Multiple Facets of NF-κB in the Heart
To Be or Not to NF-κB

Joseph W. Gordon, James A. Shaw, Lorrie A. Kirshenbaum

Abstract: The progression from cardiac injury to symptomatic heart failure has been intensely studied over the last decade, and is largely attributable to a loss of functional cardiac myocytes through necrosis, intrinsic and extrinsic apoptosis pathways and autophagy. Therefore, the molecular regulation of these cellular programs has been rigorously investigated in the hopes of identifying a potential cell target that could promote cell survival and/or inhibit cell death to avert, or at least prolong, the degeneration toward symptomatic heart failure. The nuclear factor (NF)-κB super family of transcription factors has been implicated in the regulation of immune cell maturation, cell survival, and inflammation in many cell types, including cardiac myocytes. Recent studies have shown that NF-κB is cardioprotective during acute hypoxia and reperfusion injury. However, prolonged activation of NF-κB appears to be detrimental and promotes heart failure by eliciting signals that trigger chronic inflammation through enhanced elaboration of cytokines including tumor necrosis factor α, interleukin-1, and interleukin-6, leading to endoplasmic reticulum stress responses and cell death. The underlying mechanisms that account for the multifaceted and differential outcomes of NF-κB on cardiac cell fate are presently unknown. Herein, we posit a novel paradigm in which the timing, duration of activation, and cellular context may explain mechanistically the differential outcomes of NF-κB signaling in the heart that may be essential for future development of novel therapeutic interventions designed to target NF-κB responses and heart failure following myocardial injury. (Circ Res. 2011;108:1122-1132.)

Key Words: NF-κB • ventricular myocytes • apoptosis • necrosis • autophagy • inflammatory cytokines • TNFα
to heart failure is postulated to involve the continued loss of functional cardiac myocytes through pathways of cell death, including necrosis, apoptosis, and autophagy,3,4 which results in replacement of myocytes by scar tissue or diffuse interstitial fibrosis. Early loss of cardiac myocytes is likely the combination of ischemia-induced necrosis, ischemia/reperfusion injury, and apoptosis. However, late myocyte loss is believed to occur predominately through apoptosis and is considered to be the end point of pathological remodeling triggered by cardiac injury, cytokine secretion, and enhanced autonomic activity.

Pathological remodeling occurs as a sequelae to acute myocyte loss following a myocardial infarction, or as a chronic result of the afterload imposed on the heart by arterial hypertension, and is associated with myocyte hypertrophy, and the deterioration to congestive heart failure, arrhythmias, and mortality.5 Pathological remodeling of the heart is accompanied by increased apoptosis, fibrosis, and alterations in cardiac gene expression. For instance, during pathological hypertrophy there is increased expression of genes involved in embryonic and fetal development with concurrent down-regulation of adult myocardial genes.6 This so-called “fetal gene activation” is associated with impaired contractile performance, as well as gross metabolic derangements where cardiomyocytes decrease their overall oxidative capacity with increased reliance on anaerobic glucose metabolism.5–8

Potent agonists of pathological cardiac remodeling, angiotensin II (Ang II), endothelin (ET)-1, and α-adrenergic stimulation all activate Gq-coupled receptors, which subsequently activate phospholipase C, and increase fetal gene activation.9 Transgenic mice with forced cardiac-specific expression of the α-subunit of Gq, exhibit baseline cardiac hypertrophy and contractile dysfunction, which is ultimately accompanied by cardiac myocyte apoptosis.10,11 Conversely, genetic ablation or forced expression of a dominant-negative Gq in mice can nearly completely block all aspects of pathological remodeling.12,13 Furthermore, prenatal application of a caspase-3 inhibitor in the Gq-transgenic background resulted in reduced apoptosis, hemodynamic improvement, and a complete amelioration of mortality.14 This study, along with others, highlights the critical role of cardiac myocyte death in the progression from cardiac injury to symptomatic heart failure (reviewed in15). Therefore, manipulation of the molecular pathways that regulate myocyte survival could represent important therapeutic strategy to prevent cardiac deterioration.

