Review

Advances in NF-κB Signaling Transduction and Transcription

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The molecular mechanisms for NF-κB signaling transduction and transcription have been the most attractive subjects for both basic research and pharmaceutical industries due to its important roles in both physiological and pathogenesis, particularly the close association of dysregulated NF-κB with tumorgenesis and inflammation. Several novel intracellular molecular events that regulate NF-κB activity have been described recently, including the discovery of an alternative signaling pathway that appears inducing a specific subset genes involved in adoptive immune response. Multi-level and multi-dimensional regulation of NF-κB activity by phosphorylation and acetylation modifications have unveiled and became the hottest targets for potentially tissue specific molecular interventions. Another emerging mechanism for NF-κB-responsive gene's regulation where NF-κB participates the transcriptional regulation independent of its cognate regulatory binding site within the target gene's promoter but facilitating the transaction activity of other involved transcription factors, that implicated an novel transcriptional activities for NF-κB. Thus, the current review will focus on these recent progresses that have been made on NF-κB signaling transduction and transcription. *Cellular & Molecular Immunology*. 2004;1(6):425-435.

Key Words: transcription factor, NF-κB, signaling pathway, immunology, transcriptional regulation

Introduction

The nuclear factor NF-κB stands out as an exceptionally important factor due to its pleiotropic effects, the inducible and expression patterns, its unique regulatory mechanisms, and large number of activating signaling pathways and number of genes that it controls (1-5). NF-κB factors are expressed in essentially for all mammalian cells and can be activated by a wide variety of stimuli. Although NF-κB plays an essential beneficial role in normal physiology for immune and inflammation responds (6-9), also, constitutive activation of NF-κB has been found in association with most of cancers (8, 10-14) and other diseases: cardio-vascular disease (15), diabetes (16, 17), chronic inflammation (8, 12) and CNS-related disease (18-20).

Several ground-break studies have highlighted the progress on the understandings of key steps and molecular events of NF-κB signaling pathway and the regulations of its activation activities on the NF-κB-responsive genes recently (10, 11, 21, 22). The discovery of a novel alternative signaling pathway for activation of NF-κB that distinct the classical pathway by the involved of major kinases and activation of different NF-κB complex have implicated having different regulatory functions than the classical pathway, one is involving in innate immunity and the other, in adaptive immunity (23-25). In addition to the cytoplasmic functions, IKKα, the key component in NF-κB signaling pathways, has found to also have nucleus function that introducing the site-specific phosphorylation on histone H3 that is critical for cytokine-induced gene expression (21, 22). Moreover, both NF-κB- and histone-directed acetylation has been described in the regulation of NF-κB activities, especially the mechanisms of reversible acetylation for NF-κB (26-30), implicating that acetylation-dependent regulation occurs in multiple level for NF-κB regulated gene activation that shows the molecular mechanisms of controlling the strength and duration of NF-κB regulated gene expression in tissue and gene-

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Abbreviations: BAFF, B-cell-activating factor belonging to the TNF family; BLC, B-lymphocyte chemoattractant; COX-2, cyclooxygenase 2; ELC, Epstein-Barr virus-induced molecule 1 ligand CC chemokine; ICAM-1, intercellular adhesion molecule 1; IKK, IκB kinase; iNOS, inducible nitric oxide synthase; LT, lymphotoxin; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; NIK, NF-κB-inducing kinase; PLA2, phospholipase 2; SDF-1, stromal cell-derived factor-1α; SLC, secondary lymphoid tissue chemokine; TLRs, Toll-like receptors; VCAM-1, vascular cell adhesion molecule-1. PKA_c, protein kinase A; LPS, lipopolysaccharides; PKC, protein kinase C; MSK1, mitogen- and stress-activated kinase-1; CKII, stimulate casein kinase II; TF, transcription factor; TFBS, transcription factor binding site; CRP, C-react protein.

