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FORUM REVIEW ARTICLE

Redox Regulation of Nuclear Post-Translational Modifications During NF-κB Activation

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Abstract

The transcription factor NF-κB controls the expression of hundreds of genes involved in the regulation of the immune/inflammatory response, development, and apoptosis. In resting cells, NF-κB proteins are sequestered in the cytoplasm through their tight association with IκB proteins. NF-κB activation relies on the signal-induced IκB phosphorylation and degradation, thereby allowing the nuclear translocation of NF-κB proteins. In the nucleus, several post-translational modifications of NF-κB and chromatin remodeling of target genes are mandatory for NF-κB DNA binding and full transcription. Since 1991, reactive oxygen species (ROS) have been implicated in NF-κB activation. ROS enhance the cytoplasmic signaling pathways leading to NF-κB nuclear translocation, but reduction/oxidation (redox) also controls several key steps in the nuclear phase of the NF-κB program, including chromatin remodeling, recruitment of co-activators, and DNA binding. Here we describe the redox regulation of NF-κB activity in the nucleus. *Antioxid. Redox Signal.* 11, 2209–2222.

The NF-κB/IκB Family

NUCLEAR FACTOR (NF)-κB transcription factors consist of homo- or heterodimers of a group of five proteins, namely p65 (RelA), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) (Fig. 1) (32). NF-κB proteins share a unique and evolutionary conserved domain of ~300 amino acids called Rel-homology domain (RHD). The RHD is responsible for dimerization and binding to discrete DNA sequences (know as κB elements) in promoters and enhancers of target genes. Despite high sequence variability between the NF-κB-target promoters was found, a κB element consensus sequence was defined as 5′GGGRNWYYCC3′ (N: any base; R: purine; W: adenine or thymine, and Y: pyrimidine) (38). Once bound to DNA, NF-κB proteins regulate transcription through the recruitment of co-activators or co-repressors. p65, RelB and c-Rel contain a transcription activation domain (TAD) in their C-terminal part responsible for positive regulation of gene expression. p50 and p52, which lack TAD, act as transcriptional repressor if bound to κB sites as homodimers, but activate transcription if associated with a TAD-containing NF-κB protein (Fig. 1) (32). In resting cells, NF-κB proteins are sequestered in the cytoplasm by three typical IκBs (IκBα, IκBβ, and IκBε) and the precursor's p100 and p105. Bcl-3 and IκBξ, two atypical IκB proteins, are inducibly-expressed nuclear proteins involved in the regulation of NF-κB-mediated transcription. IκB proteins contain akyrin-repeat motifs responsible for the interaction with the RHD of NF-κB proteins, thereby masking their nuclear localization sequence (NLS) (Fig. 1). Upon activation, typical IκB proteins are rapidly degraded, thereby rendering freed NF-κB proteins able to translocate into the nucleus and bind κB elements of target promoters. The prototypical NF-κB/IκB complex is composed of a p50/p65 heterodimer bound to IκBα. Crystal structure studies revealed that IκBα is able the mask only the NLS of p65, thereby allowing the complex to translocate into the nucleus thanks to the NLS of p50. However, steady-sate localization appears almost exclusively cytoplasmic thanks to the export of the p50/p65/IκBα complex back to the cytoplasm through the NES of IκBα. Upon activation of the cell, degradation of IκBα alters this shuttling and favors a nuclear localization of NF-κB (32).

NF-κB Signaling Pathways

Classical pathway

This pathway relies on the phosphorylation of IκBα on Ser32 and Ser36 and subsequent degradation of the inhibitory protein through the 26S proteasome, thereby allowing p50/p65 heterodimers to translocate into the nucleus and bind specific κB elements (32). IκBα phosphorylation is achieved by the IKK complex, which consists in three subunits: IKKα and IKKβ, two highly homologous kinases, and a regulatory subunit called NEMO/IKKγ (Fig. 2). In most cases, IKKβ is

