The proliferative cell cycle in both vertebrates and much simpler organisms entails the transduction of mitogenic signals to cyclically expressed proteins known as cyclins and, hence, to their catalytically active targets, the cyclin-dependent protein kinases (cdks). In yeast, a single kinase, cdc28, suffices to partner with all of the cyclins, which are expressed sequentially in the G1 (cln 1/2/3), S (clb 5/6), and G2/M phases of the cycle, respectively. In mammals, by contrast, D-type cyclins in G1 partner with cdk4/6, cyclins E and A sequentially partner with cdk2 in S phase, and cyclin B interacts with cdc2 for entry into M phase. Whereas one family of cdk inhibitors is specific for cdk4/6 (the INK4 family, comprising p15, p16, p18, and p19), a second, the Cip/Kip family, has much broader activity, inhibiting cdk2 and cdc2 in addition. Activation of the cell cycle through induction of cyclin D occurs in large part by the titration of Cip/Kip proteins away from cdk2 to the cyclin D–cdk complexes. Substrates of primary importance to the actions of cdk4/6 are the retinoblastoma family of pocket proteins, whereas much less is known about the substrates for cdk2, although some have been identified. One source of positive feedback within this process is that phosphorylation by cdc28 and cdk2 can promote the degradation of cdk inhibitors using the ubiquitin-proteasome pathway. A second source is that cdk2, whose enzymatic activation thus depends on cdk4/6, also phosphorylates pocket proteins in sequential or processive fashion. In addition, the mitogen-induced transcription factor Myc likewise causes S-phase entry by activating cdk2 in parallel with the pocket protein pathway. Thus, together these positive and negative regulators form a complex and interdependent network tightly regulating the cell cycle, in which the kinase activity of cdk2 is ordinarily essential for DNA replication.

In cardiovascular research, these 3 multigene families—cyclins, cdk5, and cdk inhibitors—hold fascination both for intrinsic intellectual reasons and for their potential therapeutic implications. This is more obvious, perhaps, in vascular biology than for cardiac muscle per se, because excessive growth in graft atherosclerosis, restenosis injury, and tumor angiogenesis has been disrupted experimentally in each of these contexts by manipulations of cell-cycle protein expression or function. Implications of this body of work for myocardial biology, by contrast, are less immediately clear. Ventricular muscle cells are thought by most investigators to exit the proliferative cell cycle irreversibly soon after birth, notwithstanding a known increase in DNA content in failing human myocytes and the capacity for proliferative regeneration of cardiac muscle after injury in some species, such as the newt. Such clues and the conceptual desire to augment cardiac growth therapeutically have begun to spur investigators to examine the function of cell-cycle proteins in cardiac muscle by gene delivery in cell culture and transgenic animals. In addition to substantiating proteins’ expected roles, novel pathways and genes have already been disclosed by this approach.

Two important studies of cardiac cell-cycle regulators appear in the present issue of *Circulation Research*, with a shared emphasis on cdk2, yet stressing very different aspects of its function. Liao et al demonstrate that forced expression of cdk2 in the cardiac muscle of transgenic mice results in catalytically active protein, increased cardiac muscle cell number, and an increase in DNA synthesis by adult ventricular myocytes (100-fold, albeit to a prevalence of 0.06%). Interestingly, in cdk2 transgenic mice, the normal preponderance of binucleated cardiac myocytes was shifted toward mononucleated cells, which were correspondingly smaller in size. In many respects, this model resembles the consequence of overexpressing Myc, which delayed but did not prevent cardiac cell-cycle exit and resulted in myocytes that were increased in number but smaller in size. Delayed quiescence, rather than ongoing DNA synthesis, also has been shown in cardiac myocytes lacking p27Kip1. Myc and p27 both regulate cdk2 function; however, it is not known whether cdk2 specifically is a responsible effector for these shared phenotypes. Despite the lack of functional impairment or hypertrophic growth, nominal markers of hypertrophy and heart failure were induced by the cdk2 transgene (atrial natriuretic factor and β-myosin heavy chain mRNA), which is taken as evidence for impaired maturation. Alternatively, subtle myocardial dysfunction is conceivable, escaping echocardiographic assessment in mice. Several G1/S cell-cycle regulators also were persistently expressed, and the animals had an exaggerated growth response to pressure overload, which was proportionately greater in the mononucleated cells. Thus, the model differs in its details from forcible expression of cyclin D1 in the heart, where sustainable DNA synthesis likewise was seen, but fetal gene induction was absent and multinucleation was pronounced. The observed delay in cell-cycle exit evoked by cdk2 is in keeping with...
the studies previously cited, using single regulators of S-phase entry, which delay cell-cycle exit in ventricular myocytes but do not prevent it and do not overcome the G2/M block. The second study on cdk2 in this issue deals instead with a potential role for cdk2 in cell death through apoptosis. Adachi et al. 17 demonstrate that hypoxia selectively upregulates cdk2 activity associated with cyclin A in cultured rat cardiac myocytes (but not cyclin E–cdk2), that dominant-negative cdk2 partially inhibited apoptosis, and that cyclin A was sufficient to trigger programmed cell death. One suggested mechanism for the activation of cdk2 by hypoxia was the observed cleavage of p21Cip1, potentially by caspase-3. Indeed, a causal role in apoptosis for p21 cleavage and activation of cyclin cdk2 has been reported recently in human endothelial cells as well as other systems. 18,19 Together with thematically related work by these authors on G1 cdk activity in cardiac hypertrophy, 20 this study by Adachi et al. 17 provides intriguing evidence that the function of cell-cycle regulators in ventricular muscle cells is not confined to the cell cycle alone.