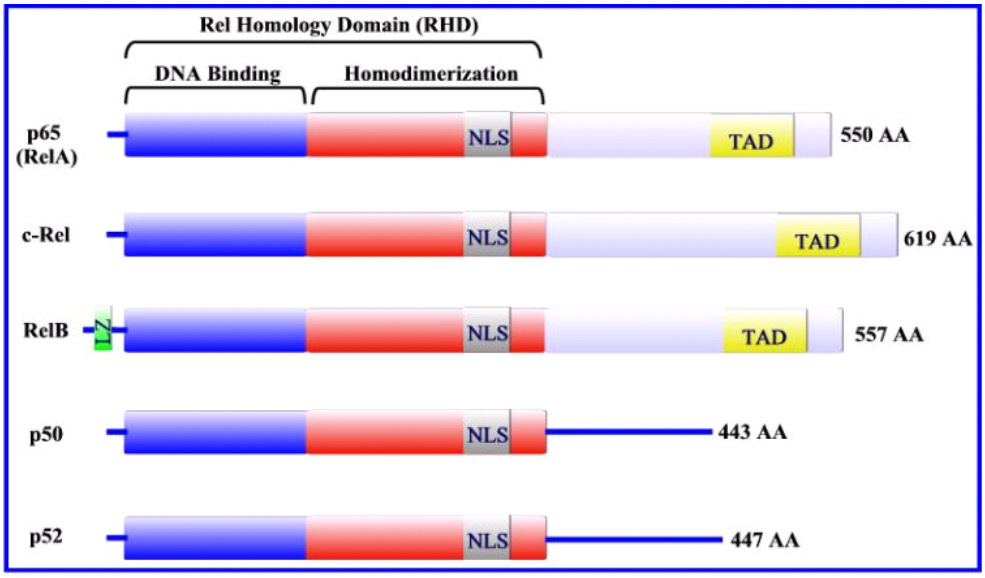

Figure 1. Members of the NF-κB (or Rel) family. NF-κB family consists of five members, including p65 (RelA), c-Rel, RelB, p50 and p52. It is characterized as containing a well-conserved N-terminal ~300 amino acid Rel-homology domain (RHD), which includes DNA-binding and dimerization domains, and a nuclear localization signal (NLS). NF-κB proteins present as either homodimers or heterodimers with its family. Three members, p65, c-Rel and RelB, contain the transactivation domain (TAD) within the C-terminal region that is required for the transcriptional activation activities, but the TAD is lacked in other two members, p50 and p52 and thereby p50:p52 homodimer function as repressors of NF-κB transcriptions. p50 and p52 are encoded as precursor as p105 and p100, and corresponding to the N-terminal half of these precursors, respectively.

specific manners. Further, several studies have also described a novel mechanisms of NF-κB in activating its target genes where NF-κB is independent of its regulatory motifs within the promoter region (13, 31-33), but instead, facilitating the activation activities of other associated transcription factors that possible through the coactivators to regulate the NF-κB-responsive gene's expression (13, 33). All these latest progresses have led to a deep insight into the intracellular signaling molecules or events involved in NF-κB activities and provided a clearer picture for molecular basis and the biological roles of NF-κB in both normal and abnormal conditions. Thus, the scope of present review will focus on those current progresses on NF-κB signaling.

The NF-κB transcription factor family

Transcription factors are proteins responsible for the coordinated expression of genes through specific binding to gene promoter and enhancer sites. The NF-κB/Rel, a family of transcription factors are known to regulate the expression of a wide spectrum of genes that participate mainly in immune and inflammation responses (8, 10, 12), and is comprised of five members: NF-κB p50 (p105/NF-κB1), NF-κB p52 (p100/NF-κB2), p65 (RelA), RelB and c-Rel (Figure 1). NF-κB p50 and NF-κB p52 are synthesized as large precursors, p105 and p100 (Figure 2), which is post-translationally processed to the DNA-binding subunits p50 and p52, respectively. The subunits p50 and p52 carry a Rel-homology domain (RHD), which is a common feature of all NF-κB proteins; the RHD contains a nuclear localization sequence (NLS) and is involved in dimerization, sequence-specific DNA binding and interaction with the inhibitory IκB proteins (34), which either mask the nuclear import signal of NF-κB/Rel proteins or render them inactive for transactivation in resting cells (Figure 1). Active DNA-binding complexes of the NF-κB/Rel family bind to a common κB site (5'-GGGPuNNPyPyCC-3'), where Pu is purine, Py is pyrimidine and N is any nucleotide (35, 36). p65 (RelA), RelB and cRel contain one or more transactivation domains in their C-terminal end, while p50 and p52 lack this domain. The most abundant member of the NF-κB family is p65:p50 heterodimer that is often used synonymously for NF-κB, and is retained in the cytoplasm in inactive form primarily through interaction with IκBs (mostly IκBα), a family of inhibitor proteins (37), while some homodimers, such as p50:p50 and p52:p52, can repress transcription of their target genes, since they lack the transactivation domain (38, 39) and, RelB does not homodimerize but it forms stable heterodimers with either p50 or p52 (40, 41).

Activation of NF-κB is a complex process. Depending on the status of NF-κB complexes, two phases of molecular events occurs during the process; including release of selective NF-κB complexes from inhibitors upon the transduction signals and results the NF-κB complexes translocating into nucleus and post-translational modifications with phosphorylation and acetylations on both NF-κB and histone proteins that determines the strength and duration of NF-κB activation activities. After activated as a transcription factor, NF-κB is recruited to the proximal promoter region and participates in the transactivation activities of NF-κB- responsive genes.

