elongation factor b [P-TEFb]).[37–39] Serine 2 phosphorylation counteracts the suppressive action of negative elongation factors and allows RNAPII to escape the promoter and perform productive elongation.[30]

Thus, promoter–proximal pausing of RNAPII is thought to be a general rate-limiting step after transcription initiation,[40] with most of transcription by RNAPII being blocked by the P-TEFb inhibitors 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole and flavopiridol.[41] One manifestation of control at this postinitiation step is the physical accumulation of paused RNAPII at a location 20 to 40 bp downstream of the transcription start site in many genes, including human *MYC*, *FOS*, and *HSP70–1*. The hyperphosphorylated state of RNAPII (IIo) is maintained during elongation and enables the recruitment of positive-acting elongation factors, splicing factors, polyadenylation/termination factors, and chromatin remodeling proteins to the elongating polymerase. Among eukaryotic genes, P-TEFb has been studied most conclusively in the induction of *Drosophila* heat shock response genes, with transcriptional effects somewhat greater for *hsp26*, and 3′ end processing the greater for *hsp70*.[42] By RNA interference, P-TEFb was shown to be required not only for serine 2 phosphorylation but also broadly, essential for expression of early embryonic genes in *C. elegans*.[43] In budding yeast, serine 2 phosphorylation is mediated by CTDK-I, a putative homologue of P-TEFb, which promotes elongation and is

