In this issue of Circulation Research, Yurkova et al. present direct evidence that a cell cycle regulatory factor, E2F-1, binds to a proapoptotic gene in hypoxic cardiac myocytes, leading to apoptosis. Life embraces death.

The role of the cell cycle regulatory protein E2F-1 in a postmitotic cardiac myocyte has remained somewhat of a mystery. In 1997, while working in the laboratory of Schneider, Kirshenbaum and colleagues uncovered a critical clue. Cardiac myocytes overexpressing E2F-1 entered the cell cycle and headed for the grave.2,3 This work was among the first to lead a wave of evidence across disciplines to support a role for E2F-1 in the cell cycle and cell death.4 However, the mechanism(s) by which E2F-1 promotes apoptosis have remained unclear. Kirshenbaum has returned. This time with the discovery that E2F-1 binds to the promoter region of Bnip3, an apoptotic gene, promoting an intrinsic cell death signaling pathway under conditions of hypoxia (Figure).1 Thus, 10 years ago, Kirshenbaum and colleagues published findings causing us to reexamine the predefined 1-dimensional role of E2F-1 as a cell cycle regulatory factor and consider E2F-1 as a multidimensional protein with the ability to regulate both cell cycle and intrinsic cell death pathways. In this issue of Circulation Research, Yurkova et al have discovered that E2F-1 binds the promoter region of a cell death gene and promotes apoptosis.

Prior evidence from other laboratories linked E2F-1 expression with signature patterns of both cell cycle proliferation genes and proapoptotic genes. However, Yurkova et al take this important step further and demonstrate that E2F-1 binds to the Bnip3 promoter under specific conditions, including hypoxia.1 Importantly, this is not seen in the setting of normoxia, nor under conditions of the enhanced presence of the retinoblastoma gene product (Rb), a pocket protein that binds and inactivates E2F-1 (Figure).1 Yurkova et al use several independent strategies to demonstrate that the intrinsic cell death pathway induced by E2F-1 is dependent on the transcriptional activation of Bnip3.1 First, overexpression of E2F-1 in ventricular myocytes induced an ≈6-fold upregulation of Bnip3 transcript expression.1 This finding is in line with the evidence showing that E2F-1 binds to the promoter region of Bnip3. Finally, E2F-1–induced cell death was inhibited by overexpression of a carboxyl-terminal deletion mutant of Bnip3, as well as short hairpin RNA directed against Bnip3.1

This work raises several new questions:

- At the onset of ischemia, what specific factors trigger the binding of Bnip3 to E2F-1?
- If Bnip3 is bound to E2F-1, is death inevitable?
- What other events must transpire in the cell to allow Bnip3 to bind to E2F-1?
- Are these events similar or different from the events that release E2F-1 from Rb?

These questions are critical in moving this landmark finding forward in several arenas, including basic science discovery, identification of druggable targets for therapy, and patient care.

What cellular events trigger the binding of Bnip3 to E2F-1? Presumably, E2F-1 must be released from the retinoblastoma gene product Rb to allow it to bind to Bnip3. Rb is thought to bind to E2F-1 and suppress its activity (Figure). Rb is oftentimes thought of as a transcriptional corepressor that is indirectly tethered to DNA. Tipping the balance of E2F-1 and Rb by increasing E2F-1 induces cell cycle entry and apoptosis in cultured adult ventricular myocytes, neonatal ventricular myocytes, and the mouse heart.2,3 However, others have demonstrated that disrupting the balance of E2F-1 and Rb by deleting Rb, specifically in the heart, had no effect on apoptosis under normoxic conditions.5 This suggests that loss of Rb under normoxic conditions is not sufficient to induce apoptosis in cardiac myocytes. Secondly, it suggests that several steps in the pathway remain unearthened. Thus, the interplay between E2F-1 and Rb and cell death remains unclear. Whereas Kirshenbaum and colleagues have unraveled key intrinsic cell death regulatory pathways in the myocyte over the past 10 years, Schneider and colleagues have begun to unravel the role of Rb and other pocket proteins.5 It was Kirshenbaum and Schneider and colleagues who first demonstrated that disrupting the balance of E2F-1 and Rb in cardiac myocytes led to apoptosis.2,3 Schneider and colleagues demonstrated that mice deficient in Rb displayed no change in heart size, myocyte cell cycle distribution, size, apoptosis, or mechanical function.5 This suggested overlapping roles for another pocket protein family member in the heart, p130. Thus a double-knockout mouse was created by crossing a germ line deletion of another pocket protein, p130, to the cardiac-specific Rb-null line.5 These adult mice exhibited a 3-fold increase in heart weight-to-body weight ratios and showed increased evidence of myocyte cycling. However, loss of both Rb and p130 did not lead to an increase in apoptosis.5 Disruption of Rb using alternative strategies, including overexpression of the SV40 large T-antigen onco-
gene in the heart (leading to Rb inactivation), also led to enhanced hyperplasia in the absence of cell death under normoxic conditions.6 In sum, this suggests that multiple levels of regulation are likely involved in the regulation of Rb during hypoxia/ischemia.