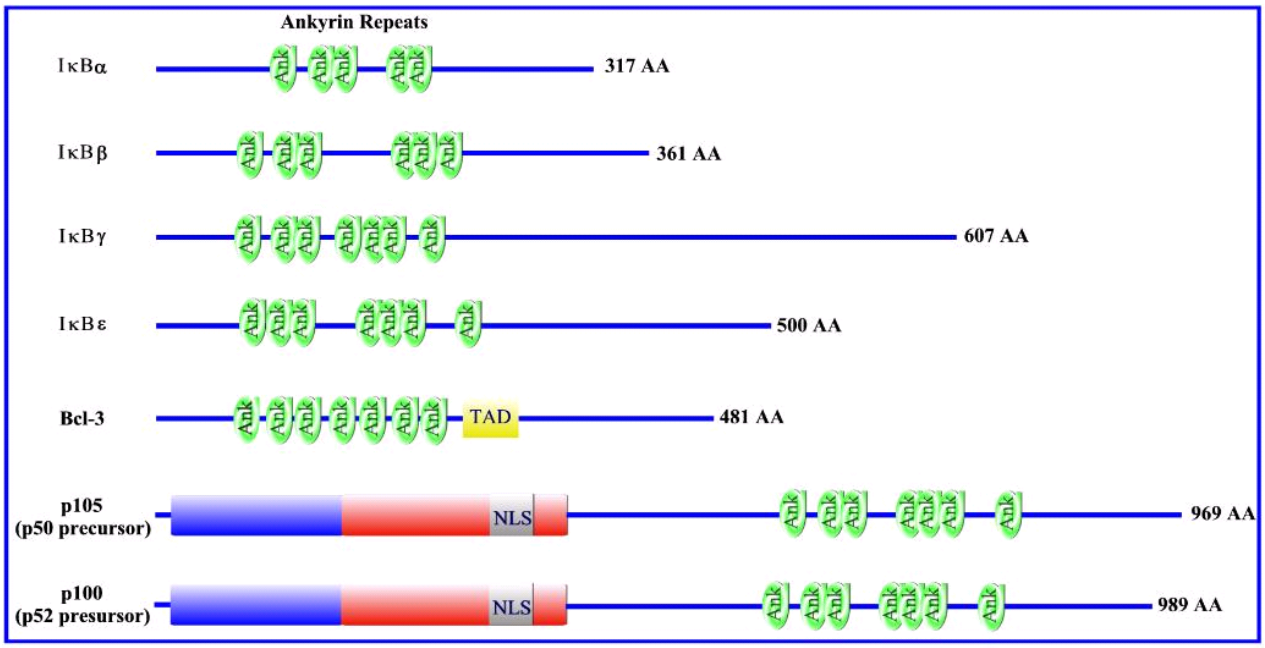

Figure 2. Members of the IκB family. The inhibitory κB (IκB) family are grouped as the structurally homology for sharing the conserved ankyrin repeat motifs (labeled as Ank) that are essential for IκB activity and binding to the RHD of NF-κB proteins, and consist of seven members: IκBα, IκBβ, IκBγ, IκBε, Bcl-3, p105 and p100. The number of ankryin repeats varies from five (IκBα), six (IκBβ) and seven (Bcl-3, IκBε, IκBγ, p100 and p105). Except Bcl-3, containing the transactivation domain (TAD) and functional in nucleus, all the other members of IκB family lack the TAD and functional in cytoplasmic. p105 and p100 are the precursors for p50 and p52, respectively.

Signaling pathways of NF-κB activation

In most normal cells, NF-κB is in an inactive state that retained in the cytoplasm by the form of complex with IκBs (Figure 2). The IκBs bind to the RHD region of NF-κB protein and interfere the NLS function to prevent NF-κB translocation into nucleus (42-45). IκB family consist of seven known mammalian members, IκBα, IκBβ, IκBε, IκBγ, Bcl-3, and the precursor Rel proteins p100 and p105 (34, 42, 45, 46) (Figure 2). Despite of Bcl-3 is the only member of IκB that forms complex with p50:p52 heterodimer and appears to facilitate the transcriptional activities to p52 target genes in nucleus instead of cytoplasmic as other members (47-49), IκBs are characterized by the presence of multiple ankyrin repeats, which are protein-protein interaction domains that interact with NF-κB *via* the RHD (45, 50-52). The structural studies revealed that the RHD forms a unique butterfly-shaped structure that is composed of β strands arranged in a pattern similar to immunoglobulin domains. The crystallographic structures of IκBα and IκBβ bound to p65/p50 or p65/c-Rel dimers revealed that the IκB proteins mask only the nuclear localization sequence (NLS) of p65, whereas the NLS of p50 remains accessible (45, 50-53). The presence of this accessible NLS on p50 coupled with nuclear export sequences (NES) that are present on IκBα and p65 results in constant shuttling of IκBα/NF-κB complexes between the nucleus and the cytoplasm, although the steady-state localization is in the cytoplasmic (43, 54-57). The dynamic balance between cytoplasmic and nuclear localization is altered upon IκBα degradation, because it removes the contribution of the IκB NES and exposes the masked NLS of p65, resulting in predominantly nuclear localization of NF-κB. IκB proteins bind with different affinities and specificities to NF-κB dimers. Thus, not only are there different NF-κB dimers in a specific cell type, but the large number of combinations between IκB and NF-κB dimers illustrates the sophistication of the system.

There are two signaling pathways, classical (or canonical) and alternative (or non-canonical) NF-κB signaling pathways (Figure 3), which lead to activation of NF-κB, have been described. The distinctions of these two pathways are the members of IKK that involved in mediating the degradation of selective IκB, and subsequently lead to the release of different dimmers of NF-κB and the activation of the selective subset of genes and their responsible biological functions (1, 58-62).

