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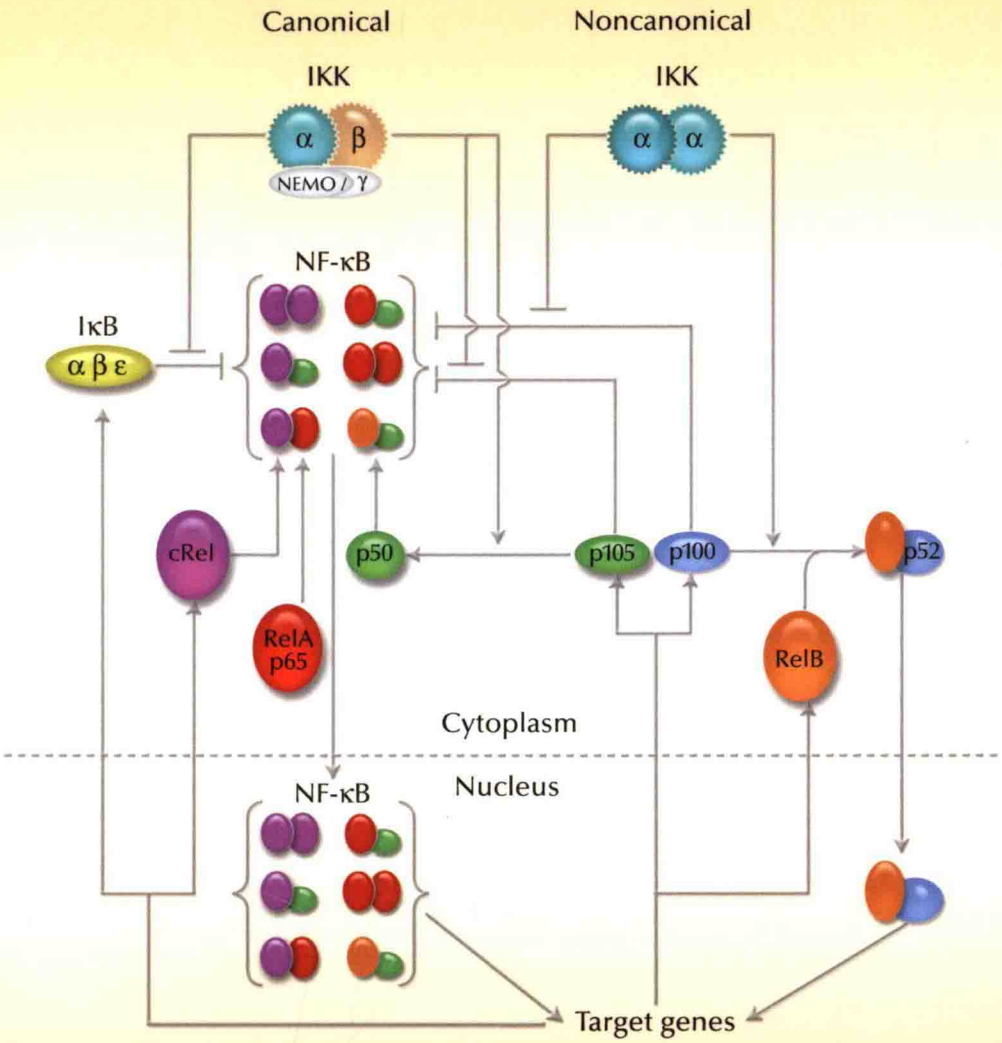
NF-κB

A Network Hub Controlling Immunity, Inflammation, and Cancer



EDITED BY Michael Karin
Louis M. Staudt

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Front cover artwork: Schematic illustration of canonical and non-canonical pathways for activation of the transcription factor NF-κB. Canonical signals activate NEMO-containing IKK complexes (green), which degrade the canonical IκB proteins (IκBα, β, ε) and IκBγ complexes with associated NF-κB dimers. Released Rel-containing dimers move to the nucleus to activate gene expression programs and further expression of IκBα, IκBε, p105, p100, and RelB proteins. Non-canonical signals activate IKKα complexes (blue), which degrade IκBλ complexes associated with NF-κB dimers. The resulting increase in synthesis of p100 and RelB, concomitant with IKKα complex activity, causes increased p100 processing to p52 and dimerization with RelB to generate active RelB:p52 dimers in the nucleus. Stress signals can activate the eIF2α kinases, causing phosphorylation of eIF2α and resulting in inhibited translation. A block in IκB synthesis, in combination with constitutive IKK activity, results in the loss of IκB proteins and subsequent NF-κB dimer activation. See chapter by Ellen O'Dea and Alexander Hoffmann in this volume.