Recently, studies have documented a potent role for the nuclear factor-κB (NF-κB) family of transcription factors in the regulation of cardiac myocyte survival through repression of apoptotic cell death triggered by hypoxia or ischemic myocardial injury.16–18 For example, transgenic mice harboring a cardiac-specific expression of a NF-κB inhibitor (IkBα) display a 50% greater infarct size with significantly higher levels of postinfarct apoptosis.18 These studies strongly suggest a protective role for NF-κB during pathological remodeling of the heart following acute cardiac injury. However, the exact role of the NF-κB family in the heart remains a source of controversy, where the role of NF-κB has been described as both adaptive and maladaptive in certain cellular contexts. Indeed, following ischemia and reperfusion in the heart, at least 2 waves of NF-κB activity has been described over the first 6 hours of reperfusion, and recent evidence suggests that NF-κB activity may be sustained for weeks following coronary ligation.19,20 In this context, chronic activation of NF-κB may induce the expression of inflammatory cytokines and produce detrimental consequences, including cardiac cell death. This review series will provide an overview of NF-κB signaling in the heart and attempt to clarify the present state of knowledge regarding the cytoprotective, or cytotoxic, role of NF-κB in acute and chronic pathological remodeling leading to apoptosis during the progression toward heart failure. In the second part of this series, Douglas Mann will discuss recent findings on the role of NF-κB in innate immunity, Toll receptor signaling, and inflammation. Finally, in part 3, Sumanth Prabhu will explore therapeutic opportunities for NF-κB manipulation in cardiovascular pathologies.

### NFκB Family Overview

NF-κB was originally identified as a nuclear factor that bound the immunoglobulin κB light chain promoter in B lymphocytes by David Baltimore and company more than 25 years ago.21 Since this initial discovery, NF-κB and -related signaling pathways have been intensely studied in multiple cell types, notably in the context of innate cellular immunity and oncogenesis.22,23 The mammalian NF-κB superfamily of transcription factors consists of at least 5 genes encoding the members RelA (p65), RelB, c-Rel, p50, and p52.24 All family members are conserved throughout evolution and share a conserved Rel homology domain (RHD) at their N terminus, which mediates DNA binding and dimerization between family members; however, the NF-κB family is divided into two subfamilies based on divergence of the C terminus.24 The “Rel” subfamily (RelA, RelB, and c-Rel; as well as the viral v-Rel and Drosophila Dorsal and Dif) all contain C-terminal transcriptional activating domains (TADs) that are capable of recruiting basal transcriptional machinery to target promoter/enhancer regions. The “NF-κB” subfamily (p50 and p52; as well as Drosophila Relish) lack a TAD and requireimerization with “Rel” family members to activate transcription, but may serve as endogenous inhibitors of target gene expression in the absence of a Rel family member. This subfamily is expressed as longer precursor proteins, p100 and p105, which

### Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>HAT</td>
<td>histone acetyl transferase</td>
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<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
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<tr>
<td>IkB</td>
<td>inhibitor of κB</td>
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<td>IKK</td>
<td>IkB kinase</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>NEMO</td>
<td>NF-κB essential modulator</td>
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<tr>
<td>NF-κB</td>
<td>nuclear factor κB</td>
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<tr>
<td>TNFα</td>
<td>tumor necrosis factor α</td>
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<tr>
<td>TRAF</td>
<td>tumor necrosis factor receptor–associated factor</td>
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contain multiple repressive ankyrin repeats that become cleaved on activation to produce p52 and p50, respectively. In cells NF-κB exists as a heterodimer comprised by the association of its subunits, p50/p50; p50/p52; p50/p65 are the most commonly found in mammalian cells with p50/p65 the predominant NF-κB complex in the heart. As with most transcription factors, nuclear localization is essential for downstream gene transcription. In this regard, in the absence of activating signal, NF-κB exists as an inactive dimer in the cytoplasm of cells bound to inhibitors of κB (IκB) proteins (IκBα, IκBβ, -ε, -κ).24 Classically, signal induced NF-κB activation involves the phosphorylation-dependent degradation of IκB proteins via a proteasomal regulated pathway. Indeed, the discovery of the IκB kinases (IKK) which comprise a complex of IκKα, IκKβ and regulatory subunit IκKγ (NEMO) by Michael Karin and company provided the missing link to explain how NF-κB becomes activated by external ligands, such as TNFα and other inflammatory cytokines. In this model the phosphorylation of two critical serine residues at positions Ser32 and Ser36 were found to be crucial for IκBα phosphorylation and its subsequent degradation by the proteasome. The loss of IκBα, ostensibly unmasks the NF-κB nuclear localization sequence, permitting nuclear targeting of NF-κB. Notably, IκBα is an NF-κB target gene and becomes activated following NF-κB activation, providing a negative feedback to limit or curtail NF-κB signaling. Despite this well accepted model for signal-induced NF-κB activation, recent data from our laboratory and others suggest that NF-κB can shuttle into the nucleus in nonstimulated cells. Though the significance of this finding is unknown, it supports the intriguing possibility that NF-κB regulates the expression of certain genes under basal conditions.