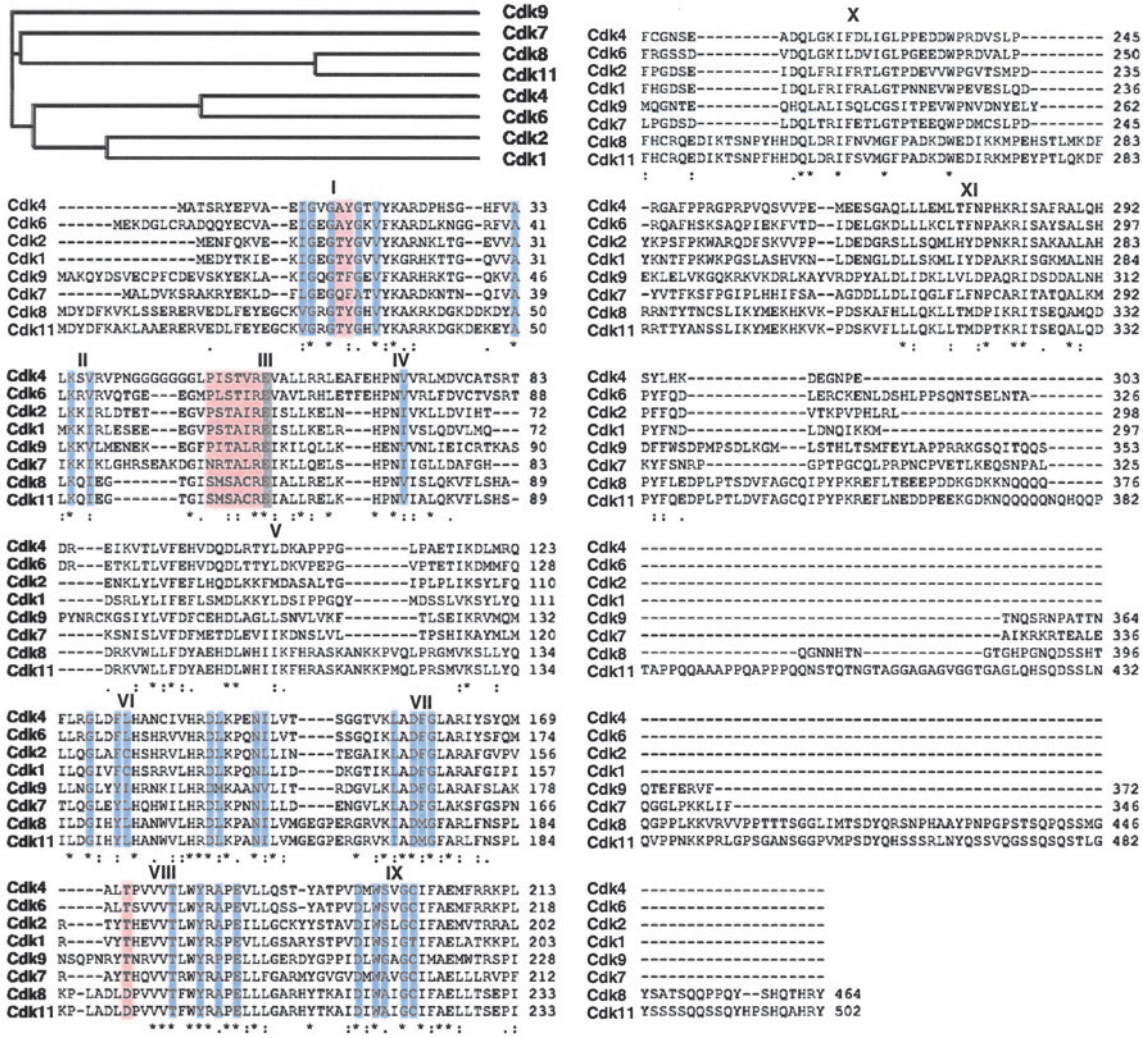

Figure 1. The four Cdks that directly mediate transcription (Cdk9, Cdk7, Cdk8, Cdk11) form a structurally distinct subclass of cyclin-dependent kinases. ClustalW was used for the cladogram and multiple sequence alignment.[105] Highly conserved residues from a phylogenetic analysis of the protein kinase superfamily (at least 62 of the 65 sequences) are highlighted in blue:[106] features shared with other protein kinase subfamilies include ATP-binding sites (subdomains I, VI, and VII), invariant lysine of the catalytic domain (subdomain II), consensus Ala-Pro-Glu (subdomain VIII), and signature of serine/threonine specificity (subdomain VI). Hallmarks of the Cdk subfamily are highlighted in red: the Thr-Tyr dipeptide that is the target of Cdc25 phosphatases (subdomain I), the cyclin-binding domain (subdomain III), and the activation (T-) loop Thr that is the target of Cdk-activating kinase (subdomain VIII).

required for certain stress responses, although not for viability per se.[27,44]

At the termination of transcription, serine 2 is dephosphorylated by the TFIIF-dependent CTD phosphatase FCP-1 and unphosphorylated RNAPII is recycled.[45] Phosphorylation of RNAPII by Cdk8/cyclin C blocks its recruitment to promoters. A second suppressive effect of Cdk8 on transcription occurs by phosphorylating cyclin H, disrupting the activation of Cdk7. Like Cdk8, the closely related kinase Cdk11 is a consensus component of the mammalian Mediator complex.[3]

Transcriptional Regulation in Stress-Induced Cardiac Hypertrophy

Growth of the heart occurs chiefly through proliferation of cardiomyocytes during embryogenesis and through cell enlargement (hypertrophy) after birth. "Physiological" hypertrophy occurs during normal postnatal maturation and in response to chronic exercise training, and classically comprises an increase in cardiac mass without apparent deleterious effects on cardiac function or prognosis. Although the dichotomy in respects is a misleading oversimplification, "pathological" hypertrophy occurs as the heart's response to various stressors, including workload (hypertension and valve disorders), loss of working myocytes (myocardial infarction, but also sporadic cell death), and mutations affecting sarcomeric, cytoskeletal, calcium-handling, or mitochondrial proteins. Operating from a teleological perspective as an initially adaptive compensatory response to offset wall stress and maintain cardiac pump function, pathological hypertrophy ultimately can degenerate into a decompensated dilated cardiomyopathy (heart failure). Heart failure, in animals and humans, is commonly accompanied by a markedly increased prevalence of myocyte apoptosis,[46] and the functional importance of cardiac apoptosis has begun to emerge from investigations of engineered mice with modified pro- or anti-apoptotic genes, as well as from pharmacological inhibition of apoptotic pathways and effectors.[46–53]