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Preface

THE NF-κB PATHWAY BROUGHT MAMMALIAN SIGNAL TRANSDUCTION into the modern era, allowing the analysis of cell signaling to become inclusive and holistic. Prior to its definition, biologists had focused on receptor-proximal events, largely through study of receptor tyrosine kinases and G-protein-coupled receptors or the study of isolated protein kinases. The gaping hole in our understanding at that point was the connection to the nucleus. It was clear that many of the physiological effects of receptor signaling were achieved by altering gene expression, but how signals were transduced from the plasma membrane to the nucleus was by-and-large a mystery. The elucidation of NF-κB signaling in all of its molecular glory provided a new paradigm for understanding how receptor signaling can elicit transcriptional responses in mammalian cells. Even more importantly, NF-κB helped transform the study of cell signaling from dealing with cultured cells to consideration of whole animal systems.

As David Baltimore points out in his introduction, NF-κB was born rather innocently in a search for transcriptional regulators of the immunoglobulin locus. At the time, this effort was directed towards understanding the orchestrated changes in gene expression that take place during lymphocyte development. However, by following their noses, scientists in the Baltimore lab soon realized that the nuclear accumulation of the NF-κB DNA binding proteins could be induced by a number of extracellular stimuli and set out to understand how this molecular connection was made. The discovery that NF-κB exists in a latent form in the cytoplasm and translocates to the nucleus in response to various stimuli changed the way mammalian signal transduction was conceptualized.

What followed was a breathtaking explosion of research that uncovered pivotal roles for NF-κB in a host of biological processes critical for normal physiology of animals and their ability to counter stress, disease, and infection. As Jules Hoffman discusses, NF-κB emerged early in animal evolution as an important regulator of development and as a defense mechanism against invading pathogens, themes that have been embellished in vertebrate evolution. Although mammals no longer count on NF-κB in the control of general development and morphogenesis, they use it to signal downstream of a host of receptors, many of which are members of extended gene families to control responses to stress, infection, and injury.

This collection includes reviews of the molecular mechanisms by which NF-κB transcription factors are activated and exert their function in the nucleus, as well as reviews that summarize certain realms of biology that are particularly influenced by NF-κB signaling. Ingrid Wertz and Vishva Dixit focus on the mechanisms whereby receptors that detect antigens, inflammatory cytokines, and foreign organisms utilize protein–protein interactions and the ubiquitin system to engage IkB kinase (IKK) to initiate NF-κB signaling. The structure of IKK complex components and the elaborate regulation of its protein kinase activity are described by Alain Israël in his contributon. Andrea Oeckinghaus and Sankar Ghosh summarize the process by which IkBs sequester NF-κB in a latent state in the cytoplasm and how IKK action relieves this inhibition. Yinon Ben-Neriah and colleagues discuss the role of ubiquitination in the regulated degradation of the IkBs. The Rel-homology DNA binding domains of NF-κB transcription factors are discussed in structural detail by Tom Huxford and Goury Ghosh. The various NF-κB heterodimers have distinct target genes, as discussed by Ranjan Sen and Steve Smale, and can be altered in their transcription regulatory activities by post-translational modifications and association with co-factors, as discussed by

Fengyi Wan and Mike Lenardo. Chromatin structure further shapes the transcriptional output of NF-κB dimers, as reviewed by Gioacchino Natoli. The overall biological output of NF-κB signaling must be viewed from a systems biology perspective, as argued by Ellen O'Dea and Alex Hoffmann, whereas Steve Gerondakis and Uli Siebenlist recount the manifold ways in which NF-κB signaling controls lymphocyte differentiation, activation, and function in vivo. Equally important is the regulation of innate immunity and inflammatory responses, as presented by both Toby Lawrence and Michael Karin. Inflammation can promote cancer development, and Michael Karin outlines the compelling evidence for the critical pathogenic role played by NF-κB signaling in this process. Not surprisingly, cancers of many varieties accumulate genetic lesions that subvert NF-κB signaling to protect against cell death and promote proliferation, offering many possible avenues for therapy, as discussed by Lou Staudt. Barbara and Christian Kaltschmidt remind us that without NF-κB in our neurons, we would suffer learning disabilities and memory loss and would gain little from reading collections like this!

