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NEMO Nuances NF- κ B

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Stress-induced adverse remodeling of the myocardium is a major mechanism leading to heart failure, a leading and rapidly escalating source of morbidity and mortality worldwide.^{1,2} As a result, much work is underway to dissect molecular mechanisms governing cardiac remodeling in hopes of identifying novel therapeutic targets. In recent years, much of this work has focused on the hypertrophic growth response of the cardiac myocyte. Initially adaptive, cardiac hypertrophy compensates for declines in cardiac performance and increases in wall stress; sustained hypertrophy, however, is a major risk factor for emergence of systolic dysfunction and clinical heart failure.³ On the bright side, numerous preclinical studies have demonstrated that abrogation of the hypertrophic response is well tolerated, and even beneficial.⁴

One potential target of therapy in the pathologically remodeled, hypertrophied heart is the transcription factor nuclear factor (NF)- κ B. First discovered more than 20 years ago, NF- κ B has been linked to numerous neurohormonal, pathophysiological, and stress stimuli responses, and it has been characterized most extensively in the immune system. In the heart, activation of NF- κ B-dependent transcription has been detected in numerous disease contexts, including hypertrophy, ischemia/reperfusion injury, myocardial infarction, allograft rejection, myocarditis, apoptosis, and more.^{5,6} Within coronary vessels, NF- κ B has been implicated in atherosclerosis and restenosis.^{5,6} However, parsing the specific role(s) of NF- κ B in these diverse disease processes has been hampered by the embryonic lethality of inactivation of several NF- κ B components.^{7–9}

In heart, the NF- κ B family of transcription factors comprises 4 members: p50, p52, p65, and RelB. All are capable of multimerization, forming either homo- or heterodimers, but the ubiquitously expressed p50 and p65 (herein termed NF- κ B) are responsible for the majority of NF- κ B binding activity in the myocardium. Activation of cytoplasmic NF- κ B requires phosphorylation and subsequent proteasome-dependent degradation of its repressor, inhibitor of κ B (I κ B). The essential step in this so-called canonical pathway, degradation of I κ B, is initiated by a multicomponent I κ B kinase (IKK) complex, comprising a regulatory scaffolding subunit NEMO (NF- κ B essential modifier) (also known as IKK γ) and 2 catalytic subunits, IKK α and IKK β . Then, the p50/p65 complex, released from the

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repressive influence of I κ B, migrates to the nucleus, where it binds cognate DNA sequences (κ B sites) in target genes.

The NF- κ B response is often transient, because it is governed by at least 2 negative feedback loops. In one, the protein I κ B α , a product of NF- κ B transcription, binds NF- κ B, and the newly formed I κ B α /NF- κ B complex is translocated out of the nucleus, obviating NF- κ B-dependent gene transcription. In another, the NF- κ B-responsive, zinc finger protein A20 inhibits initiation of the NF- κ B cascade by inactivating IKK in the cytoplasm. In addition, p50/p50 homodimers can attenuate NF- κ B transactivation.^{10,11} Thus, expression of NF- κ B-dependent genes is tightly regulated by multiple, interlacing control processes.

In the myocardium, it remains puzzling the extent to which NF- κ B promotes cell survival or cell death. Some evidence points to important cardioprotective effects. For example, NF- κ B activation attenuates the hypertrophic response to pressure overload,¹² minimizes infarct size during late-phase ischemic preconditioning,¹³ and lowers tumor necrosis factor- α -dependent apoptotic myocyte death.¹⁴ By contrast, other evidence suggests that cardioprotection can be brought about by blocking essential components of the NF- κ B pathway; suppression of the NF- κ B cascade decreases cardiac hypertrophy^{15–17} and prevents stress-induced ventricular dilation.^{15,16,18,19} In addition, NF- κ B activation is required for doxorubicin-induced cardiomyocyte apoptosis.²⁰ Clearly, the multifaceted roles of NF- κ B in the heart require clarification.

NF- κ B is so pleiotropic that it has traditionally been considered a “general,” nonspecific transcription factor: a diverse array of stimuli activates NF- κ B, and NF- κ B, in turn, regulates more than 200 genes. More recent evidence, however, suggests that NF- κ B serves as a nodal point of signaling, governing a network of circuits to integrate sundry inputs and elicit precise outputs via specific downstream targets. Consistent with this is our ever-growing understanding of the complexity of NF- κ B feedback and feedforward control loops. Very recently, microRNAs have entered the NF- κ B control picture.²¹ This work has uncovered an entire network of genes involved in cardiovascular development and reprogramming enriched for NF- κ B binding sites in their proximal promoter regions.

Much of the work to delineate NF- κ B functions in heart has been conducted using transgenic models of cardiac-specific expression of mutant p50 and/or unphosphorylatable (undegradable) I κ B. However, conclusions drawn from these studies have been conflicting.^{12,13,15–18} For example, 2 independent groups studying mice exposed to ischemic stimuli and harboring degradation-resistant I κ B mutants reported that NF- κ B can be either maladaptive²² or cardioprotective.²³ Although this discrepancy may stem from differences in severity of the stresses used (ischemia/reperfusion versus permanent coronary artery occlusion),⁵ the diametrically opposing results are nonetheless puzzling. In light of this, development of novel genetically manipulated animal models targeting other components of the cardiac NF- κ B machinery is welcome.