Hence, the question of transcriptional control in cardiac hypertrophy is really no fewer than three separable questions. Plasticity ("reprogramming" gene expression), growth, and predisposition to cell death must each be explained. First, then, how does stress induce the typical program of stress-induced cardiac genes? Here, progress has been uncanny, including the delineation of calcium-dependent pathways that potentiate the zinc finger protein GATA-4 by importing a coactivator, NF-AT, from the cytoplasm[54] and unlock the transcriptional activity of myocyte enhancer factor-2 (MEF2) by expelling a class II histone deacetylase from the nucleus.[55] NF-AT trafficking to the nucleus involves the calmodulin-dependent protein phosphatase, calcineurin; HDAC export from the nucleus involves a calmodulin-dependent protein kinase that is yet to be identified.[1] These and other insights establish novel therapeutic opportunities.[56]

Second, how, exactly, are stress-induced pathways coupled to growth? For the moment at least, the known GATA4 and MEF2 targets seem easier to reconcile with the hypertrophic gene program than with the global increase of RNA and protein per cell that constitutes hypertrophic cell enlargement. Surprisingly, ribosomal S6 kinases—presumptive mediators of translational control—were found to be dispensable for cardiac growth imposed by the insulin-like growth factor 1-phosphoinositide 3-kinase hypertrophic pathway.[57] However, pathways for protein synthesis exist that are independent of S6 kinases and even of their activator, mTOR.[58]

Third, what early events during cardiac hypertrophy create the later predilection to cell death in heart failure? Molecular signatures of "physiological" versus "pathological" hypertrophy have begun to emerge from genome-wide expression profiling.[59,60] For example, the pro-apoptotic protein Nix (a lethal relative of Bcl-2) was incriminated through a "genetically unbiased" genome-wide survey of apoptosis-prone, hypertrophic hearts and has been validated experimentally.[61] However, it is logical to surmise that other grounds also exist for the predisposition to cell death in ailing myocardium.

Previously, we speculated that transcriptional activation in hypertrophy might be accomplished by modulating the basal transcriptional machinery itself, not merely gene-specific factors. We demonstrated that hypertrophic signals, both in vitro and in vivo, cause RNAPII CTD phosphorylation.[62] Moreover, integrity of the CTD was essential for the global activation of transcription by hypertrophic stimuli,[62] although these early experiments did not specify the responsible CTD kinase. Later, we focus on our recent findings to show more exactly how hypertrophic signals impinge on CTD phosphorylation, how CTD phosphorylation by Cdk9 is essential for cardiac growth in culture, how hypertrophic signals induce Cdk9 activity, and how the genetic activation of Cdk9 to pathophysiological levels implicates this kinase in both hypertrophic growth and vulnerability to apoptotic cues. The apoptosis provoked by Cdk9 was attributable to a selective defect in the expression of genes for mitochondrial function, resulting, in turn, from the selective suppression of a master gene for mitochondrial biogenesis and function, PGC-1.

Cdk9 Is an Essential CTD Kinase for Hypertrophic Growth in Culture

As discussed, CTD phosphorylation is required for productive transcription elongation and pre-mRNA processing and, thus, is a criterion of the actively transcribing RNAPII. In cultured cardiomyocytes, diverse hypertrophic agonists including endothelin-1 (ET-1), phenylephrine, and heparin-binding epidermal growth factor all increased the extent of phosphorylated RNAPII within 15 minutes.[63] Thus, the amount of active RNAPII increases rapidly in response to hypertrophic stimuli. Under these circumstances, this phosphorylation appears to be mediated preferentially by Cdk9. ET-1 induced Cdk9 kinase activity, with no parallel change in Cdk7. Hypertrophic agonists increased the phosphorylation of Ser^2 (the substrate residue preferred by Cdk9). Both a selective pharmacological inhibitor (5,6-dichloro-1-b-D-ribofuranosylbenzimidazole) and a catalytically inactive, dominant-interfering mutation of Cdk9-negative Cdk9 (D167N) blocked the ET-1 effect, whereas a dominant-interfering mutation of Cdk7 did not. Consistent with these data, and with other evidence for the importance of Cdk9 in living cells,[64] inhibition of Cdk9 activity blocked ET-1–induced hypertrophic growth as determined by global RNA synthesis, global protein synthesis, and cell enlargement. Inhibition of Cdk7 had, at best, a much smaller effect. In mice, whereas long-term load or genetic triggers of hypertrophy (Gq[65] and calcineurin[54]) activated both kinases, short-term mechanical load activated just Cdk9 (Figure 2). Given this concordance of observational and functional results, with Cdk9 as common to each of the many models that we studied—including human heart failure[66]—we next applied our efforts toward defining the molecular basis for Cdk9 activation and the biological consequences of this event.