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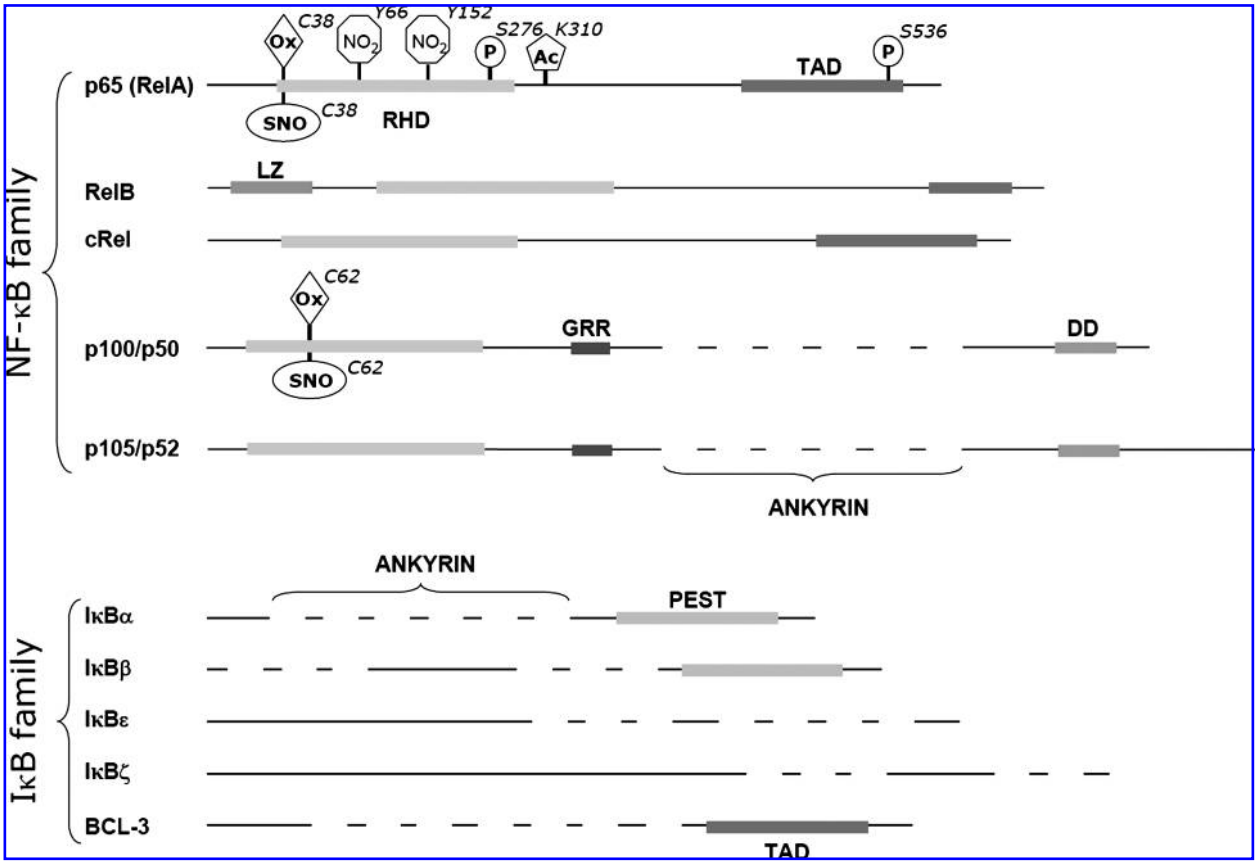

FIG. 1. Members of the NF-κB and IκB protein families and their redox-regulated post-translational modifications. The precursor proteins p100 and p105 function as both IκB proteins and, when processed, NF-κB family members. Redox-dependent post-translational modifications and target residues are indicated. Ac, acetylation; ANKYRIN: ankyrin repeat; DD: death domain; GRR, glycine-rich region; LZ, leucine-zipper; NO_2, nitration; Ox, oxidation; P, phosphorylation; PEST, proline-, glutamic acid-, serine-, and threonine-rich; RHD, REL homology domain; SNO, S-nitrosylation; TAD, transactivation domain.

responsible for IκBα phosphorylation, whereas IKKα plays a key role in the alternative pathway and in the regulation of NF-κB-mediated transcription (see below) (72). The classical pathway is activated in response to a very large array of stimuli, all culminating in IKK complex activation. These include pro-inflammatory cytokines (TNFα, IL-1β), bacterial products (LPS), TCR or BCR engagement, DNA damage and, in some cell types, reactive oxygen species (Fig. 2). NF-κB activation through the classical pathway controls the immune and stress responses (particularly inflammation), cell proliferation, differentiation, and apoptosis (62).

Alternative pathway

The alternative pathway is activated by a most restricted number of stimuli, including some members of the TNFR superfamily (such as LTβR), BAFF, or CD40L (10, 18). This pathway depends on the activation of IKKα by NIK. Activated IKKα phosphorylates p100, leading to its ubiquitination and processing to p52 which, in association with its partners (like RelB), translocates into the nucleus and binds specific κB elements (Fig. 2). The alternative pathway is important for secondary lymphoid organ development and homeostasis and adaptive immunity (10, 18).

Atypical pathways

Atypical NF-κB activation pathways are IKK-independent and rely on phosphorylation of IκBα on Tyr42 or on Ser residues in IκBα PEST domain. IκBα Tyr42 phosphorylation occurs upon stimulation of tyrosine kinase receptors (such as EGFR, CNTFR, or NGFR) or after oxidative challenge such as hypoxia/reoxygenation or pervanadate stimulation (11, 25, 31, 50, 62, 76). H_2O_2 also induces IκBα Tyr42 phosphorylation in some cell lines (Fig. 2) (28, 31). Upon tyrosine phosphorylation, IκBα is degraded or dissociates from NF-κB (9, 31). Upon UV irradiation or *HER2* expression, NF-κB is activated through phosphorylation of IκBα PEST domain by CK2, which induces its degradation through a calpain-mediated mechanism (Fig. 2) (43, 68, 73, 77).

Nuclear Phase of NF-κB Activation

Once in the nucleus, NF-κB must undergo a series of post-translational modifications that are required for the complete activation of NF-κB-dependent gene transcription (63). Moreover, DNA is packaged with histones into chromatin, and a relaxation of the chromatin is needed for increased DNA accessibility to NF-κB (27). Chromatin remodeling is in large part regulated by acetylation/deacetylation of lysine residues