Tantalizing ambiguities remain, including resolution of the cdk2 substrates essential for the growth- and death-promoting effects in cardiac muscle, respectively. In particular, the reported cdk2 substrates do not provide an easy explanation for the role of the protein in apoptosis. One possibility is E2F–1–dependent transcription after pocket protein inactivation, but only a very small increase was detected by Adachi et al. 17 The latter authors put forward the case that caspase cleaves p21 in hypoxic cardiac myocytes, disinhibiting cdk2, but other gaps in the present understanding are worth noting, too. What is the status of p27, which coexists in cardiac muscle with p21 10 and has comparable activity against cdk2 but is relatively resistant to caspase cleavage? 21 Even if p27 were also degraded, what is the molecular basis for selective activation of cdk2 bound to cyclin A and not cyclin E? Assuming the degradation of p27 is as important in cardiac muscle as in other settings, an interpretation alternative to the authors’ is suggested by the fact that p21 can promote survival independently of affecting the cell cycle: the former may require cytoplasmic p21, whereas the latter requires nuclear localization. 22 Is cdk2 germane just to hypoxia-induced apoptosis or also to other cardiac death signals? Does this pathway operate in the intact, adult myocardium? Is inhibition of cdk2 a potential therapeutic target in cardiac muscle that might be advantageous by comparison with chronic use of more general apoptosis inhibitors?

Conversely, additional questions also remain with respect to cdk2 in promoting cardiac growth. First, at a genetic level, will there be synergy between cdk2 and other interventions, such as other positive-acting regulators of S-phase entry, the deletion of cell-cycle inhibitors, or mediators of the G2/M transition? Overcoming replicative senescence can specifically require the enzyme telomerase reverse transcriptase, suggesting that a combinatorial approach may be necessary. Second, from a translational perspective, would the net effect of such interventions be beneficial or adverse? Hypertrophy was augmented in mice overexpressing cdk2 in the heart, but dysfunction was augmented as well. 12 Might this approach be better applied instead to engineering cells for implantation? Third, are these 2 studies linked mechanistically rather than just by coincident submission? Apoptosis can result from mechanical load, is clearly susceptible to genetic modifiers, and can be a consequence of forced cell-cycle reentry in the myocardium. Hence, it will be interesting to show whether cdk2 leads to impaired function after load in part by impaired cell survival given the potential concerted effect of gene and physiology.

As investigators increasingly begin to scrutinize the function of cell-cycle proteins in postmitotic cardiac muscle in relation to this novel context and novel functions beyond the cell cycle alone, unique insights into cardiac biology and new therapeutic possibilities are anticipated.

References


Cyclin-Dependent Kinase-2 in the Birth and Death of Cardiac Muscle Cells
Michael D. Schneider and W. Robb MacLellan

Circ Res. 2001;88:367-369
doi: 10.1161/01.RES.88.4.367
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/88/4/367

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/