Dissociation of a Noncoding Small Nuclear RNA by Hypertrophic Signals Unleashes Cdk9 Function

How do hypertrophic signal transduction pathways activate Cdk9? Obvious possibilities were quickly excluded, because neither the expression of Cdk9, nor the expression of its activator cyclin T, nor the assembly of P-TEFb (the Cdk9/cyclin T heterodimer) changed acutely in response to ET-1 in culture or to aortic banding. An all-important clue to solving this dilemma was the discovery that P-TEFb is maintained in an inactive form by an associated factor, 7SK, a 330-nucleotide small nuclear RNA.[67,68] (Given a number of recent additional examples, regulation of RNAPII by noncoding RNAs is very likely a more general phenomenon.[69,70]) Importantly, certain stress signals such as UV irradiation release 7SK from P-TEFb, and in so doing disinhibit the catalytic activity of Cdk9.[67,68] With this as an instructive context, we asked whether cardiac Cdk9/cyclin T complexes contain 7SK, whether hypertrophic agonists dissociate 7SK from Cdk9/cyclin T in cardiac myocytes, whether this also holds true for hypertrophic signals in the intact heart, and whether loss of 7SK might suffice for Cdk9 activation and hypertrophy in cardiac muscle cells. For each question, the answer was "yes." As we had extrapolated, ET-1 caused the rapid

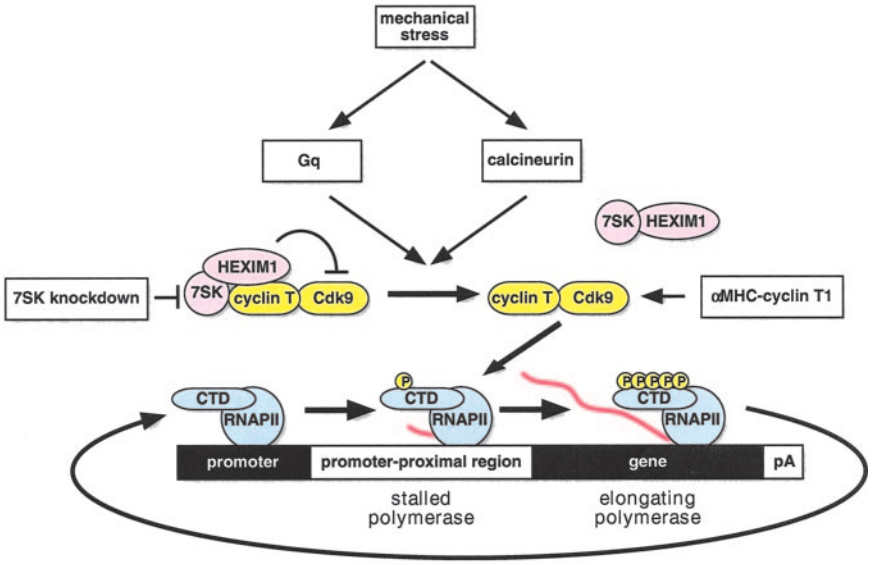

Figure 2. Hypertrophic signals perturb the RNAPII phosphorylation–dephosphorylation cycle. Mechanical load, Gq, calcineurin, and endothelin all activate endogenous Cdk9/cyclin T, and do so through dissociation of an endogenous inhibitor, 7SK, a noncoding small nuclear RNA. Interference with 7SK expression and overexpression of cyclin T each suffice to activate endogenous Cdk9 and cause hypertrophic growth, in vitro and in vivo, respectively.[63]

dissociation of 7SK from Cdk9/cyclin T1, Gq and calcineurin provoked this dissociation in transgenic mice, and mechanical load did the same. Thus, hypertrophic signals result in the liberation of functional P-TEFb from its endogenous inhibitor. (To the best of our knowledge, the release of 7SK snRNA from cardiac P-TEFb by load, calcineurin, and Gq is the only example to date of this dissociation taking place in any intact organism.) Furthermore, an antisense knock-down of 7SK snRNA specifically induced Cdk9 activity and global RNA synthesis in cultured cardiomyocytes (Figure 2).[63]