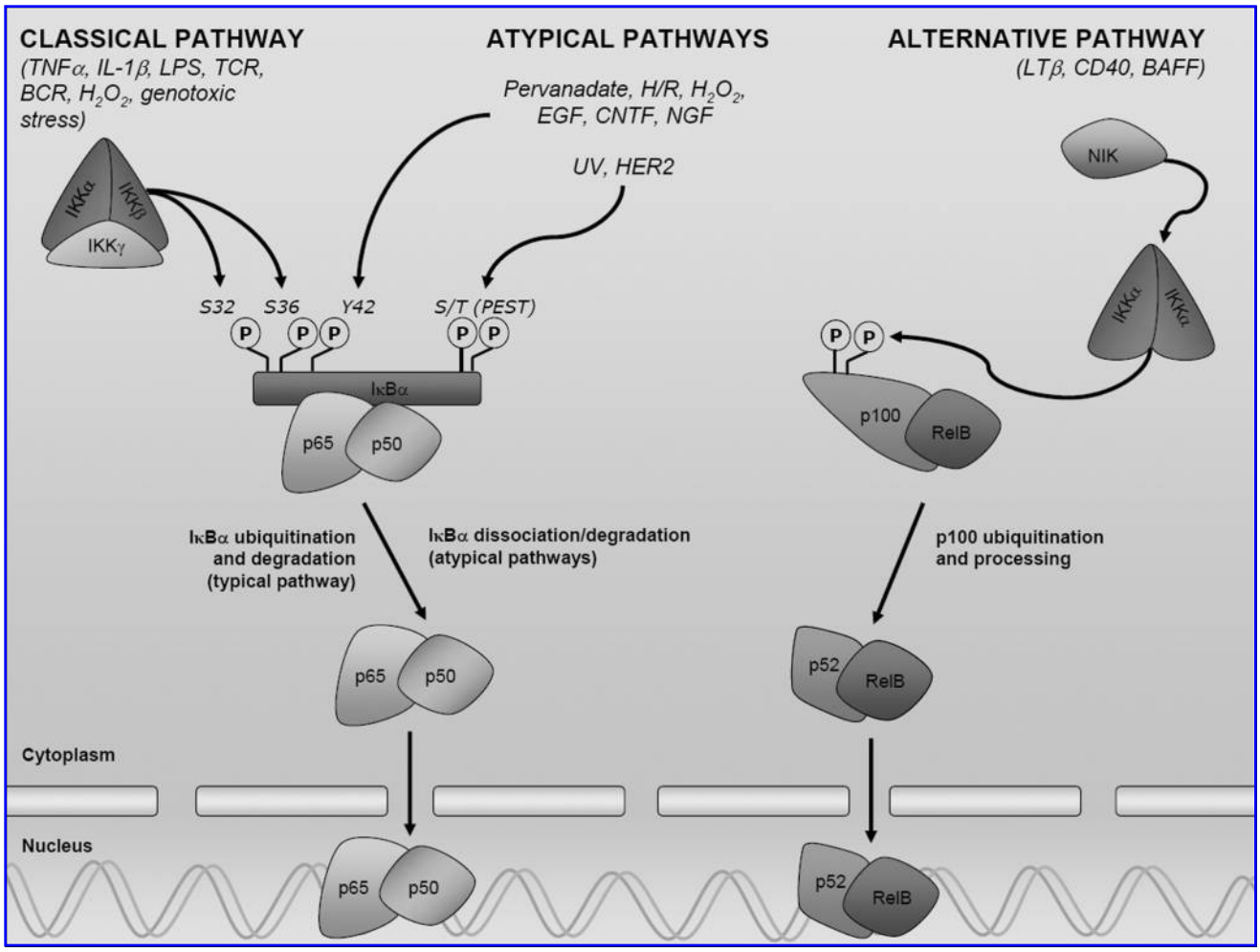

FIG. 2. Classical, atypical, and alternative pathways of NF-κB activation. The classical NF-κB pathway relies on IKK-mediated IκBα phosphorylation on Ser32 and 36, leading to its ubiquitination and degradation through the proteasome, which allows NF-κB nuclear translocation. Atypical pathways target IκBα Tyr42 or Ser and Thr in IκBα PEST region. The alternative pathway relies on NIK and IKKα-mediated p100 phosphorylation and processing to p52, which translocates into the nucleus with RelB.

in histone N-terminal tail (12). Histone acetylation is achieved by histone acetyltransferases (HATs) that transfer an acetyl group from acetyl coenzyme A to a specific lysine residue (notably Lys9 and Lys14 from histone H3), thereby modifying the electrostatic properties of the histone protein, resulting in uncoiling of the DNA. HATs are antagonized by histone deacetylases (HDACs), which remove acetyl groups from acetylated histones, thereby increasing DNA compaction and inhibiting transcription (Fig. 3) (95). However, the situation is actually more complex. Some promoters, called constitutively and immediately accessible (CIA), possess an open conformation and do not require stimulus-induced chromatin remodeling. On the contrary, other promoters with regulated and late accessibility (RLA) are dependent on chromatin modification for DNA accessibility (56). Among NF-κB proteins, post-translational modification of p65 are the best characterized (59). Phosphorylation of p65 Ser276 occurs after IκBα degradation and promotes the interaction of p65 with CBP (CREB binding protein) and p300, two transcriptional co-activators which possess acetyltransferase activity, thereby increasing transcription. p65 Ser276 phosphorylation is achieved by the catalytic subunit of protein kinase A (PKAc) or MSK-1 and -2 (87, 99). Phosphorylation of p65 Ser311 by PKCζ and Ser536 by IKKα and IKKβ were also reported to increase p65 binding to CBP and CBP/p300, respectively (15, 21). Furthermore, phosphorylation of p65 Ser276, and, to a lesser extent, Ser536, promotes direct p65 acetylation by CBP/p300 at Lys310 (15). Acetylation of p65 occurs at multiple sites and regulates different biological functions (12). Notably, Lys310 acetylation promotes full p65 transcriptional activity (14). p65 is deacetylated through specific interactions with HDAC-1, -2, and -3 or SIRT1 (Fig. 3) (12, 96). Thus, full NF-κB-mediated transcription is dependent on both chromatin remodeling events and direct modifications of p65 through the activity of protein kinases and histones acetyltransferases and deacetylases (Fig. 3). Recently, IKKα has been reported to regulate and co-ordinate these two events (27). Despite its major cytoplasmic localization, IKKα was also shown localized into the nucleus thanks to an NLS located in the kinase domain of the protein (29, 30, 79). A recruitment of IKKα, together with p65 and CBP, onto NF-κB target genes promoters was observed (3, 92). This association induces