Twenty-four years after the discovery of mammalian NF-κB and more than 25,000 publications later, there remain mysteries and challenges in the NF-κB field. The contribution by Tao Lu and George Stark demonstrates that unbiased genetic screens continue to yield new regulators of NF-κB, so it will be years before we have a complete parts list for this system and a full understanding of its working. Given the dysregulation of NF-κB in inflammatory and autoimmune diseases, as well as in cancer, it is imperative that precise methods to manipulate NF-κB are developed. This will be a challenge given the baroque regulation of the NF-κB signaling system and its diverse biological functions, but one that can be met by building on the strong edifice of knowledge presented in this volume.

Finally, we wish to thank the Cold Spring Harbor Laboratory Press project manager, Mary Cozza, for her excellent support in pulling this book together, as well as Alex Hoffmann, whose NF-κB wiring diagram formed the basis of the front cover illustration.

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Contents

Discovering NF-κB

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NF-κB is a protein transcription factor that can orchestrate complex biological processes, such as the inflammatory response. It was discovered in a very different and very limited context, and only over time has its protean nature become evident.

It was actually a very logical process that led us to NF-κB. Although the discovery was very exciting, NF-κB was not the protein we were seeking at the time. To explain this, I must go back to when my laboratory first became interested in immunology.

My first independent position was at the Salk Institute, where I arrived in the spring of 1965. For the previous 4 years that I had been in research, my interests had revolved around the biochemistry of viruses. At Salk, although my work continued to be on viruses, I was exposed to the fascinating questions of immunology. The main issue was how the enormous diversity of antibodies is generated from a limited amount of genetic information. Like so many others, I thought about the question, but it took the experimental attention of Susumu Tonegawa, in 1976, to crack the problem and show that the solution involved DNA rearrangement.

In 1974, the methods of recombinant DNA technology were first developed and it was clear that previously intractable complex systems, like the immune system, could be examined with these methods. In 1976, knowing that the methods were available and that the paradigm of DNA rearrangement had been established, some postdoctoral students in my laboratory and I decided to plunge into this field. I wanted to apply our biochemical skills to this suddenly tractable system. We were already working on one enzyme that was involved in immunoglobulin gene specification, terminal transferase, and had a useful viral transformation system in the laboratory that affected lymphoid cells, the Abelson mouse leukemia virus. So, immunology was not totally new to us.

We had to develop our skills with recombinant DNA methods, become familiar with the awful lingo of immunology, and define some questions for ourselves, but all of that came to pass. In time, I began to see the question of how immune cells develop as the key one for my laboratory. It seemed likely that the problem would come down to understanding the control of transcription factors. So, we focused on transcription of immune cell genes as our primary interest. We had produced evidence that in the development of B lymphocytes, the heavy-chain locus is first to rearrange its DNA, followed by the light-chain locus (Siden et al. 1981). Cary Queen joined the laboratory and studied the transcription of the κ light-chain gene and demonstrated that it contains an intragenic transcriptional enhancer (Queen and Baltimore 1983). These

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developments led us to ask whether it might be possible to understand the transition of a cell from heavy-chain only to heavy-plus-light chain by understanding the transcription factors that bind to the κ light-chain enhancer. Understanding the proteins that bind to the regulatory sites in both the heavy- and the light-chain genes became the project of a new postdoctorate, Ranjan Sen. He worked closely with people in Phil Sharp's laboratory, who had similar interests.

Ranjan and Harinder Singh, from the Sharp laboratory, worked out how to use mobility shift assays to find transcription factors, and first published on the existence of the Oct factors (Singh et al. 1986). Then Ranjan applied the methods to enhancers and found multiple factors binding to both the heavy- and κ light-chain enhancers (Sen and Baltimore 1986a). Among the factors he discovered was one that bound only to the κ light-chain enhancer—it covered the sequence GGGACTTTCC. We called it NF-κB because it was a nuclear factor that bound selectively to the κ enhancer and was found in extracts of B-cell tumors but not other cell lines (Sen and Baltimore 1986a).