However, 7SK does not inhibit Cdk9 directly. Later work has ascribed this property to HEXIM1 (hexamethylene bisacetamide-induced protein 1), a protein that is recruited to P-TEFb via 7SK as a bridge, and is released from P-TEFb by a variety of cell stresses including ultraviolet irradiation and low levels of global transcriptional inhibitors.[71,72] It is speculated that the resulting increase in free, active P-TEFb is necessary for normal stress-induced transcription. It remains unproven how stress signaling pathways dissociate HEXIM1–7SK from P-TEFb in any biological setting, although it is suspected this may involve the phosphorylation of Cdk9 on Thr186 in the T-loop.[73]

Cdk9 Activation by Cyclin T1 Induces Cardiac Hypertrophy in Mice

Normally, cardiac Cdk9 activity is highest in embryonic life and decreases after birth. Downregulation of Cdk9 and cyclin T1 occurs and presumably contributes to this decline. We created cardiac-specific gain-of-function models that maintain Cdk9 or cyclin T1, respectively, at their embryonic levels, to test if one or both might be limiting.[63] Expressing cyclin T1 increased both Cdk9 kinase activity and the phosphorylation of endogenous RNAPII; expressing Cdk9 did neither. This suggests that cyclin T1 is limiting in cardiac muscle after birth and is necessary to overcome the inhibition of Cdk9 by HEXIM1–7SK.

In proportion to their increased levels of Cdk9 activity, independent lines of αMHC-cyclin T1 mice showed hypertrophic growth at the cell and organ level. In virtually all conventional respects, hypertrophy induced by cyclin T1 appeared benign (or "physiological"), with normal ventricular systolic function and normal life span under unstressed conditions. Lacking were fibrosis, myocyte apoptosis, and induction of the prototypical hypertrophic markers. Together with the fact that Cdk9 was both required and sufficient for hypertrophy in culture, the induction of Cdk9 by all hypertrophic signals that we tested and the induction of hypertrophy in mouse myocardium by pathophysiologically relevant levels of Cdk9 activity implicates this pathway as a highly plausible mediator of hypertrophic growth.

Although the baseline phenotype of cyclin T1 transgenic mice appeared benign in the absence of a superimposed stress, the suppression of mitochondrial biogenesis and function by Cdk9/cyclin T interacts adversely and dramatically with the well-characterized triggers of pathological hypertrophy, partial aortic constriction, and Gq.[66] Both pressure-overload and chronic Gq signaling precipitated increased apoptosis, myocardial fibrosis, a transition to heart failure, and early demise. Mice inheriting both transgenes never survived beyond 1 month, whereas no early mortality occurred with either transgene singly. Analogously, two-thirds of cyclin T1 mice (and none of the littermate controls) were dead within 3 weeks of mechanical load. We speculated that gene dysregulation by Cdk9/cyclin T might provide an explanation for this much more than additive phenotype.

Cdk9 Activation Impairs the Expression of Mitochondrial Proteins

Beyond its role as a basic transcription elongation factor, Cdk9/cyclin T can also regulate elongation if bound to gene-specific factors. Most notoriously, the Cdk9/cyclin T1 heterodimer P-TEFb acts as a cofactor for HIV-1 Tat protein.[74–76] In the absence of Tat, the assembled RNAPII complex cannot elongate efficiently on the viral DNA template. Binding of Tat to a stem-loop transactivation response element at the 5′ end of the nascent viral transcripts recruits P-TEFb, and the Cdk9 kinase activity of P-TEFb is essential for stimulating the production of full-length HIV transcripts

and productive viral replication.[74,75] Tat also alters the substrate preference of Cdk9 within the CTD, enabling it to phosphorylate serine 5, which then potentiates cotranscriptional mRNA capping.[76] Perhaps the first example of eukaryotic genes requiring P-TEFb kinase activity for their correct regulation is the induction of glycogen synthase and cytosolic catalase in yeast during the transition from fermentative to respiratory growth.[77] In mammals, eukaryotic sequence-specific activators that physically recruit P-TEFb include the class II transactivator for transcription of major histocompatibility complex class II genes,[78] androgen receptor,[79] Myc,[80] and nuclear factor kappa B.[81]