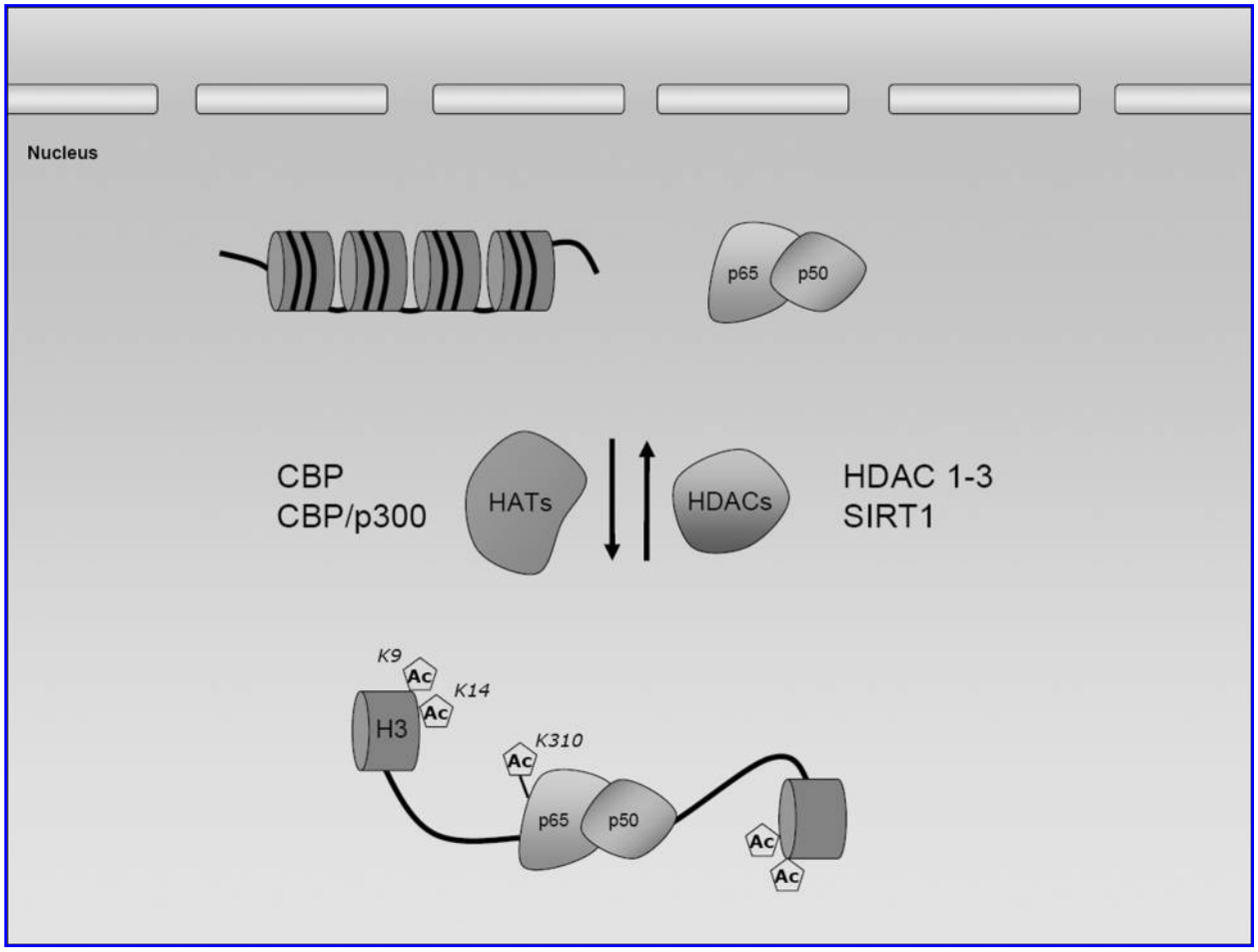

FIG. 3. Regulation of NF-κB activation and chromatin remodeling by HATs and HDACs. Chromatin compaction and unwinding is regulated by the combined action of HAT and HDAC. HATs, such as p300 and CBP, favor transcription through direct acetylation of histones and NF-κB. Acetylations of Lys9 and Lys14 of histone H3 and of Lys310 of NF-κB have been reported. HDACs repress transcription through their deacetylase activity. HADC1-3 and SIRT1 have been implicated in the control of NF-κB-mediated transcription.