Although numerous NF-κB dimer-pairs could exist, the most common and therefore most studied NF-κB dimer is between p65 and p50.25 However, from a functional point of view the p65 subunit contains the TAD and is believed to be the critical subunit required for transcriptional modulation. Indeed, genetic ablation of both p65 alleles is embryonic lethal at day E8.5 presumably due to excessive apoptosis.26 The failure of the other NF-κB family members to functionally compensate for the loss of p65 in cardiac myocytes (see below). On ligation, the TNFα receptor 1 (TNFR1) recruits several adaptor proteins, such as the TNF-associated death domain protein (TRADD) and the TNF receptor–associated factor (TRAF) family. Once assembled, the TRADD-containing complex activates several well-studied signaling pathways, including JNK and p38 MAP kinases, caspase 8, and the canonical NF-κB pathway.32 TRAF2 and -5 appear to have redundant roles in NF-κB signaling; however, lipopolysaccharide (endotoxin) and several hypertrophic agonists have also been shown to activate p65 in cardiac myocytes (see below). On ligation, the TNFα receptor 1 (TNFR1) recruits several adaptor proteins, such as the TNF-associated death domain protein (TRADD) and the TNF receptor–associated factor (TRAF) family. Once assembled, the TRADD-containing complex activates several well-studied signaling pathways, including JNK and p38 MAP kinases, caspase 8, and the canonical NF-κB pathway.32 TRAF2 and -5 appear to have redundant roles in NF-κB signaling; however, lipopolysaccharide (endotoxin) and several hypertrophic agonists have also been shown to activate p65 in cardiac myocytes (see below). On ligation, the TNFα receptor 1 (TNFR1) recruits several adaptor proteins, such as the TNF-associated death domain protein (TRADD) and the TNF receptor–associated factor (TRAF) family. Once assembled, the TRADD-containing complex activates several well-studied signaling pathways, including JNK and p38 MAP kinases, caspase 8, and the canonical NF-κB pathway.32 TRAF2 and -5 appear to have redundant roles in NF-κB signaling; however, lipopolysaccharide (endotoxin) and several hypertrophic agonists have also been shown to activate p65 in cardiac myocytes (see below).
NF-κB regulator, in association with TRAF proteins, is the TGFβ-associated kinase 1 (TAK1). Although somewhat controversial, TAK1 appears to activate canonical NF-κB signaling at the level of RIP1 and IKK. Termination of canonical signaling occurs through p65-dependent induction of IκBα expression, which alters the dynamic shuttling of the p65/p50 dimer with substantial restoration of the cytosolic inactive pool. In addition, NF-κB induces the expression of IKK inhibitors, such as A20, which attenuate IκB phosphorylation. Finally, it should be noted that NF-κB also induces the expression of other inhibitor proteins that attenuate additional components of the TNFα signaling. For example, the NF-κB target gene cFLIP has been shown to prevent apoptosis by inhibiting JNK and p38 MAP kinase signaling. In addition, NF-κB-dependent induction of c-IAPs inhibits apoptosis through attenuation of caspase 8 at the TNFα receptor.