The classical NF-κB pathway (canonical pathway) (Figure 3A), upon stimulation by a wide range of stimuli including the proinflammatory cytokines and pathogen-associated molecules, the activated IKK complex (63), predominantly acting through IKKβ in an IKKγ-dependent manner. The released NF-κB dimers that are most commonly the p50:p65 dimmers in this pathway, translocate to the nucleus and activate the target genes (58, 64). The activation of p50:p60 heterodimmer has been found in association with increased transcription of genes encoding chemokines (65, 66), cytokines (66, 67), adhesion molecules (68, 69); enzymes that produce secondary inflammatory

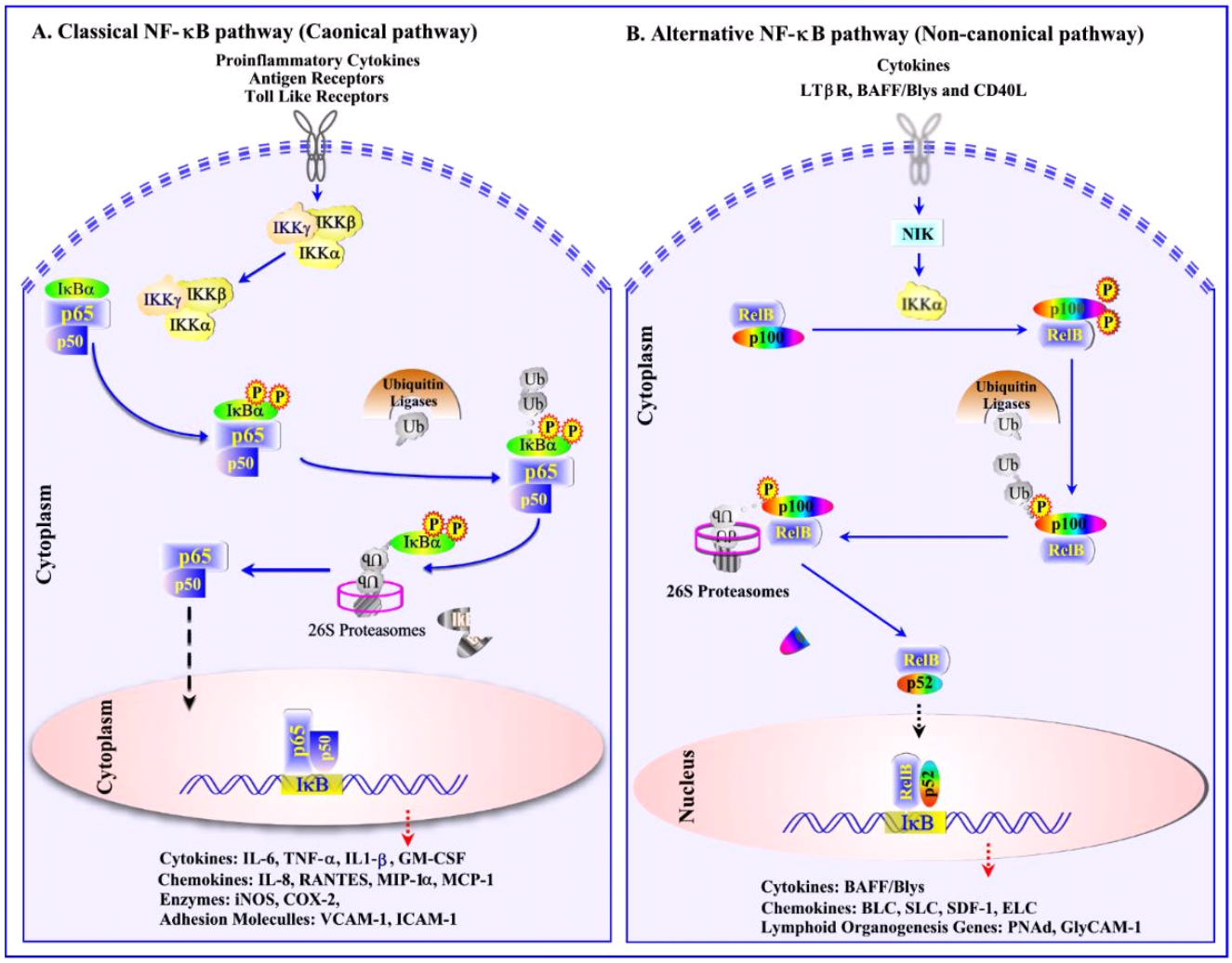

Figure 3. NF-κB signaling pathways. The classical NF-κB pathway (A) is activated by a variety of inflammatory signals including proinflammatory cytokines and antigens from microorganisms. The signals lead to the activation of IKK complex and degradation of IκBα in an IKKβ-dependent manner. The NF-κB complex that releasing by this classical pathway is mainly the p65:p50 heterodimerics and results in coordinate expression of sets of genes that involving in inflammatory and innate immune genes. By contrast, the alternative NF-κB pathway (B) is activated by the engagement of subset of TNF receptor family, including LTβR, BAFF and CD40L, and leads to the precursor processing of p100 and releases of p52:RelB heterodimerics through an NIK-mediated IKKα-dependent manner. The genes that selectively activated by p52:RelB dimmer are involved in adoptive immune.

mediators and inhibitors of apoptosis (11, 66) (Figure 3A). This classical NF-κB pathway has been implicated being an important pathway in coordinating expression of multiple inflammatory and innate immune genes through maintaining the survival of professional immune cells during bacterial infections or acute inflammatory stimuli (64, 70-74).

The alternative pathway (or non-canonical) (Figure 3B), by contrast with the classical NF-κB pathway, is mainly stimulated by ligation of LTβR, BAFFR and CD40R (60), mediated by NIK and strictly dependent on IKKα homo-dimmer, but independent of IKKβ and IKKγ (1, 23, 75-77). The target for IKKα homodimers is NF-κB p52/p100 protein. C-terminal phosphorylation is essential for p100 processing to p52, which is also dependent on polyubiquitination and proteasomal degradation. However, the phosphorylation-dependent ubiquitination of p100 results degradation of only C-terminal, but leaves the N-terminal portion intact, and that produces the p52 polypeptide (77-82). As the RHD of p100 is most commonly associated with RelB, activation of this 'alternative' pathway results in nuclear translocation of p52-RelB dimers (79, 81, 83) and has been implicated playing a central role in the expression of genes involved in development and maintainance of secondary lymphoid organs (83, 84).