IKKα-mediated phosphorylation of histone H3 on Ser10, triggering its subsequent acetylation on Lys9 and Lys14 by CBP, a crucial step in modulating chromatin accessibility at NF-κB responsive promoters. IKKα also phosphorylates various other substrates involved in chromatin remodelling, including SMRT and CBP (36, 37, 39). SMRT is a component of large co-repressor complexes often associated with HDAC3. SMRT phosphorylation promotes its nuclear export together with HDAC3 and degradation via the proteasome pathway, thus allowing transcriptionally active p50/p65 complexes to stimulate transcription (37). IKKα-mediated phosphorylation of CBP enhances CBP-binding affinity for p65 and its HAT activity (39). As aforementioned, IKKα also phosphorylates chromatin-bound p65 Ser536, which prevents HDAC3 recruitment to chromatin and allows p300 to acetylate p65 at Lys310 (36). Accordingly, we showed that IKKα directly regulates p65 DNA binding on some, but not all, NF-κB-target promoters (30). This gene-specific function may account for the NF-κB-mediated transcription specificity under different biological conditions.

NF-κB target-genes mainly encode regulators of the immune/inflammatory response, like cytokines (TNFα, IL-1, IL-6), chemokines (MCP-1, IL-8, MIP-1α), adhesion molecules (ICAM-1, VCAM-1), enzymes (COX-2, iNOS), and immune receptors (MHC, IL-2 receptor, IFN-γ receptor). NF-κB also enhance the transcription of anti-apoptotic proteins (XIAP, GADD45β), antioxidant enzymes (MnSOD), and proteins involved in the negative feedback of NF-κB activation, such as IκBα (60). Indeed, after its degradation, IκBα is rapidly neosynthesized in an NF-κB-dependent fashion and terminates NF-κB activation by transporting NF-κB dimers back into the cytoplasm (4, 5, 86).

NF-κB and Reactive Oxygen/nitrogen Species

Reactive oxygen species (ROS) derive from the reduction of molecular oxygen, a molecule crucial for sustaining life processes of nearly all living organisms. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) (84). Superoxide anion comes from monovalent reduction of oxygen and can be rapidly dismutated to H_2O_2 by superoxide dismutase. ·OH, a highly reactive species, is formed through the reaction of H_2O_2 with metallic cations following the Fenton/Haber–Weiss reaction (90). There are exogenous and endogenous sources of cellular ROS. Exogenous sources include irradiation (UV, X- and γ-rays) and

chemicals, such as redox-active metals (such as Fe and Cu) that enhance the Fenton/Haber–Weiss reaction (44, 84). Several endogenous sources of ROS have been described. O_2^- can be generated by electron leakage from mitochondrial complex I and III of the electron transport chain (54). O_2^- can also be produced be specialized enzymes called NADPH oxidases (NOX). These enzymes are mainly expressed in phagocytic cells and help destroying pathogens through ROS production (47, 69). Nonphagocytic NOX expressed by various cell types have also been described and serve in a series of biological functions, such as cellular signaling and host defense (47). Reactive nitrogen species (RNS) comprise nitric oxide (NO·) and peroxinitrite ($ONOO^-$). NO· generation occurs through the activation of nitric oxide synthase (NOS). $ONOO^-$ is formed by reaction of NO· with O_2^- (80). To maintain redox homeostasis, cells have developed a series of enzymatic and nonenzymatic antioxidants. Enzymatic antioxidants comprise superoxide dismutase (SOD), which catalyze the dismutation of O_2^- to H_2O_2. Further degradation of H_2O_2 to H_2O is achieved by catalase, peroxiredoxins, or the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) by glutathione peroxidase (GPX). GSH is the most abundant tripeptide thiol in the cell and represents a major nonenzymatic antioxidant. It is able to scavenge directly ·OH and singlet oxygen by reaction with its thiol group. The thioredoxin system, composed of thioredoxin (TRX) and thioredoxin reductase, is another important antioxidant that act by reducing oxidized thiol-containing proteins (84).

NF-κB was the first transcription factor recognized to be redox-regulated in eukaryotic cells (31, 74). Reduction/oxidation control both cytoplasmic and nuclear steps in NF-κB activation, including IκBα degradation, NF-κB DNA binding, NF-κB transcriptional activity, and chromatin remodeling (42). Redox regulation of cytoplasmic events in the NF-κB program is thoroughly reviewed by Oliveira–Marques and colleagues in this issue. Here we describe the redox regulation of nuclear events during NF-κB activation.

Redox Control of Chromatin Remodeling During NF-κB Activation: Implications in Lung Inflammation