In contrast to canonical NF-κB signaling, very few studies have investigated the role of noncanonical signaling in the heart. In other cell systems, the major ligands of the alternative pathway include lymphotoxin-β (LTβ), B-cell activating factor (BAFF), and the CD40 ligand. Ligation of these respective receptors results in phosphorylation of p100 at Ser866 and Ser870 and subsequent processing, resulting in an active p52:RelB dimer. The mechanism responsible for initiating p100 processing is the activation of the NF-κB inducing kinase (NIK), which is directly responsible for phosphorylating and activating IKKα. Thus, IKKα is the critical kinase that initiates p100 processing to p52 in the alternative pathway, and not IKKβ. Interestingly, the ligands of the noncanonical pathway, have also been shown in some cell systems to activate the canonical NF-κB signaling pathway, suggesting a degree of cross-talk between the two classically identified pathways.

**Posttranslational Modification and Interacting Partners**

Although nuclear/cytosolic shuttling of the NF-κB dimer has been shown to be the primary mechanism regulating target-gene expression, additional post-translational modifications (e.g., phosphorylation and acetylation) and physical interaction with coactivators and corepressors has also been identified as a mechanism through which NF-κB transcriptional regulation can be modulated (Figure 2). Of particular interest, phosphorylation events have been shown to regulate the physical interaction, and transcription activity of NF-κB, with histone
modifying enzymes, such as the histone acetyltransferases (HATs) and histone deacetylases (HDACs). NF-κB is activated by a physical interaction with the HATs p300 and CBP. As reviewed in by Hayden and Ghosh,32 this physical interaction is modulated by phosphorylation of p65 by PKA at Ser276.45 In this series of experiments, the catalytic subunit of PKA was found to physically associate with a cytosolic IκB:p65 containing complex. On stimulation, IκB is degraded, which activates PKA resulting in phosphorylation of Ser276. Phosphorylation at this residue disrupts and intramolecular fold between the N terminus and C terminus of p65, which makes the NF-κB dimer more permissive to physical interaction with the coactivator HATs, such as CBP and p300.45–47 These results are particularly interesting to the field of cardiovascular biology given the importance of PKA and adrenergic signaling in pathological cardiac remodeling.48,49 In addition, both p65 and p300 have been shown to induce cardiac hypertrophy,30,50,51 and p300-null mice display a lethal cardiac defect related to impaired proliferation.52 Therefore, it