Post-translational modifications in NF-κB activation

Direct post-translational modification of the NF-κB complex or the histones that is recruited to NF-κB target genes occurred following the release from inhibitors. These molecular events, including phosporylation and acetylation,

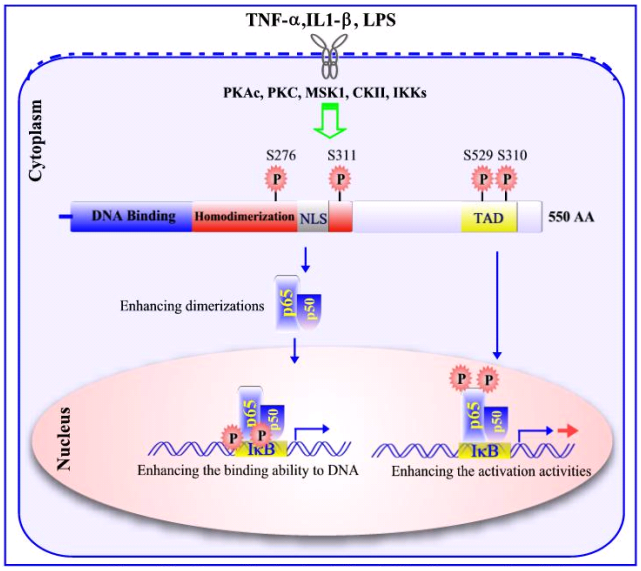

Figure 4. Protein-specific phosphorylation of NF-κB p65 and corresponding effects. At least four serines (S) within NF-κB p65 can be phosphorylated by various kinases that induced with proinflammation cytokins and LPS. The phosphorylation modifications of the two serines in homodimerization domain of p65, including S276 and S311, lead to enhancing of dimerization between NF-κB subunits, while phosphorylations of the serines in TAD, S529 and S310, enhance the transcriptional activation of p65 for its target genes.

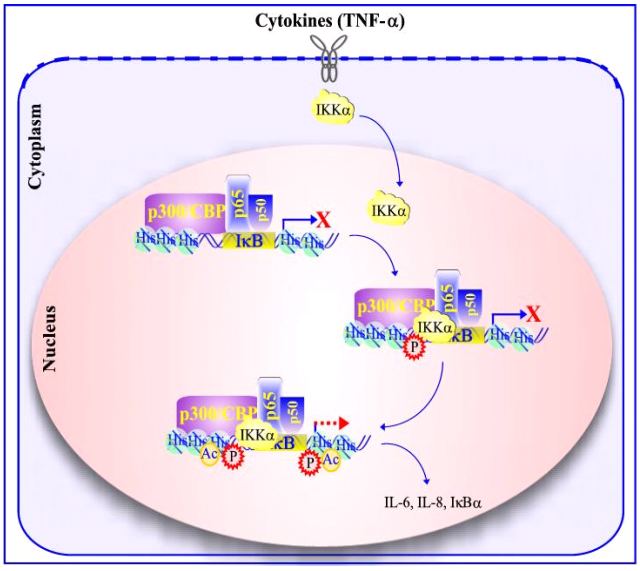

Figure 5. Cytokine induced nucleus functions of IKKα. The IκB-kinase α (IKKα) translocates into nucleus in response to cytokines stimulations, such as TNF-α. It is then recruited to the promoter regions of NF-κB-responsive genes in association with NF-κB p65 and the coactivator p300/CBP, and mediates the protein-specific phosphorylation and acetylation of histone H3 in S10 and K14, respectively. Such post-translational modifications of histone H3 by IKKα are critical for NF-κB-responsive gene expression.

determine the strength and duration of the NF-κB transcriptional response (85-87).

Phosphorylation modification for the regulation of NF-κB-responsive genes

The increased transactivation activity of NF-κB by phosphorylation has been described and at least four different serine phosphoacceptor sites have been identified in NF-κB p65 (Figure 4), including serines S276 and S311 in the REL-homology domain and S529 and S536 in the transactivation domain (TAD) (88-91) by various kinase, including phosphatidylinositol 3-kinase (PI3K)/AKT(90, 92), glycogen-synthase kinase-3β (GSK3β) (93), protein kinase family members (PKA_c, PKC^{ξ}) (89, 94), and mitogen- and stress-activated kinase-1 (MSK1) (95). Phosphorylation of a transcription factor provides a powerful mechanism for the rapid and reversible integration of intracellular signals (89, 90, 96, 97). It is not surprising that the phosphorylation of p65 also facilitates the recruitment of various transcriptional cofactors. For example, p65 phosphorylation at serine 276 by PKA_c or MSK1, or at serine 311 by PKC^{ξ}, enhances the binding of CBP and p300 to RELA (95, 98-102). This modified NF-κB complex is more effective at displacing transcriptional repressive histone deacetylase (HDAC) complexes, specifically p50-HDAC1 complexes, that are frequently bound to the κB enhancers of target genes under the stead conditions (64, 86, 94, 102). Whether phosphorylation at serine 536 similarly enhances the assembly of p65 with p300 are unclear (103, 104). Nevertheless, it seems likely that the phosphorylation of the TAD of p65 at serines 529 and 536 facilitates the assembly of p65 with other components of the basic transcriptional machinery, thereby enhancing the transcriptional responses (98, 102-104).