The impact of oxidants on chromatin remodeling of NF-κB-dependent gene has been extensively studied in the airway epithelium. Due to its unique structure and function, the lung is the major target for oxidative injury due to endogenous or exogenous ROS (66). Endogenous ROS production involves in most cases immune/inflammatory responses against inhaled pathogens and the recruitment into the airspace of numerous ROS-producing cells, such as macrophages, neutrophils, and eosinophils. On the other hand, various inhaled pollutants, such as cigarette smoke, ozone, or automobile exhausts contribute to the production of ROS into the lung. Excessive ROS production has been associated with many chronic inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary fibrosis (65). Cigarette smoke (CS) is a clearly established etiological agent of COPD. CS contains an estimated 10^{17} free radicals and many ROS-producing chemical agents per puff, and is thus one of the major exogenous sources of ROS in the lung (16, 66). CS-mediated oxidative stress not only activates NF-κB through activation of the IKK complex but also induces important chromatin modifications that enhance the transcription of pro-inflammatory NF-κB-dependent genes. As aforementioned, the regulation of NF-κB-mediated transcription is tightly regulated by HDACs-containing repressor complexes. Notably, HDAC-1, -2, and -3 were found to repress NF-κB transcriptional activity though various mechanisms (12). HDAC1 interacts directly with p50 or p65 subunits. In resting cells, HDAC1/p50 complexes bind to the DNA and repress transcription by local histone H3 deacetylation, which prevents RNA PolII recruitment. Upon stimulation of the cell, p65 is phosphorylated by PKA, enters into the nucleus, and associates with CBP/p300, which leads to the displacement of p50/HDAC1 repressive complexes and allows transcription (97). HDAC2 can regulate NF-κB activity through its association with HDAC1 (7), and HDAC3 directly deacetylates p65, thereby promoting its association with IκBα and its nuclear export (13). It was shown that cigarette smoke extract (CSE)-derived oxidants profoundly alter the expression level and activity of HDAC1-3 in macrophages, accounting for increased pro-inflammatory gene transcription (93). This decline of activity is restored by pre-treating cells with GSH or the dietary polyphenols curcumin, highlighting a role for ROS in HDAC loss of function (53, 93). Particularly, HDAC2 expression and activity is decreased in smokers, COPD, and asthma patients. The reduced HDAC2 activity is due to CSE-induced post-translational modifications such as nitration, carbonylation, and nitrosylation (Fig. 4) (66, 93). Degradation of HDAC2 is due to phosphorylation of HDAC2 in a CK2-dependent fashion, thereby inducing its proteasomal degradation (1). CSE-induced degradation of HDAC2 is though to be associated with steroid resistance in severe COPD subjects and asthmatic patients who smoke. Indeed, anti-inflammatory effects of corticosteroids rely on the fact that ligand-bound steroid receptors recruit HDAC2 to target promoters of pro-inflammatory genes, thereby leading to transcriptional repression. The absence of HDAC2 in COPD may thus lead to corticosteroid unresponsiveness (17, 40, 66). IKKα has also been implicated in chromatin modification of pro-inflammatory genes in lung inflammation. CSE exposure to mice leads to an increase of IKKα expression level in alveolar macrophages and airway epithelial cells. IKKα was found associated to promoters of IL-6 and MIP-2 pro-inflammatory genes upon CSE exposure, leading to phosphorylation of Ser10 and acetylation of Lys9 on histone H3. Increased acetylation of p65 on Lys310 was also observed (Fig. 1). Transfection of a dominant negative mutant of IKKα reduced CSE-induced chromatin modifications and expression of pro-inflammatory genes in macrophages (Fig. 5) (94).

p65 Serine 276 Phosphorylation and the Control of NF-κB-Mediated Transcription

Despite several phosphorylable serine residues within the p65 protein have been identified so far, the phosphorylation of p65 Ser276 by PKAc incontestably plays the most important role in the control of NF-κB-mediated transcription (Fig. 1). PKAc was found to co-purify with NF-κB/IκB complexes in the cytoplasm and to phosphorylate p65 Ser276 upon stimulus-induced IκBα degradation (98, 99). Mutation of Ser276 to Ala abolishes the transcriptional capacities of p65 without interfering with nuclear translocation and DNA binding. It was shown that p65 Ser276 phosphorylation permits its interaction with the co-activator CBP/p300 and the