<table>
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<tr>
<th>Gene</th>
<th>Function</th>
<th>Regulation</th>
<th>Transgenic Phenotype</th>
<th>Reference</th>
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<tr>
<td>RelA (p65)</td>
<td>Transcriptional activator in the canonical NF-κB dimer</td>
<td>Regulated by nuclear/cytoplasmic shuttling, post-translational modification (see Figure 2)</td>
<td>KO</td>
<td>Embryonic lethal due to liver apoptosis</td>
</tr>
<tr>
<td>NFκB1 (p50)</td>
<td>Component of the canonical NF-κB dimer</td>
<td>Generated from p105; phosphorylated at S337 by PKA and acetylated by p300 (reviewed by Perkins44)</td>
<td>KO</td>
<td>Immune defects; resistance to arthritis; neural degeneration</td>
</tr>
<tr>
<td>IκBα</td>
<td>Inhibitor of the canonical NF-κB dimer</td>
<td>Phosphorylated by IKKβ at S32 and S36; ubiquitinated at K21 and K22 (reviewed by Hayden and Ghosh32)</td>
<td>KO</td>
<td>Early neonatal lethal with granulopoiesis and dermatitis</td>
</tr>
<tr>
<td>IKKβ</td>
<td>Phosphorylates and promotes proteasomal degradation of IκBα</td>
<td>Recruited with NEMO; phosphorylated by TAK1 (reviewed by Perkins44)</td>
<td>KO</td>
<td>Embryonic lethal due to liver apoptosis</td>
</tr>
<tr>
<td>IKKγ (NEMO)</td>
<td>Modulates IKKβ activity</td>
<td>Activated by K63-linked polyubiquitination and interaction with RIP1 (reviewed by Perkins44)</td>
<td>KO</td>
<td>Embryonic lethal due to liver apoptosis</td>
</tr>
<tr>
<td>IKKγ (NEMO)</td>
<td>Modulates IKKβ activity</td>
<td>Above</td>
<td>Cardiac-specific expression of phospo-resistant mutant</td>
<td>Enhanced apoptosis following acute MI; Chronic protection from apoptosis and ER stress following ligation-induced HF; Protection from hypertrophy</td>
</tr>
<tr>
<td>IKK</td>
<td>Phosphorylates and promotes proteasomal degradation of IκBα</td>
<td>Recruited with NEMO; phosphorylated by TAK1 (reviewed by Perkins44)</td>
<td>KO</td>
<td>Embryonic lethal due to liver apoptosis</td>
</tr>
<tr>
<td>IKKγ (NEMO)</td>
<td>Modulates IKKβ activity</td>
<td>Above</td>
<td>Cardiac-specific deletion</td>
<td>Age-related myopathy with enhanced apoptosis</td>
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Figure 2. Schematic of major p65 (RelA) structural domains, posttranslational modifications, and interacting factors. The structural domains of the p65 subunit are shown including, the N-terminal Rel homology domain (RHD) and its associated nuclear localization sequence (NLS), and the C-terminal transcriptional activation domain. A, Previously identified posttranslational modifications of p65 subunit and their relative locations are shown and designated by the following: A indicates acetylation; K, lysine residue; P, phosphorylation; S, serine residue; T, threonine residue. B, Selective p65-interacting factors and their relative binding positions in the RHD or transcriptional activation domain. *Binding domain remains in question.
seems likely that the p65:p300 interaction and its regulation by PKA could play a critical role in pathological remodeling of the heart, yet further investigation is required to determine the exact role.

Conversely, the interaction of NF-κB with class I HDACs has been studied in greater detail in cardiovascular biology. RelA (p65) has been shown to interact with HDAC1, -2, and -3, leading to target gene repression. In cardiac myocytes, NF-κB and HDAC1 cooperatively inhibit the expression of the proapoptotic gene, Bnip3. Furthermore, forced expression of HDAC1 significantly attenuated the induction of Bnip3 by acute hypoxia in a cis element-dependent manner. In other cell systems, the interaction between p65 and HDAC1 has been shown to be dependent on phosphorylation of Thr-505 by the tumor suppressor Checkpoint kinase 1 (Chk1) in association with the PIKK-family kinase ATR. These findings are consistent with the cardiac-specific HDAC1 and -2 null mouse which displays elevated postnatal cardiac myocyte apoptosis and pathological hypertrophy. It is interesting to speculate that the elevated rate of cell death was attributable to derepression of NF-κB and enhance Bnip3 expression; however, this remains to be determined experimentally.

Acetylation of p65 also affects its biological function. Acetylation on lysine residue 221 is important for DNA binding and for inhibiting the association between p65 and IkBα. Accordingly, deacetylation of this residue promotes p65-IκBα binding, cytoplasmic localization of p65, and the termination of NF-κB activity. Furthermore, acetylation at lysine 310 was shown to increase the transcription potential of p65. Interestingly, it appears that the phosphorylation of p65 at S276 and S536 may "prime" p65 for acetylation at K310 because of increased association with the acetyl transferase p300, and this sequential modification of p65 is required for its full transcriptional activity. It is also suspected that phosphorylation of S311 may have a similar role in the induction of acetylation. Finally, microarray analysis suggests that expression of specific NF-κB target genes may be selectively modulated by acetylation of p65. Therefore, there is strong evidence that these post-translational modifications of p65 have biological significance. The discrepancies in the literature regarding the functional significance of NF-κB signaling in the heart might be the consequence of using concatemerized reporter-genes and individual NF-κB site-containing oligonucleotides in electromobility shift assays that do not detect the nuances of NF-κB target gene expression.