Thus, to ascertain the spectrum of the effects of Cdk on gene expression in adult myocardium, we compared cyclin T1 transgenic mice with nontransgenic littermates using DNA microarrays and quantitative RT-PCR.[66] If all transcripts' accumulation increased equally, then no differences whatever would result. If the "hypertrophic program" were induced, regardless if directly or indirectly, then genes for fetal contractile proteins would be anticipated, along with the typically concordant markers like BNP. However, neither of these scenarios was the outcome. Hsp70 was markedly upregulated, as foreseen from its known strict dependence on P-TEFb,[43] yet the "hypertrophic program" was not. Several potentially consequential adult cardiomyocyte-specific genes were downregulated (αMHC, SERCA2). The largest functional cluster of suppressed genes encompassed nuclear and mitochondrial genes for multiple classes of mitochondrial proteins. Notably, the cyclin T1-suppressed genes encode mitochondrial superoxide dismutase 2, enzymes for respiratory chain complexes, the tricarboxylic acid cycle, and β-oxidation of fatty acids, mitochondrial import proteins, mitochondrial ribosomal proteins, and transcription factors—mitochondrial transcription factor A (TFAM), mitochondrial transcription factors B1 and B2 (TFB1M, TFB2M), nuclear respiratory factors 1 and 2 (NRF1, NRF2), and peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1). Interestingly mitochondrial dysfunction from tissue-specific deletion of TFAM is sufficient to cause a lethal cardiomyopathy.[82] At the protein level, in the cyclin T1 mice, we confirmed a partial loss of NADH dehydrogenase (complex I), NADH cytochrome c reductase (complex I + III), and succinate dehydrogenase (complex II); furthermore, mitochondrial ultrastructure was abnormal, with loosely packed, disorganized cristae.[66]

Transcriptional Repression of PGC-1 by Cdk9/Cyclin T1

As summarized in several noteworthy reviews,[83,84] mitochondrial content and respiratory capacity both are regulated by energy demand and modulated by pathophysiological conditions, requiring the concerted expression of nuclear and mitochondrial genomes to maintain the correct complement of mitochondrial proteins. In this process, several nuclear transcription factors play an especially critical role: NRF-1 and NRF-2, which are sequence-specific DNA-binding proteins, and PGC-1, a transcriptional coactivator. NRFs directly transactivate numerous mitochondrial genes for respiratory chain complexes, mtDNA transcription and replication, mitochondrial translation, and mitochondrial protein import. Consistent with this indispensable role of NRFs in mitochondrial function, targeted disruption of NRF-1 in mice caused peri-implantation lethality with marked depletion of mtDNA.[85]

Originally identified as a regulator of adaptive thermogenesis in brown tissue, PGC-1 subsequently has been proven to act as a master regulator of mitochondrial function and biogenesis, with especially striking roles in striated muscle.[83,84] PGC-1 is highly expressed in mitochondria-rich tissues that have highly energy demands, such as heart, skeletal muscle, brown fat, kidney, liver, and brain, and is coupled through NRF-1 for many of its effects including the induction of TFAM, which in turn is essential for mitochondrial DNA replication and transcription.[86] Consistent with these findings, PGC-1 overexpression suffices to stimulate mitochondrial biogenesis in cardiac muscle,[87] and a dominant-interfering mutation dnNRF-1 blocks this effect at least in cultured cardiac muscle cells.[88] These findings establish that activation of mitochondrial biogenesis requires the functional interaction between PGC-1 and NRF-1. PGC-1 also has other important partners including PPARα and MEF2.[89–92] Another key characteristic of PGC-1 is its inducibility. PGC-1 is upregulated during normal postnatal muscle growth of the heart,[93] and is upregulated in skeletal muscle by physical exercise,[94] likely via a calcium-dependent/calmodulin-dependent/CaM kinase-dependent MEF2 pathway.[91] In this latter context, PGC-1 functions to drive the adaptive conversion of type II (fast-twitch) fibers to type I (slow-twitch) fibers, which express greater levels of genes for mitochondrial biogenesis and oxidative phosphorylation.[95] Conversely, though, PGC-1 expression was downregulated in cardiac muscle by aortic banding for 1 week,[96] and in chronic heart failure models the downregulation of PGC-1 correlates well with the decrease in nuclear- and mitochondrial-encoded genes for respiratory chain complexes.[97]