The next step was supposed to be the killer experiment. 70Z/3 cells were known to have a rearranged κ light chain but not to express it and did not have detectable NF-κB. We knew also that treatment of the cells with lipopolysaccharide (LPS) induced transcription of the κ gene. The killer result would be that LPS induced NF-κB. Sure enough, it did (Sen and Baltimore 1986b). Furthermore, it did so without the need for new protein synthesis. Thus, we concluded that NF-κB is a factor that pre-exists in an apparently inhibited state and is released from that inhibition by LPS treatment. It looked like we had found a factor that might cause cells to go from making only heavy chain to making heavy and light chains, which could thus explain a step in differentiation. However, history has treated this optimistic conclusion with total disrespect, as is shown below.

I will not describe all that we have done on the NF-κB system but will only take this story one step further. That step was taken by Patrick Baeuerle, who joined my laboratory as a postdoctoral fellow. He found that the inactive form of NF-κB is in the cytoplasm of 70Z/3 cells and can be liberated from its inhibited form by treatment of cytoplasmic extracts with a detergent (Baeuerle and Baltimore 1988a). This discovery allowed us to purify the inhibitor, which we named IκB (Baeuerle and Baltimore 1988b). That set the stage for a detailed biochemical study of the activation process, an effort that has involved many investigators and is not complete to this day.

The seeds of doubt about the role of NF-κB as a regulator of B-cell development were sown in these early papers. We showed that the inhibited NF-κB is not specific to B-lineage cells: it was evident in T cells and even HeLa cells (Sen and Baltimore 1988b)—we know now that virtually all cells have it. Another paper showed this even more directly (Baeuerle and Baltimore 1988a). Thus, it was evident that NF-κB could be active in a wide range of cells and further work has borne this out.

A later postdoctorate, Yang Xu, provided the *coup de grace* for the notion that NF-κB is critical to κ-chain transcription. He knocked out the intronic κ enhancer—containing the NF-κB binding site—in mice and showed that, in those cells that rearrange κ, the gene is transcribed at a normal rate (Xu et al. 1996). There is a second enhancer, lacking an NF-κB binding site, that can control κ gene transcription. Each enhancer plays a quantitative role in κ-gene rearrangement, but not a qualitative one (Inlay et al. 2002).

Meanwhile, over the 24 years since its discovery, NF-κB has been implicated in a wide range of normal and disease processes. No transcription factor has attracted more experimental attention. Its role in inflammatory processes is especially important. Yet, its role in the transcription of the κ light chain, for which it was named, remains uncertain.

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The NF-κB Family of Transcription Factors and Its Regulation

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Nuclear factor-κB (NF-κB) consists of a family of transcription factors that play critical roles in inflammation, immunity, cell proliferation, differentiation, and survival. Inducible NF-κB activation depends on phosphorylation-induced proteosomal degradation of the inhibitor of NF-κB proteins (IκBs), which retain inactive NF-κB dimers in the cytosol in unstimulated cells. The majority of the diverse signaling pathways that lead to NF-κB activation converge on the IκB kinase (IKK) complex, which is responsible for IκB phosphorylation and is essential for signal transduction to NF-κB. Additional regulation of NF-κB activity is achieved through various post-translational modifications of the core components of the NF-κB signaling pathways. In addition to cytosolic modifications of IKK and IκB proteins, as well as other pathway-specific mediators, the transcription factors are themselves extensively modified. Tremendous progress has been made over the last two decades in unraveling the elaborate regulatory networks that control the NF-κB response. This has made the NF-κB pathway a paradigm for understanding general principles of signal transduction and gene regulation.

Following the identification of NF-κB (nuclear factor-κB) as a regulator of κB light chain expression in mature B and plasma cells by Sen and Baltimore, inducibility of its activity in response to exogenous stimuli was demonstrated in various cell types (Sen et al. 1986b; Sen et al. 1986a). Years of intense research that followed demonstrated that NF-κB is expressed in almost all cell types and tissues, and specific NF-κB binding sites are present in the promoters/enhancers of a large number of genes. It is now well-established that NF-κB plays a critical role in mediating responses to a remarkable diversity of external stimuli, and thus is a pivotal element in multiple physiological and pathological processes. The progress made in the past two decades in understanding how different stimuli culminate in NF-κB activation and how NF-κB activation is translated into a cell-type- and situation-specific response has made the NF-κB pathway a paradigm for understanding signaling mechanisms and gene regulation. Coupled with the large number of diseases in which dysregulation of NF-κB has been implicated, the continuing interest into the regulatory mechanisms that

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govern the activity of this important transcription factor can be easily explained.