In addition to histone modifying enzymes, such as CBP and HDACs, NF-κB has been shown to interact with numerous transcription factors and cofactors that may be of interest to the cardiovascular biologist (see www.nf-kb.org for a complete list). For example, p65 has been shown to interact with SRF, MEF2, myocardin, STAT3, and menin in other cell types. Importantly, each of these interacting partners has been shown to play a critical role in cardiovascular development and/or pathological remodeling of the heart, yet the precise role of these interactions with p65 needs to be fully characterized to define their in vivo function with respect to cardiac biology and site directed NF-κB transcriptional control. Finally, a recent ELM analysis of p65 has revealed several uninvestigated SH2, SH3, and WW domains, which could represent additional phosphorylation-dependent interaction domains where interacting partners could modulate NF-κB transcriptional control.

The Role of the NFκB Family in the Heart: Hypertrophy

In cultured cardiac myocytes, hypertrophic agonists that activate Gq/11-coupled receptors, such as Ang II, endothelin-1, and phenylephrine, all promote IκB degradation, as well as p65 nuclear translocation and transcriptional activity (Figure 1). In addition, NF-κB activity is required for the hypertrophic phenotype induced by these agonists and for the increased expression of atrial natriuretic factor (ANF) in postnatal ventricular cells, an important marker of fetal gene activation and pathological remodeling. In vivo studies have confirmed in vitro results, in that cardiac-specific expression of a mutant IκBα super-repressor attenuates the hypertrophic cardiac phenotype induced by Ang II and isoproterenol infusion, or low-grade aortic banding; as well as the induction of a subset of fetal cardiac genes. Furthermore, p50−/− mice display reduced cardiac hypertrophy following myocardial infarction or during TNFα-induced cardiomyopathy. However, NF-κB inhibition could not overcome hypertrophy in vivo studies using higher gradient aortic banding, nor did NF-κB inhibition alter the course of stress-induced remodeling, which has raised the question of whether NF-κB is associated with adaptive or maladaptive hypertrophy. In addition, it remains to be demonstrated how NF-κB induces cardiac hypertrophy and fetal gene expression, because, to our knowledge, NF-κB binding sites have not been identified in the promoter regions of adult or fetal cardiac genes associated with cardiac growth. Therefore, we speculate that NF-κB induces cardiac remodeling through a physical interaction with another hypertrophic transcription factor (see above), or through an indirect effect, perhaps by activating the expression of another regulator of cardiac remodeling, such as PKCδ, BMP-2, or FGF8, which have been shown to be NF-κB target genes in other cell systems and have been demonstrated to regulate cardiac growth.

Interestingly, NF-κB signaling is also activated by the interleukin (IL)-6 family of cytokines, such as IL-6 itself, leukemia inhibitory factor (LIF), and cardiotropin-1 (CT-1) in association with cardiac myocyte growth (Figure 1). The mechanism by which the IL-6 family induces NF-κB-dependent cardiac growth has not been established; however, LIF induces cardiac hypertrophy by activating ERK5 signaling, which has been shown to activate NF-κB through RSK2-mediated phosphorylation and degradation of IκBα. Thus, although NF-κB activity has been demonstrated to be required during postnatal cardiac growth, many questions remain unanswered regarding the mechanisms by which NF-κB induces a hypertrophic response and whether the physiological consequences of NF-κB induced cardiac growth are adaptive or maladaptive.
The Role of the NFκB Family in the Heart: Cardioprotection