The molecular mechanisms underlying the loss of PGC-1 expression in cardiac hypertrophy and the consequent decrease in mitochondrial function are poorly understood. Downregulation of PGC-1 might even been seen as paradoxical, given the gene's dependence on MEF2 and the known activation of MEF2 by many hypertrophic signals. Interestingly, cyclin T1 had a greater effect on PGC-1 expression than did Gq, either in cultured cells or in transgenic mouse myocardium.[66] Hence, the balance between MEF2 and Cdk9/cyclin T pathways might be expected to be a determinant of this critical energy switch.

Several questions were especially important in validating the suppression of endogenous PGC-1 by Cdk9 activity to be functionally consequential and in providing a concrete basis for this gene's diminished expression.[66] First, is loss of PGC-1 in fact the limiting step for expression of mitochondrial proteins? We found exogenous PGC-1 was sufficient to rescue cardiac myocytes from Cdk9/cyclin T1, not only as measured by the expression of TFAM and Cox5b but also, more importantly, as reflected by preservation of mitochondrial membrane potential and a successful block to apoptosis. Second, is a direct effector of PGC-1 is necessary for cardiac myocyte survival? Interference with NRF-1 was tested; as

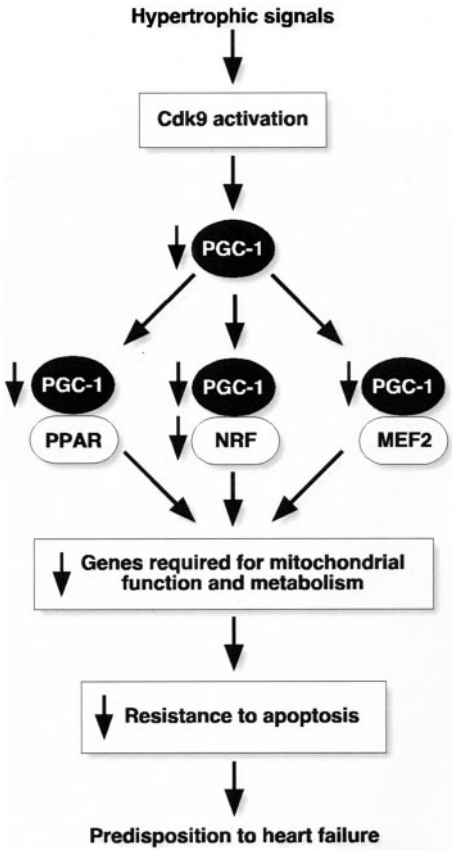

Figure 3. Activation of Cdk9 to pathophysiological levels leads to mitochondrial dysfunction, apoptosis, and heart failure via suppression of PGC-1, an essential co-activator for the transcription of nuclear and mitochondrial genes that encode mitochondrial proteins.[66]

anticipated, dominant-negative NRF-1 suppressed the previously reported NRF target genes, caused dissipation of mitochondrial membrane potential, and promoted apoptosis both singly and in synergy with Gq. Third, is PGC-1 expression impaired via interference with transcription elongation (contrary to the general effect of Cdk9/cyclin T1 at this step), or via another mechanism entirely? Interestingly, as determined by chromatin immunoprecipitation assays, Cdk9/cyclin T1 blocks the physical recruitment of the general transcription factor TATA box-binding protein and of RNAPII to the endogenous PGC-1 promoter, that is, it blocks assembly of the PGC-1 pre-initiation complex (Figure 3).

Future Directions

Our investigations began as an effort to define the transcriptional mechanism for the global increases in RNA and protein synthesis that are fundamental features of hypertrophic growth. To recap our most relevant findings, we found that hypertrophic signals in vitro and in vivo increase the hyperphosphorylated (active, productive, elongating) form of RNAPII, that Cdk9 activity was needed for the increase in RNA synthesis and myocyte size, and that Cdk9 activation by cyclin T1 in transgenic mice sufficed to provoke seemingly benign cardiac hypertrophy. However, activation of Cdk9 by cyclin T1 led to more selective effects than would be expected from nonspecifically enhancing the efficiency of transcription elongation.