In addition to the phosphorylation on NF-κB protein itself that lead to alteration of activation activity of transcriptional regulations, phosphorylation of histone proteins that surrounds the NF-κB target genes promoter is also demonstrated to enhance the activation activity of NF-κB responsive genes (98, 105, 106). Indeed, the molecular mechanisms that introducing such phosphorylation event has been emerged from the discovery of nucleus functions of IKKα (21, 22).

The recently discovered novel function of IKKα has demonstrated its nucleus functions (Figure 5) in addition to its previously described role on the processing of p100 to p52 in cytoplasmic (21, 22). Upon cytokines stimulation, e.g., TNFα, IKKα interacts with CREB-binding protein (CBP) and in conjunction with p65 is recruited to NF-κB-responsive promoters and mediates the cytokine-induced phosphorylation at histone H3 S10 and subsequent acetylation of specific residues in K14. The IKKα, but not IKKβ or IKKγ, mediated phosphorylation of histone H3 S10 has been demonstrated to be essential for activation of NF-κB responsive genes (Figure 5). The results defined the novel molecular mechanisms and importance of phosphorylation modification on histones for NF-κB-responsive genes, although the mechanisms of linking the phosphorylation enhanced subsequent acetylation are still unknown. Furthermore, it is likely that cytokine-induced histone H3

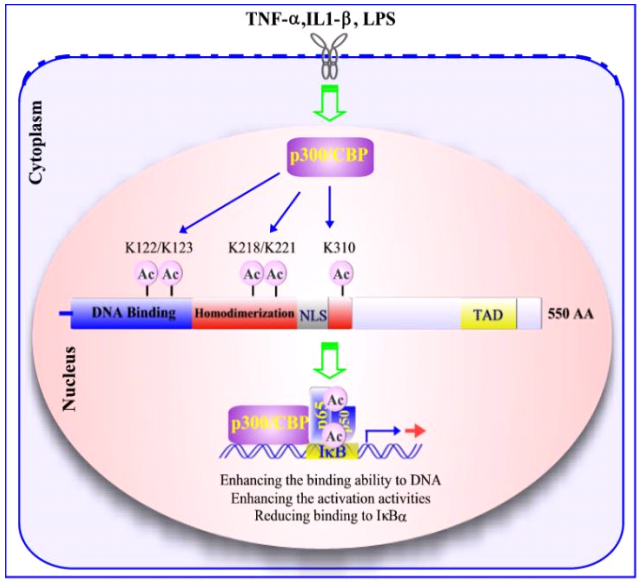

Figure 6. Protein-specific acetylation of NF-κB p65 and corresponding effects. The coactivator p300 or CREB-binding protein (p300/CBP) is the major mediator of acetylation (Ac) modifications for NF-κB p65 once p65 translocates into nucleus. Acetylation of lysines K122 and K123 reduces the binding ability of p65 to the NF-κB binding site and impairs the overall transcriptional activity, while acetylation of K218 and K221 increases the DNA-binding affinity of p65 and prevents the association of p65 with IκBα. By contrast, acetylation of K310 does not control DNA binding nor IκBα assembly, but instead is required for the full transactivation activity of p65.

phosphorylation might be regulated by multiple kinases in a cell-type specific and stimulus-dependent manner. For example, inhibitors of p38 activation can prevent increases in histone H3 phosphorylation of specific cytokine promoters in dendritic cells following treatment with inflammatory cytokines such as lipopolysaccharide (LPS) (107, 108) and IKKα kinase-deficient mice do not appear to have global defects in the activation of NF-κB-regulated genes. These evidences suggest that potentially redundant mechanisms to phosphorylate histone H3 may exist and different kinases could be preferentially recruited to the promoters of different NF-κB target genes in a stimulus-specific manner (91). However, there are still questions remaining such as: how IKKα is recruited to NF-κB-regulated promoters and whether IKKα recruitment is required for only NF-κB-regulated genes or is a general regulator of histone function.

Acetylation modification for regulation of NF-κB responsive genes

Like phosphorylation, acetylation of NF-κB itself and the surrounding histones have been described (Figure 6), which are important for regulating the nuclear function of NF-κB (87, 109). NF-κB p65 is acetylated in a signal-coupled manner in responding to TNFα or phorbol myristate acetate (PMA) stimulations (85, 110). It is now well established that NF-κB-dependent transcription requires multiple coactivators possessing HAT activity (22, 27, 29, 111-113), including cAMP- response-element-binding protein (CREB)-binding protein (CBP) or p300 and steroid receptor coactivator-1 (SRC-1). Five main acetylation sites have been identified within p65 —lysines 122, 123, 218, 221 and 310 (109) (Figure 6). The modification of these sites modulates distinct biological responses. For example, acetylation at lysine 221 enhances DNA binding by p65 and impairs its assembly with IκBα. Conversely, acetylation of lysine 310 is required for full transcriptional activity of p65, but modification of this site does not affect DNA binding or IκBα assembly. It is possible that acetylated lysine 310 forms a binding site for a transcriptional coactivator, possibly one that contains a bromodomain (28). Bromodomains specifically recognize acetylated lysine residues and are present in many chromatin-associated proteins as well as nearly all known nuclear histone acetyltransferases (114). The acetylation of lysines 122 and 123 has also been described, although modification of these sites seems to exert contrasting negative effects on NF-κB-mediated transcription by reducing p65 binding to the κB enhancer and consequently reducing p65-mediated transcriptional activation (115). In addition, NF-κB p50 is also posse stimulus-induced acetylation of the lysine residues 431, 440 and 441. These modifications seem to enhance the DNA-binding activity of p50 and increase transcriptional activation by the heterodimeric NF-κB complex (116-118). However, it is unclear whether or not the other NF-κB/REL-family members are similarly regulated by acetylation.