The cardioprotective role of NF-κB signaling has been evident for a number of years. In this regard we previously reported that Bcl-2 suppresses cell death of ventricular myocytes through a mechanism involving IKKβ-mediated activation of NF-κB.82,83 Furthermore, in cultured cardiac myocytes, forced expression of a nonphosphorylatable 1xIκBα designed to inactivate NF-κB signaling resulted in a heightened sensitivity to TNFα-induced apoptosis.16 Cardiac-specific expression of a similar 1xIκBα mutant increased the susceptibility of myocytes to apoptosis following acute coronary occlusion in vivo,18 whereas p50−/− mice display enhanced cardiac dysfunction following myocardial infarction.84 This finding is supported by recent evaluation of a natural occurring human polymorphism in the p50 gene that is associated with increased functional deterioration in patients with heart failure.85 These findings are further supported by the cardiac-specific deletion of the NEMO gene, in which mice develop an age-related cardiomyopathy accompanied by increased oxidative stress and apoptosis, which is exacerbated by pressure overload.86 Indeed, work from our laboratory has previously shown that hypoxia-induced intrinsic cell death and mitochondrial defects can be completely overcome by forced expression of IKKβ in cultured cardiac myocytes.87 Furthermore, IKKβ was found to promote cell survival in hypoxic conditions by repressing the expression of the proapoptotic Bcl-2 family member, Bnip3, through a physical interaction with HDAC1.55,88 Detailed analysis of this molecular “switch” regulating cell death and cell survival revealed that hypoxic conditions induces the expression of the cell cycle transcription factor E2F-1, which is released from retinoblastoma (Rb) repression. Active E2F-1 binds to a consensus cis element within the bnip3 proximal promoter region to induce Bnip3 expression and promote cell death.89 These results were validated in the context of intact chromatin in primary cardiac myocytes using chromatin immunoprecipitation, where hypoxia induced E2F-1 binding to the bnip3 promoter, whereas forced expression of Rb eliminated E2F-1 binding and reduced Bnip3 expression.89 Most recently, our research group demonstrated that p65 competes for E2F-1 binding at the bnip3 promoter through an adjacent κB site.90 In this series of experiments, forced expression IKKβ protected cardiac myocytes from E2F-1-induced death by displacing E2F-1 from the bnip3 promoter region. Furthermore, using chromatin immunoprecipitation assays, we identified that p65 occupies the κB site within the bnip3 promoter under basal normoxic conditions and Bnip3 expression is repressed; however, when cardiac myocytes are stress with hypoxia, p65 is displaced by E2F-1 leading to increased expression of Bnip3 and intrinsic apoptotic death.90 Not only do these experiments confirm the cardioprotective role of p65 during acute hypoxia, but they also suggest that at least two distinct pools of p65 exist within cardiac myocytes. One pool within the cytosol bound to 1xIκBα, which undergoes basal nuclear/cytosol shuttling, yet is sensitive to stimulation through IKKβ. The other pool, which is likely less abundant, readily shuttles between cytoplasm and the nucleus to regulate gene expression. Though unproven, the basal nuclear presence of p65 may be important for regulating death promoting genes, such as Bnip3.

The Role of the NFκB Family in the Heart: Cardiotoxicity

The controversy surrounding the proapoptotic nature and detrimental features of NF-κB signaling in the heart are equal only to the controversial role of TNFα signaling in the heart (reviewed elsewhere27). For example, early studies investigating the role of TNFα in heart failure concluded that TNFα was involved in a maladaptive response leading to heart failure,93 yet gene-targeting studies using null-mutations in the TNFR1 and/or -2 genes concluded that TNFα protects the heart from ischemia and reperfusion induced apoptosis.17,92 Furthermore, p50−/− mice display protection from TNFα-induced cardiomyopathy and improved cardiac function following myocardial infarction.73,74 However, this later result has been disputed by another research group.44 Importantly, and as noted above, TNFα receptor signaling has been shown in multiple cell systems to simultaneously activate both proapoptotic JNK, p38, and caspase 8 cascades, along with canonical NF-κB signaling, and that TNFR1 and -2 may have opposing effects on cardiac remodeling.93 Therefore, it seems likely that the cellular response will depend on additional criteria, such as the timing and duration of signaling, as well as other environmental cues including hypoxia, and other inflammatory signals. For example, genetic ablation of TNFα in mice reduced infarct size following ischemia/reperfusion and reduced the expression of proinflammatory chemokines, such as IL-6 and the monocyte chemoattractant protein-1 (MCP-1).17 Interestingly, DNA-bound NF-κB was also reduced following ischemia/reperfusion in the TNFα-null mice, and the authors suggest that reduced NF-κB activation might be a mechanism for the improved outcome and reduced inflammatory response in this model.17

The hypothesis that NF-κB activation could produce a chronic inflammatory response from macrophages and inflammatory cells with detrimental consequence is not new and is supported by the fact that numerous NF-κB target genes are proinflammatory, such as IL-1β and IL-6,95 whereas others have been shown to induce intrinsic apoptosis in many cell types, such as TNFα,96 and p53.97 Indeed, activation of NF-κB down-stream of the TNFα is known to provoke apoptosis.