Yurkova et al demonstrate that hypoxia induces enhanced expression of the caspase-cleaved isoform of Rb.7 Hypoxia-induced cleavage of Rb has not been reported. Earlier published work has shown cleavage of Rb by upstream caspases in the setting of tumor necrosis factor-α.2 The upstream caspases that are cleaving Rb are not sufficient to kill the cells.7 Once cleaved, the degradation of Rb is thought to activate E2F-1 and lead to apoptosis.7 It is not clear from previously published work whether this 68-kDa caspase cleaved form of Rb differs in its ability to bind E2F-1. Tan and Wang provide an excellent overview of the multiple steps in Rb regulation, including cleavage, dephosphorylation, and hyperphosphorylation.7 Future studies will need to unlock the regulation of Rb and how this may influence the binding of Bnip3 to E2F-1 to induce cell death in the setting of hypoxia/ischemia/reperfusion.

E2F-1 is part of an E2F family that is being increasingly recognized for regulating unique functions in gene transcription, the cell cycle, and intrinsic cell death pathways.8 A question 10 years ago, when Kirshenbaum and colleagues first reported that overexpression of E2F-1 reactivated cell cycle progression and induced apoptosis, was how to promote the cell cycle progression without inducing apoptosis. Work by Field and colleagues has demonstrated that overexpression of Cyclin D1, D2, or D3 in the mouse using the α cardiac myosin heavy chain promoted increased rates of DNA synthesis without apoptosis.9 This suggests that adult cardiac myocytes can reenter the cell cycle and not undergo cell death. Thus, manipulation of specific cell cycle regulatory genes promotes turnover in the absence of apoptosis. Field and colleagues also have demonstrated that the response seen in the mice after cardiac injury differs depending on the unique cyclin D transgene being overexpressed, further emphasizing the intricate balance of this network.9 Cyclin Ds phosphorylate members of the Rb protein family, thereby displacing Rb and permitting E2F-1–mediated transcription.

This illustrates one of the many E2F-1/Rb regulatory loops within the cell cycle and raises the question of whether these regulatory loops lie yet unnoticed in the intrinsic cell death genes including Bnip3. Furthermore, it raises the issue of how these coregulated transcription, cell cycle, and cell death networks are controlled under conditions of hypoxia. Finally, Bnip3 itself is likely under tight transcriptional and posttranscriptional control in normoxic and hypoxic conditions. In fact, Kirshenbaum and colleagues have recently demonstrated that Bnip3 is silenced by nuclear factor κB and histone deacetylase 1.10 In sum, the work by Yurkova et al in this issue of Circulation Research has opened a vision to the future of this intricate balance and coregulation of transcription, cell cycle, and cell death in cardiac myocytes.

The role of Bnip3 in regulating intrinsic cell death pathways in the myocyte is clearly of interest for potential translation of this finding to the clinical setting. However, the potential role of Bnip3 and other potential binding partners to E2F-1 that regulates both life and death is also of considerable interest. It is unclear whether the regulation of Bnip3 in other cell types, including fibroblasts, endothelial cells, and stem cells, is similar to that seen in cardiac myocytes. Diwan et al have demonstrated that targeted ablation of Bnip3 in the cardiac myocyte leads to inhibition of myocyte apoptosis after surgical ischemia/reperfusion injury in the peri-infarct region.
and remote regions and leads to preserved left ventricular systolic performance, along with diminished left ventricular dilation. Forced and conditional overexpression of Bnip3 increased myocyte apoptosis and infarct size. An excellent review by Whelan et al discusses the potential role of Bnip3 in cardiovascular remodeling postinfarct. It is possible that the size of the infarct and the remodeling seen in these models is a result of the expression being targeted to the cardiac myocyte alone and not fibroblasts and other cell types. Thus, further understanding the role of Bnip3 outside of the myocyte is an area for future work. In light of these new findings put forward by Yurkova et al, it would be helpful to use these genetic models to further tease out the regulation and interaction of E2F-1, Rb, and Bnip3 under conditions of normoxia, hypoxia, and other stimuli.

In summary, Yurkova et al have demonstrated multiple lines of evidence to show that E2F-1 binds to the promoter of Bnip3, thereby providing a direct mechanism for earlier studies in which E2F-1 overexpression drove cardiac myocytes first into the cell cycle followed by death. This finding opens a new window into the intricate networks of gene transcription that regulate cell cycle and intrinsic cell death. A strength of new discoveries is the next set of questions it generates. This study has pushed the field forward and has generated a series of important new questions to advance our basic understanding of: (1) how E2F-1 regulates life and death in cardiac myocytes and other cell types; (2) how the identification of genetic variants or changes in gene expression with chronic heart failure, hypertrophy, and/or inflammation may alter this signaling cascade; and (3) whether the pathway provides druggable targets for therapy.

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References

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