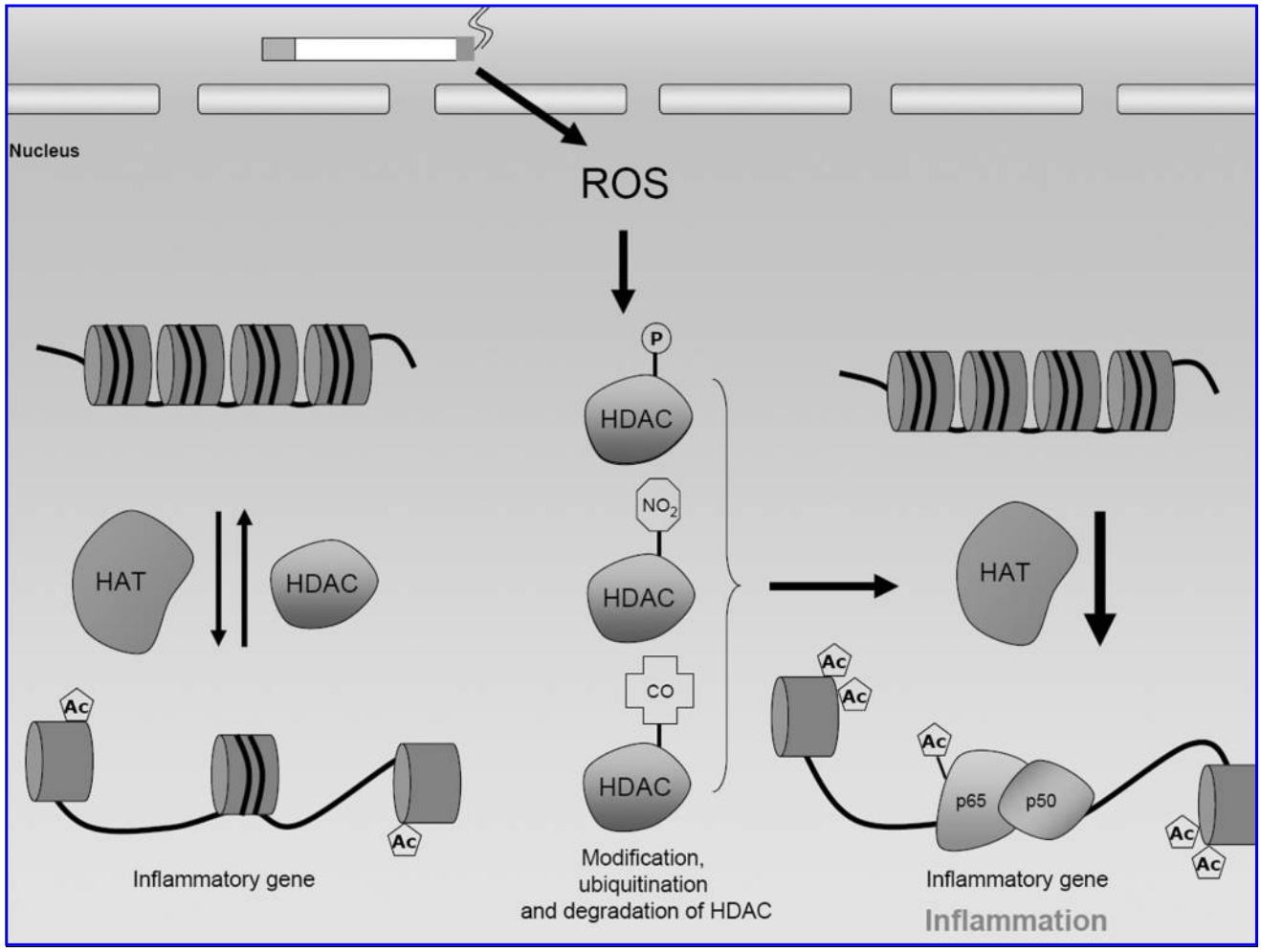

FIG. 4. ROS-mediated degradation of HDACs leading to increased expression of pro-inflammatory genes. Histones acetylation/deacetylation by the combined activity of HATs and HDACs is a fine tuning mechanism allowing a controlled transcription of pro-inflammatory genes. ROS produced by cigarette smoke extracts exposure induce various post-translational modifications of HDACs, such as phosphorylation (P), nitration (NO_2), and carbonylation (CO), leading to ubiquitination and degradation of HDACs. Decreased HDACs leads to an increased acetylation of both chromatin and NF-κB and uncontrolled transcription of pro-inflammatory mediators. Adapted from (66).

displacement of HDACs, thereby allowing RNA PolII binding and full transcription (97). There are several lines of evidence that this PKAc-mediated Ser276 phosphorylation is redox-regulated (Fig. 1) (41). TNFα stimulation of monocytes/macrophages gives rise to a rapid production of ROS that were shown to be required for transcription of IL-8, a well-known NF-κB target gene. Treatment with two unrelated antioxidants, DMSO (which quenches superoxide formation) (71) and NAC (an H_2O_2 quencher) (6) inhibits TNFα-induced IL-8 expression, even though IκBα degradation and NF-κB nuclear translocation are preserved. Further studies indicated that antioxidants inhibit TNFα-induces p65 Ser276, but not Ser536, phosphorylation, implying that PKAc catalytic activity is redox-dependent. Accordingly, the association of p65 with p300 and RNA PolII recruitment on the endogenous IL-8 promoter is impaired by antioxidant treatment (Fig. 6) (41). It was recently reported that p65 Ser276 phosphorylation is required for activation of only a subset of NF-κB-dependent genes (58). Accordingly, introduction of a S276A mutant in the mouse genome give rise to complex and variegated developmental defects (20). Important pro-inflammatory genes, such as TNFα, MIP-2 and MCP-1, were found downregulated upon p65 Ser276A expression, whereas IκBα or Cox-2 expressions were unchanged. Pax6, a transcription factor implicated in eye morphogenesis, was found dramatically downregulated in p65 Ser276 Ala knock-in mice, accounting for aberrant eye development. This repression was due to epigenetic repression of the Pax6 gene caused by the binding of the mutant p65, together with HDAC3-containing repressive complexes, in the vicinity of the Pax6 promoter (20). In that context, it is conceivable that antioxidants would inhibit the transcription of specific NF-κB-target genes, which could be of interest for specific therapeutic strategies.

Cysteine Oxidations/S-Nitrosylation and the Control of NF-κB DNA-Binding

The redox state of cysteine residues influences many properties of proteins, including enzymatic activity, protein folding, and DNA binding capacities (2, 8, 83, 85). Tran-