Because of its ability to influence expression of numerous genes, the activity of NF-κB is tightly regulated at multiple levels. The primary mechanism for regulating NF-κB is through inhibitory IκB proteins (IκB, inhibitor of NF-κB), and the kinase that phosphorylates IκBs, namely, the IκB kinase (IKK) complex. A number of post-translational modifications also modulate the activity of the IκB and IKK proteins as well as NF-κB molecules themselves. In this article, we introduce the major players in the NF-κB signaling cascade and describe the key regulatory steps that control NF-κB activity. We highlight the basic principles that underlie NF-κB regulation, and many of these topics are discussed in depth in other articles on the subject.

NF-κB STIMULI AND κB-DEPENDENT TARGET GENES

NF-κB transcription factors are crucial players in an elaborate system that allows cells to adapt and respond to environmental changes, a process pivotal for survival. A large number of diverse external stimuli lead to activation of NF-κB and the genes whose expression is regulated by NF-κB play important and conserved roles in immune and stress responses, and impact processes such as apoptosis, proliferation, differentiation, and development.

Bacterial and viral infections (e.g., through recognition of microbial products by receptors such as the Toll-like receptors), inflammatory cytokines, and antigen receptor engagement, can all lead to activation of NF-κB, confirming its crucial role in innate and adaptive immune responses. In addition, NF-κB activation can be induced upon physical (UV- or γ-irradiation), physiological (ischemia and hyperosmotic shock), or oxidative stresses (Fig. 1) (Baeuerle and Henkel 1994; Hayden et al. 2006).

Consistent with the large number of signals that activate NF-κB, the list of target genes controlled by NF-κB is also extensive (Pahl 1999). Importantly, regulators of NF-κB such as IκBα, p105, or A20 are themselves NF-κB-dependent, thereby generating auto-regulatory feedback loops in the NF-κB response. Other

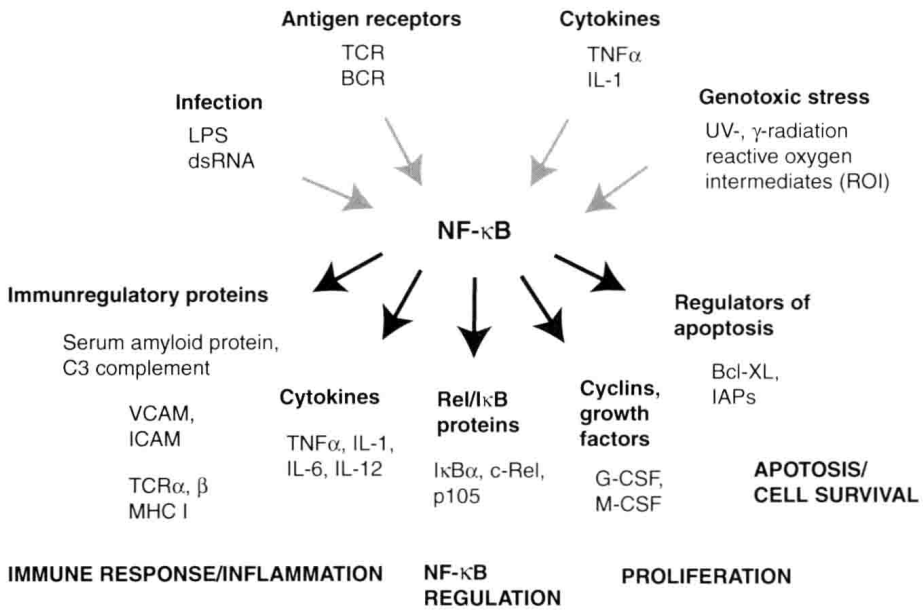

Figure 1. NF-κB stimuli and target genes. NF-κB acts as a central mediator of immune and inflammatory responses, and is involved in stress responses and regulation of cell proliferation and apoptosis. The respective NF-κB target genes allow the organism to respond effectively to these environmental changes. Representative examples are given for NF-κB inducers and κB-dependent target genes.