The reversible acetylation has been identified as a critical post-translational modification of non-histone proteins, including general and specific transcription factors (29, 87, 119, 120). Depending on the functional domain that is modified, acetylation can regulate different functions of these non-histone proteins such as DNA recognition, protein stability, protein-protein interaction and subcellular localisation. NF-κB is able to interact directly with several deacetylases (29, 102, 115, 121-124) and posses the reversible acetylation property that plays a very important biological role in regulating the assembly of p65-IκBα complexes. The interaction of p65 with IκBα regulates the NF-κB transcriptional response by controlling its duration. *De novo* synthesis of IκBα is induced by NF-κB and replenishes the intracellular stores of this inhibitor that are depleted during the course of stimulus-coupled NF-κB activation. IκBα displays nucleocyto-plasmic-shuttling properties and can retrieve NF-κB complexes from the nucleus (43, 56, 57). Interestingly, the effectiveness of this retrieval function is regulated through acetylation that p65 is acetylated on lysines 218 and 221 only weakly interacts with IκBα, however, deacetylation of p65 at these sites by HDAC3 greatly enhances the binding to IκBα (30). This interaction will in turn, lead to a rapid chromosomal-region maintenance-1 (CRM1)-dependent nuclear export of the NF-κB complex, effectively terminating the transcriptional response. This export response help replenish the cytoplasmic pool of latent NF-κB–IκBα complexes, thereby readying the cell for the next NF-κB-inducing stimulus. Therefore, the reversible acetylation of p65 serves as an intranuclear molecular switch that is important for shaping the strength and duration of the

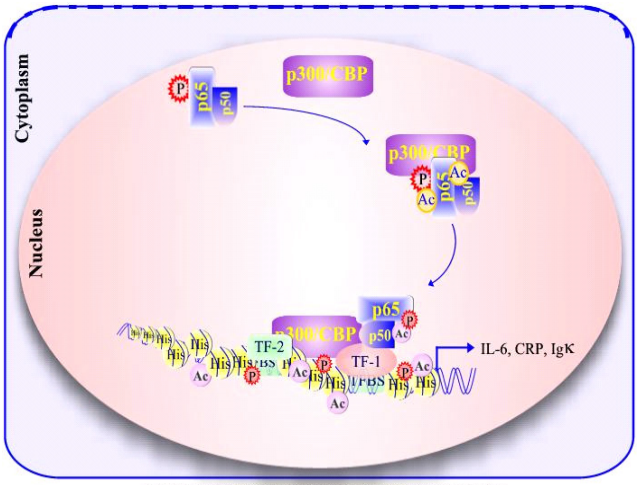

Figure 7. Hypothesis model for alternative transcriptional activity of NF-κB. Once NF-κB p65 containing dimmer enters into nucleus, it forms the steady complex with coactivators, such as p300/CBP and is modulated through acetylations to gain the transactivation abilities. Then the complex is recruited to the promoters and interacts with selective transcription factors by direct or indirect interactions to participate the activation regulation of the target genes.

NF-κB response (29).

Thus, the acetylation of different lysines in p65 and p50 regulates different functions of NF-κB, including transcriptional activation, DNA-binding affinity, and IκBα assembly. Acetylated forms of p65 are subjected to deacetylation by histone deacetylase 3 (HDAC3). Acetylation of p65 enhances the duration of TNFα-induced NF-κB translocation in the nucleus, thereby participating in the strong transcriptional synergism (125, 126). Furthermore, two distinct classes of NF-κB-responsive genes exist: those constitutively and immediately accessible to NF-κB and those that have to be conformationally modified to become accessible to NF-κB. The second classes of NF-κB-responsive genes are hyperacetylated after stimulation, before NF-κB recruitment (27, 28, 127). Acetylation could thereby increase the accessibility to these latter genes and thus favors their NF-κB-dependent transcription. Taking together the different effects of acetylation of NF-κB proteins, they do not converge to a simple link between protein acetylation and NF-κB-dependent regulation, but rather demonstrate that acetylation regulates NF-κB action at multiple levels.