In this issue of *Circulation Research*, Kratsios et al²⁴ report the effects of cardiomyocyte-specific ablation of NEMO, an essential activator of NF- κ B. Their elegant study demonstrates that inactivating NEMO in cardiac myocytes depletes cells of NF- κ B-dependent antioxidant machinery. As a consequence, cells undergo spontaneous pathological remodeling, and load-induced changes are accelerated. Inactivation of NF- κ B signaling by cardiac-specific ablation of NEMO led to attenuation of several antioxidant genes and associated accumulation of reactive oxygen species. The study went on to provide additional support for the role of oxidant stress in NEMO-deficient hearts with experiments in which mutant mice were fed chow supplemented with the antioxidant molecule butylated hydroxyanisole (BHA). BHA-supplemented diet afforded partial protection to NEMO-

deficient cardiomyocytes. However, it failed to completely abrogate apoptotic cell death, cardiac fibrosis, and contractile dysfunction.

This study has provided important new insights into the oftentimes puzzling world of NF- κ B biology in the heart. Importantly, the findings are consistent with earlier reports demonstrating that Mn-superoxide dismutase, an essential antioxidant protein, is negatively regulated by p53, a downstream target repressed by NF- κ B.²⁵ However, important questions remain to be resolved.

First, the actions of the NEMO protein itself are multifaceted. For example, NF- κ B-mediated responses to DNA damage depend on their activation by nuclear NEMO.²⁶ Furthermore, an essential feature of the role of NEMO in this, as well as in canonical NF- κ B pathway activation, is its potential for posttranslational modifications. At present, Lys285, Lys321, Lys325, Lys326, and Lys399 within NEMO have been identified as sites modified with Lys63-linked polyubiquitin chains in response to various stimuli.²⁷ In contrast to well-characterized Lys48 ubiquitin linkages, which serve as a signal for proteasomal degradation, Lys63-linked polyubiquitin chains function in signaling, protein-protein interactions and recognition, and DNA repair.^{27,28} Furthermore, Lys277 and -309 can be modified by either ubiquitin or SUMO-1 (small ubiquitin-like modifier-1).

The biology of NEMO is yet more complex in light of recent reports demonstrating that it can undergo modifications by so-called “linear” polyubiquitin chains.^{27,29} Interestingly, linearly polyubiquitinated NEMO is stable and not degraded by the proteasome, and evidence suggests that this posttranslational modification may function as a platform for the binding of additional proteins.^{27,29} Also, it has been demonstrated recently that some proteins can be conjugated with multiple polyubiquitin chains with different ubiquitin-linkages.³⁰ Therefore, it seems plausible that specific combinations of these polyubiquitin chains may modulate the function of NEMO to determine and direct specific NF- κ B signaling outputs in a given context.

In light of these facts, the NEMO mutant complements the arsenal of existing mouse models with abrogated NF- κ B signaling in the heart (ie, I κ B degradation-resistant mutants). Because NEMO acts upstream of I κ B in NF- κ B activation, this model may provide new insights into the integration of NF- κ B-activating signals and the selectivity of the output(s) of the NF- κ B-dependent transcriptional network. Additional complexity in NEMO-dependent NF- κ B activation is highlighted by the fact that NEMO harbors at least 7 reported sites for posttranscriptional modification. Up to the present time, the majority of studies have been based on systems where NF- κ B activation was abolished. Moving forward, studies designed to decipher more granular aspects of this critical pathway will be welcome.

Thus, it is not certain that ablation of NEMO, and the consequent effects on cardiac remodeling, derive exclusively from the NF- κ B-silencing actions of NEMO mutants, as it cannot be excluded that NEMO has actions on other, yet unknown, pathways. As a case in point, some evidence suggests that NEMO has IKK/NF- κ B-independent functions besides its role in DNA damage responses. For example, nuclear-localized NEMO can bind competitively to the important coactivator CBP (CREB binding protein)³¹; NEMO promotes interaction of CBP with hypoxia-inducible factor (HIF)2 α , thereby enhancing transcriptional activity of HIF2 α .³² An additional novel role for NEMO in blocking cell death, independent of its role in NF- κ B signaling, is NEMO-dependent restraint of RIP1 (receptor interacting protein kinase 1), a potent apoptotic inducer protein, from engaging caspase 8.³³ Finally, in studies of this nature, it is impossible to exclude the existence of secondary, compensatory responses to NEMO inactivation that alter combinatorial interactions between NF- κ B and other transcription factors.

Twenty years of research has revealed a plethora of important actions of NF- κ B in the governance of numerous cellular functions. Yet, elucidating its effects in the myocardium has remained elusive. Now, the report²⁴ by Kratsios et al has moved the field forward, providing important new insights into the multi-layered network of NF- κ B–dependent transcription in the heart. This new information takes us one step further toward the ultimate goal of harnessing the cardioprotective effects of NF- κ B for therapeutic gain.

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Non-standard Abbreviations and Acronyms

IκB	inhibitor of κ B
IKK	inhibitor of κ B kinase
NEMO	nuclear factor κ B essential modifier
NF-κB	nuclear factor κ B

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