During preparation of this review, Prabhu and colleagues published a critical research article that has expanded our knowledge regarding the chronic inactivation of NF-κB during chronic pathological cardiac remodeling.20 This group reports sustained activation of p65 following coronary ligation in mice. Furthermore, using a transgenic mouse model expressing a nonphosphorylatable 1xIκBα in the heart, this group followed mice postcoronary ligation for up to 4 weeks, as opposed to a 24-hour end point used previously by Misra et al from the Mann laboratory.18 With chronic inhibition of NF-κB signaling, mice displayed less mortality from heart failure, reduced inflammatory cytokine secretion (IL-1β, IL-6, and TNFα), and reduced levels of apoptosis.20 These findings strongly support the hypothesis that chronic activation of NF-κB signaling results in a prolonged inflammatory response.
state resulting in increased apoptotic cell death and progression toward heart failure. Based on the above, we provide a tenable explanation to explain the varied and dichotomous actions of NF-κB in the heart. Notwithstanding, we speculate early NF-κB activation following acute myocardial injury may be an important adaptive mechanism to prevent apoptosis and preserve myocyte number. In contrast chronic or persistent NF-κB activation would be considered maladaptive by perpetuating the inflammatory process. This would effectively preclude the removal of damaged or dying cardiac myocytes ultimately leading to infarct expansion and demise in cardiac function. This notion is concordant with elevated NF-κB and resistance to apoptotic cell death commonly seen in human tumor cells. This could also explain the observed beneficial effects of agents such as sulfasalazine or ASA (acetylsalicylic acid) that inhibit NF-κB signaling on cardiac function in patients postmyocardial infarction.

**Discussion**

Although NF-κB signaling in the heart has been controversial, the findings summarized in this review are consistent with the hypothesis that NF-κB regulates at least 3 genetic programs that depend greatly on the cellular context and the timing of activation (Figure 3). These programs include hypertrophy, 10 acute cytoprotection from hypoxia/ischemia, 18 and chronic cytotoxicity promoted by a prolonged inflammatory response. 20 However, many questions remain unanswered regarding the regulation of these genetic programs. For example, what is the nature of the transcriptional mechanism that determines which program will be executed, while others are repressed. Although it seems likely that distinct signaling pathways and a chromatin level of epigenetics are involved, the fact remains that very little is known regarding site-directed transcriptional regulation of NF-κB in the heart. Further insights into physiological and pathological role of NF-κB and its timing of activation or inactivation will be gained through the use of inducible genetic mouse models that allow for temporal control of transgene activation or recombination. Furthermore, of the more than 150 known interacting partners identified for the NF-κB family (see www.nf-kb.org for a complete list), very few of these have been evaluated in cardiac cells. Critical answers may be found with investigations evaluating the specific NF-κB cofactors assembled at target genes of these distinct genetic programs, as has been the case with the well-studied cardiovascular transcription factor, SRF. 98,99 This concept is intriguing given that different promoter architectures often recruit specific protein complexes, even when they share a common cis element. This could explain how a signaling pathway can activate one target gene and not another. In addition, the specific NF-κB dimer formed at distinct promoters, evaluated by chromatin immunoprecipitation analysis, as well as posttranslational modification and alternative pathway signaling to NF-κB may provide explanations as to how a single family of transcription factors can regulate such opposing genetic programs within the heart under physiological and pathophysiological conditions. Given the multifaceted features of NF-κB, we posit that therapies designed to modulate NF-κB activity in the heart temporally and spatially hold promise for mitigating aberrant cell death and inflammation following injury.

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**Disclosures**

None.
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