Alternative transcriptional regulation mechanisms for NF-κB

Studies of transcriptional regulation of genes that have been long considered as NF-κB-responsive genes, including interleukin-6 (IL-6), C-reactive protein (CRP), and immunoglobulin κ chain (Igκ), have shown that although the participation of NF-κB transcription factor is required, the binding of NF-κB to its regulatory sites is not required under the certain conditions (13, 31-33, 128), instead, appear through the co-operation with other DNA-binding proteins that involved in the regulation of target genes. For example, the regulation of IL-6 gene expression by NF-κB is achieved by co-operation with c-Jun that binding to the AP-1 regulatory site within the IL-6 promoter in multiple myeloma cells (13), while similarly, with C/EBP-β in prostate cancers (33). For the activation of CRP, NF-κB is able to directly interact with C/EBP-β protein to enhance the expression of CRP to 600 folds than the untreated control in responding to IL-6, although NF-κB binding motif is absent in the promoter of CRP (128). Furthermore, multiple NF-κB binding sites have been identified and the requirement of NF-κB has been intensively studied previously, the recent studies using transgene mice that containing the mutations of the NF-κB sites on Igκ promoter have demonstrated that the NF-κB binding sites are definitely not required for Igκ production in the mice, as well as in the isolated splenic B, even after it was induced with IL-4 (31, 32). All these findings have indicated a novel alternative transcriptional regulatory mechanism for NF-κB (Figure 7), in which NF-κB is capable in participating in the transcriptional regulation of the target genes independent of its cognized DNA binding sites within the regulated promoters, the molecular basis of such actions of NF-κB appears through co-operation with the selected transcription factors that are presented, or it is more likely mediated through coactivators, such as p300/CBP (Figure 7).

More than 120 proteins that were able to interact with NF-κB either directly or indirectly, also, at least 150 genes that can be regulated by NF-κB in various cells and conditions have been described so far (129, 130). It has been demonstrated that NF-κB can not activate the expression of genes by itself alone; it requires coordination with other transcription factors to fully or spontaneously activate the target gene's transcription. Clearly, not all of the NF-κB-regulated genes are activated in different cell types, even when responding to the same stimuli, indicating another layer of transcriptional regulation of NF-κB-responsive genes that their activation are also depending on the availability of the NF-κB-interacted proteins in the cells in addition to the complex controlling mechanisms for the signaling transduction pathways of NF-κB, and this regulation is in both gene specific and activation strength controlling (131-134).

Additionally, the gene expression studies that pursuing NF-κB regulated gene profile using microarray technology have found numerous genes that were respond to the status of NF-κB, but they are lacking the presence of NF-κB regulatory binding sites in their gene promoter (129). These observations can be well explained by the alternatively transcriptional regulatory mechanisms of NF-κB, and indeed, strongly support the hypothesis of alternatively transcriptional regulation mechanisms for NF-κB that described above.

Conclusions and perspectives

Nuclear factor NF-κB discovered in 1986 (135), induces more than 150 genes and many of these genes play critical

role in both the regulation of immune and inflam- matory responses as well as in the protection of cells from apoptosis (130). NF-κB factors can be activated by a wide variety of stimuli such as proinflammatory cytokines, bacterial lipopolysaccharide, negative strand RNA viruses, double stranded RNA (dsRNA), immunostimulatory DNA sequences (ISS-DNA) and various stress factors such as ionizing radiation, toxins, UV-B radiation, and oxidative stress. The NF-κB proteins therefore initiate a highly coordinated and rapid response in multiple cell types to effectively counteract the threat to the health of the organism (136). Moreover, the link between chronic inflam- mation and origination of cancers has been long proposed since 1860's by Rudolf Virchow. Although more evidences from both population- and cellular-based studies have strongly tied these two events together, but the solid evidences that validate the transformation of normal cells into malignant under the condition of chronic inflammation has been demonstrated until recently, and the cellular key player that mediated this transformation is pin-pointed to the nuclear factor NF-κB from several independent studies (4, 11, 12). These latest findings have further elucidated the important roles of NF-κB in tumorgenesis. Thus, understanding the molecular basis of the signaling transduction pathways and transcriptional mechanisms of NF-κB-responsive genes is definitely important for elucidating the roles of NF-κB in both physiological and pathogenesis conditions.

The advanced studies: discovery of classical and alternative signaling pathway, proposal of alternative transcriptional activity, and particularly the involvement of acetylation and phosphorylation in the regulation of NF-κB proteins, has further demonstrated that the controlling of NF-κB is a précised network in multi-level and multi-dimensional. Different stimulus induces the selective signaling cascades and the NF-κB complexes, and subsequently targeting selected subset genes. Thus, the signaling transduction appears to determine the specificity of the genes that was being targeted, such as the classical and alternative pathways induce the different dimmer of NF-κB complex for p65:p50 and p50:p52, respectively. By contrast, once the NF-κB complex translocated into the nucleus, post modifications including acetylation and phosphorylation and additionally, the availability of co-operational transcription factors of NF-κB determine the activation strength and durations of NF-κB. Not surprisingly, this multi-level, multi-dimensional network can result in a wide range and diverse actions by NF-κB, that what we have observed and wondered from long time and intensive studies.

Most studies on signaling and mechanisms of transcriptional activations of NF-κB were under the condition of a single stimulus, most frequently is proinflammation cytokines like TNFα, or stresses like radiations. However, how the signaling network of NF-κB response to multi-signaling transductions that is definitely the true world have almost nothing known, yet.

Abnormal activation of NF-κB results in a variety of diseases and brings great attention as therapeutic target. However, because the complexes of the controlling network and the important roles of NF-κB in both physiological and pathogenesis, the strategies to totally block the activation pathway of NF-κB have to be re-considered, indeed, pile of molecules that investigated by pharmaceutical industrials have shown serious side effects. Thus, further exploring the underlying molecular mechanisms of NF-κB regulations is needed.

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