NF-κB target genes are central components of the immune response, e.g., immune receptor subunits or MHC molecules. Inflammatory processes are controlled through NF-κB-dependent transcription of cytokines, chemokines, cell adhesion molecules, factors of the complement cascade, and acute phase proteins. The list of κB-dependent target genes can be further extended to regulators of apoptosis (antiapoptotic Bcl family members and inhibitor of apoptosis proteins/IAPs) and proliferation (cyclins and growth factors), thereby substantiating its role in cell growth, proliferation, and survival (Chen and Manning 1995; Kopp and Ghosh 1995; Wissink et al. 1997). Furthermore, crucial functions of NF-κB in embryonic development and physiology of the bone, skin, and central nervous system add to the importance of this pleiotropic transcription factor (Hayden and Ghosh 2004).

Because so many key cellular processes such as cell survival, proliferation, and immunity are regulated through NF-κB-dependent transcription, it is not surprising that dysregulation of NF-κB pathways results in severe diseases such as arthritis, immunodeficiency, autoimmunity, and cancer (Courtois and Gilmore 2006).

THE NF-κB TRANSCRIPTION FACTOR FAMILY

The NF-κB transcription factor family in mammals consists of five proteins, p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1), and p100/52 (NF-κB2) that associate with each other to form distinct transcriptionally active homo- and heterodimeric complexes (Fig. 2). They all share a conserved 300 amino acid long amino-terminal Rel homology domain (RHD) (Baldwin 1996; Ghosh et al. 1998), and sequences within the RHD are required for dimerization, DNA binding, interaction with IκBs, as well as nuclear translocation. Crystal structures of p50 homo- and p50/p65 heterodimers bound to DNA revealed that the amino-terminal part of the RHD mediates specific DNA binding to the NF-κB consensus sequence present in regulatory elements of NF-κB target genes (5′ GGGPuNNPyPyCC-3′), whereas the carboxy-terminal part of the RHD is mainly responsible for dimerization and interaction with IκBs (Ghosh et al. 1995; Muller et al. 1995; Chen et al. 1998).

In most unstimulated cells, NF-κB dimers are retained in an inactive form in the cytosol through their interaction with IκB proteins. Degradation of these inhibitors upon their phosphorylation by the IκB kinase (IKK) complex leads to nuclear translocation of NF-κB and induction of transcription of target genes. Although NF-κB activity is inducible in most cells, NF-κB can also be detected as a constitutively active, nuclear protein in certain cell types, such as mature B cells, macrophages, neurons, and vascular smooth muscle cells, as well as a large number of tumor cells.

Through combinatorial associations, the Rel protein family members can form up to 15 different dimers. However, the physiological existence and relevance for all possible dimeric complexes has not yet been demonstrated. The p50/65 heterodimer clearly represents the most abundant of Rel dimers, being found in almost all cell types. In addition, dimeric complexes of p65/p65, p65/c-Rel, p65/p52, c-Rel/c-Rel, p52/c-Rel, p50/c-Rel, p50/p50, RelB/p50, and RelB/p52 have been described, some of them only in limited subsets of cells (Hayden and Ghosh 2004). RelB seems to be unique in this regard as it is only found in p50- or p52-containing complexes (Ryseck et al. 1992; Dobrzanski et al. 1994). p50/c-Rel dimers are the primary component of the constitutively active NF-κB observed in mature B cells (Grumont et al. 1994b; Miyamoto et al. 1994). The NF-κB family of proteins can be further divided into two groups based on their transactivation potential because only p65, RelB, and c-Rel contain carboxy-terminal transactivation domains (TAD) (Fig. 2). RelB is unique in that it requires an amino-terminal leucine zipper region in addition to its TAD to be fully active (Dobrzanski et al. 1993). p50 and p52 are generated by processing of the precursor molecules p105 and p100, respectively. The amino-termini of these precursors contain the RHDs of p50 or p52, followed by a glycine rich region (GRR) and multiple