Among the questions still unanswered regarding Cdk9 in cardiac hypertrophy, two stand out in particular. First, are all the functions of Cdk9 in transcriptional control mediated through CTD phosphorylation? Second, given its adverse effects on mitochondrial function and on myocyte susceptibility to apoptosis, is Cdk9 a potential target for treatment in heart failure?

Thus far, all known Cdk9 effects on transcription are explained via phosphorylation of the RNAPII CTD. However, gene-specific DNA-binding transcriptional activators may selectively recruit the Cdk9/cyclin T complex and stimulate transcription elongation in a gene-specific manner. Conversely, a *C. elegans* transcriptional repressor (pharynx and intestine in excess protein 1) possesses a nonphosphorylatable alanine-containing heptapeptide repeat that sufficiently resembles the RNAPII CTD repeat to decoy Cdk9/cyclin T away from RNAPII and block transcription elongation.[98] Hence, even when selective effects of Cdk9 exist, altered CTD phosphorylation can be the cause. Here, the finding that Cdk9 activation directly downregulates PGC-1 provides important and unexpected clues. Repression of PGC-1 promoter activity was observed even in the absence of the "promoter–proximal region" by which Cdk9 stimulates promoter escape. Furthermore, Cdk9/cyclin T1 selectively inhibited the physical recruitment of TBP and RNAPII to the endogenous PGC-1 promoter.

Consequently, the suppressive action of Cdk9/cyclin T occurs at or before pre-initiation complex formation, rather than at the later postinitiation step of transcription elongation. Given this step as the site of control, many potential targets exist for Cdk9 apart from just RNAPII. PGC-1 promoter activity is regulated by the MEF2/HDAC pathway,[91] and PGC-1 coactivates MEF2.[99] Consistent with a block to the function of one or more of these proteins, Cdk9/cyclin T1 suppressed the PGC-1 promoter even when driven by exogenous PGC-1 plus exogenous MEF2. Interestingly, a number of the genes suppressed in cyclin T1 myocardium are known to be coordinately regulated by PGC-1 and MEF2, including GLUT4 and myoglobin.[95] PGC-1 is physically associated with the RNAPII/Cdk9/cyclin T complex.[100] Whether PGC-1 or an associated factor is a substrate of Cdk9 remains to be determined.

Finally, does Cdk9 offer a potential target of therapeutic interventions in heart failure? A priori reasoning had argued that hypertrophic growth was teleologically adaptive and that attenuating hypertrophy might therefore put the heart at risk, but this concern has been allayed.[101,102] Rather, and perhaps more so, than suppression of growth itself as the goal, a block to the adverse transcriptional program can be beneficial to prevent the transition to heart failure. Although it is still far from clear which downstream genes are the pivotally responsible culprits for this transition, energy production, calcium homeostasis, and myocyte survival loom large among the defects, and mitochondria have a critical role in each of these functions. Our results suggest the Cdk9/PGC-1/NRF1 transcriptional module as a candidate therapeutic target, underlying and underscoring the defects in mitochondrial function

in failing myocardium: cardiac Cdk9 is activated acutely by diverse hypertrophic stimuli and continues to be activated throughout the later decompensation (including human dilated cardiomyopathy). Our loss-of-function and gain-of-function studies implicate Cdk9 as a pivotal regulator of pathophysiological heart growth; furthermore, chronic activation of Cdk9 adversely changes the gene expression profile in myocardium, suppressing PGC-1, inciting mitochondrial dysfunction, predisposing hearts to apoptosis, and provoking early mortality. Hence, given the dual transcriptional role of Cdk9 in hypertrophic growth and mitochondrial dysfunction, a partial blockade of Cdk9 activity—to the normal baseline level—is cogent, and likely salutary. In that case, where should the hunt for drug discovery begin? Most obviously, pharmacological inhibitors of Cdk9 should be considered by analogy to their investigational use to impede HIV replication[103] or tumor cell growth,[104] other biological settings in which this CTD kinase is thought to be especially important. Besides the kinase activity of Cdk9, however, the pathway leading to Cdk9 activation might be scrutinized fruitfully to resolve more exactly what stress-dependent signal dissociates the endogenous inhibitor 7SK-HEXIM1 from Cdk9/cyclin T in the heart.

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