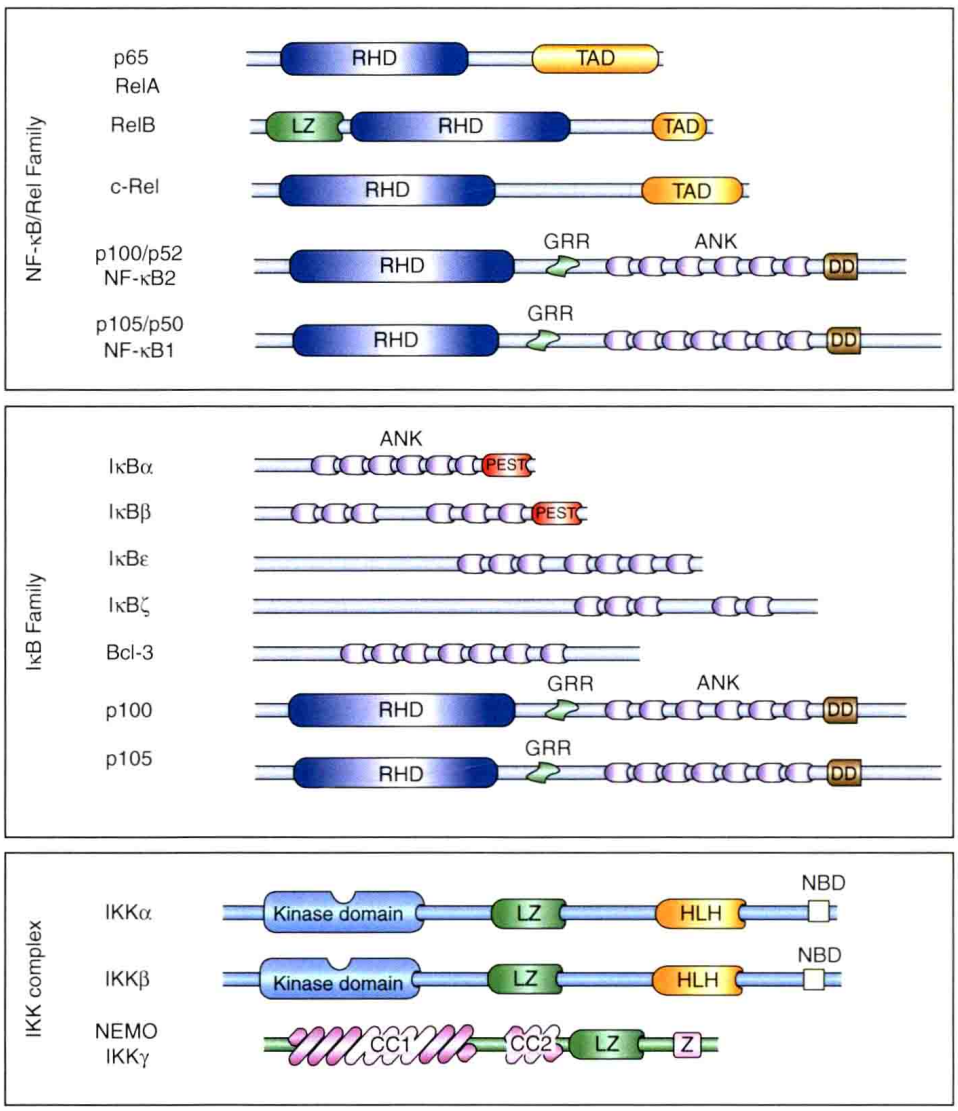

Figure 2. Members of the NF-κB, IκB, and IKK protein families. The Rel homology domain (RHD) is characteristic for the NF-κB proteins, whereas IκB proteins contain ankyrin repeats (ANK) typical for this protein family. The precursor proteins p100 and p105 can therefore be assigned to and fulfill the functions of both the NF-κB and IκB protein families. The domains that typify each protein are indicated schematically. CC, coiled-coil; DD, death domain; GRR, glycine-rich region; HLH, helix-loop-helix; IKK, IκB kinase; LZ, leucine-zipper; NBD, NEMO binding domain; PEST, proline-, glutamic acid-, serine-, and threonine-rich region; TAD, transactivation domain; ZF, zinc finger.

copies of ankyrin repeats that are characteristic for the IκB protein family. Therefore, not all combinations of Rel dimers are transcriptionally active: DNA-bound p50 and p52 homo- and heterodimers have been found to repress κB-dependent transcription, most probably by preventing trancriptionally active NF-κB dimers from binding to κB sites, or through recruitment of deacetylases to promoter regions (Zhong et al. 2002). An intriguing feature of p50 and p52 is their ability to associate with atypical IκB proteins such as IκBζ and Bcl-3, which most likely provide transcriptional activation properties. Because of their carboxy-terminal ankyrin repeats, the precursors p105 and p100 can also inhibit nuclear localization