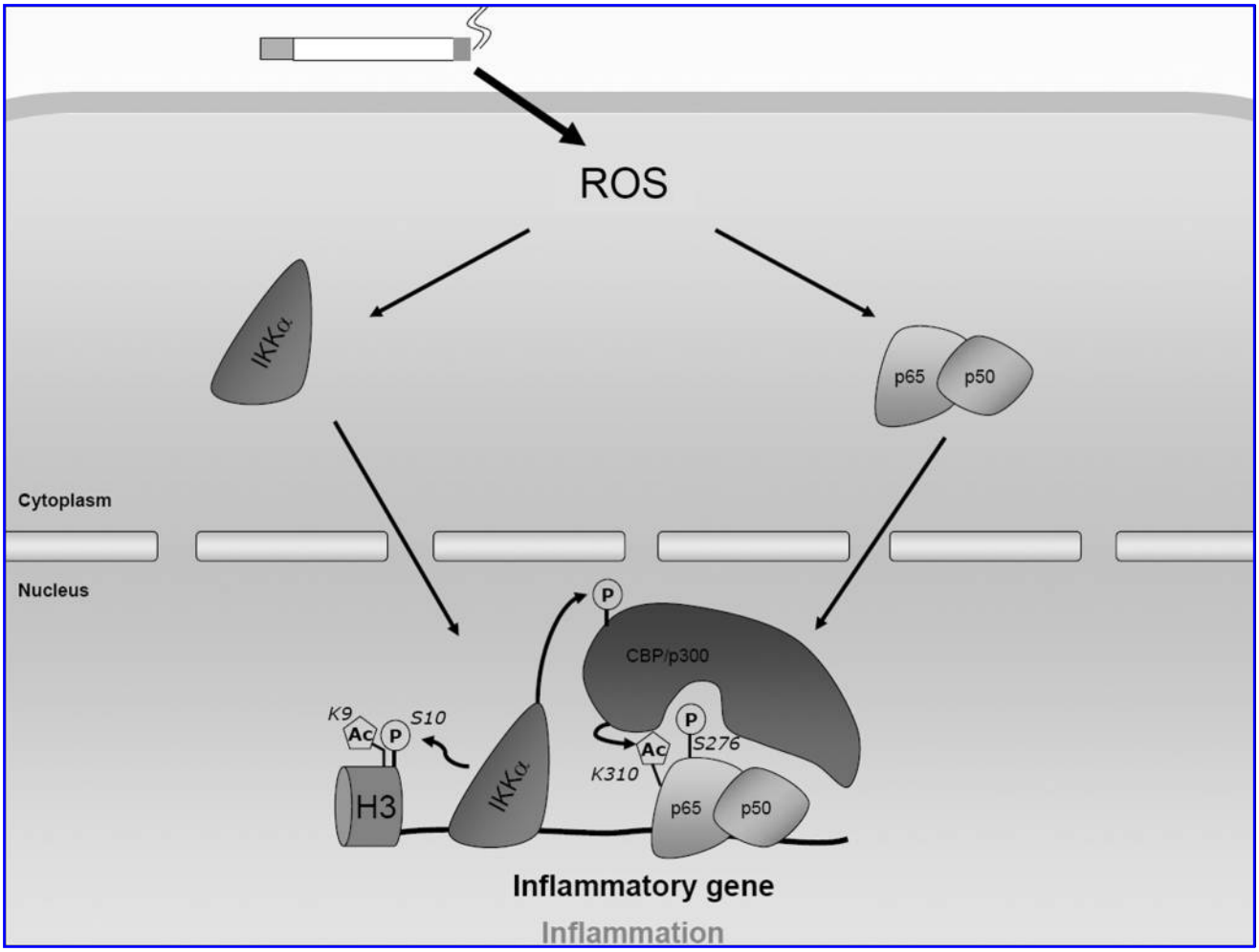

FIG. 5. IKKα causes chromatin modifications on pro-inflammatory genes upon cigarette-smoke extract-derived ROS exposure. Cigarette-smoke exposure to mice results in an increased of nuclear level of IKKα in lung epithelial cells and macrophages, leading to the phosphorylation and acetylation of histone H3 Ser10 and Lys9 on the promoter of pro-inflammatory genes, accounting for a chromatin unwinding and increased accessibility for transcription factors. Increased nuclear IKKα is also associated with increased acetylated p65 on Lys310, which enhance NF-κB-mediated transcription and inflammation.

scription factors often contain cysteine residues within their DNA-binding region whose redox state plays a crucial role in the control of DNA binding (2). It has been shown that, whereas oxidants enhance NF-κB nuclear translocation, oxidation of NF-κB decreases its DNA binding activity (82). Indeed, a critical cysteine residue within the RHD domain of p50, Cys62 (Fig. 1), needs to be reduced for efficient NF-κB DNA binding (81). p50 Cys62 is highly oxidized in the cytoplasm, but is rapidly reduced once NF-κB has migrated into the nucleus (57). Several enzymes have been reported to control the reduction of nuclear p50 Cys62. In resting cells, thioredoxin (TRX) is mainly localized in the cytoplasm, but translocates into the nucleus upon cellular stimulation by TNFα, where it reduces p50 Cys62 to promote NF-κB DNA binding (35, 52). AP endonuclease 1/redox factor 1 (APE1/Ref-1) is a DNA repair enzyme with apurinic/apyrimidic (AP) endonuclease activity important for the base excision repair pathway (19, 67). It was shown that APE1/Ref-1 is also involved in the reduction of oxidized cysteine residues within several transcription factors, including Cys62 of p50, thereby promoting DNA binding and transcriptional activities (57). APE1/Ref-1 appears to act via two mechanisms. First, APE1/Ref-1 can act as a redox factor by directly reducing proteins in a manner depending on critical cysteine residues located within the N-terminal region of APE1/Ref-1, such as Cys65 (89). Second, it was recently reported that APE1/Ref-1 can regulates the DNA binding activity of NF-κB by promoting the reduction of p50 Cys62 by other antioxidant proteins, such as GSH and TRX (2). In support of this, APE1/Ref-1 was shown to interact physically with target transcription factors and TRX (34). An APE1/Ref-1 C/S mutant, in which the seven cysteine residues were substituted to serine, lacks intrinsic redox activity but is still able to promote GSH- or TRX-mediated reduction of p50. It thus appears that APE1/Ref-1 both acts as a redox factor and a redox chaperone (Fig. 7) (2). Nitric oxide (NO) can also modify p50 C62 by S-nitrosylation (Fig. 1), thereby inhibiting NF-κB DNA binding activity (51). It is though that p50 C62 S-nitrosylation may represent a negative regulation mechanism of NF-κB activation since NO synthase (NOS), which produces NO, is an

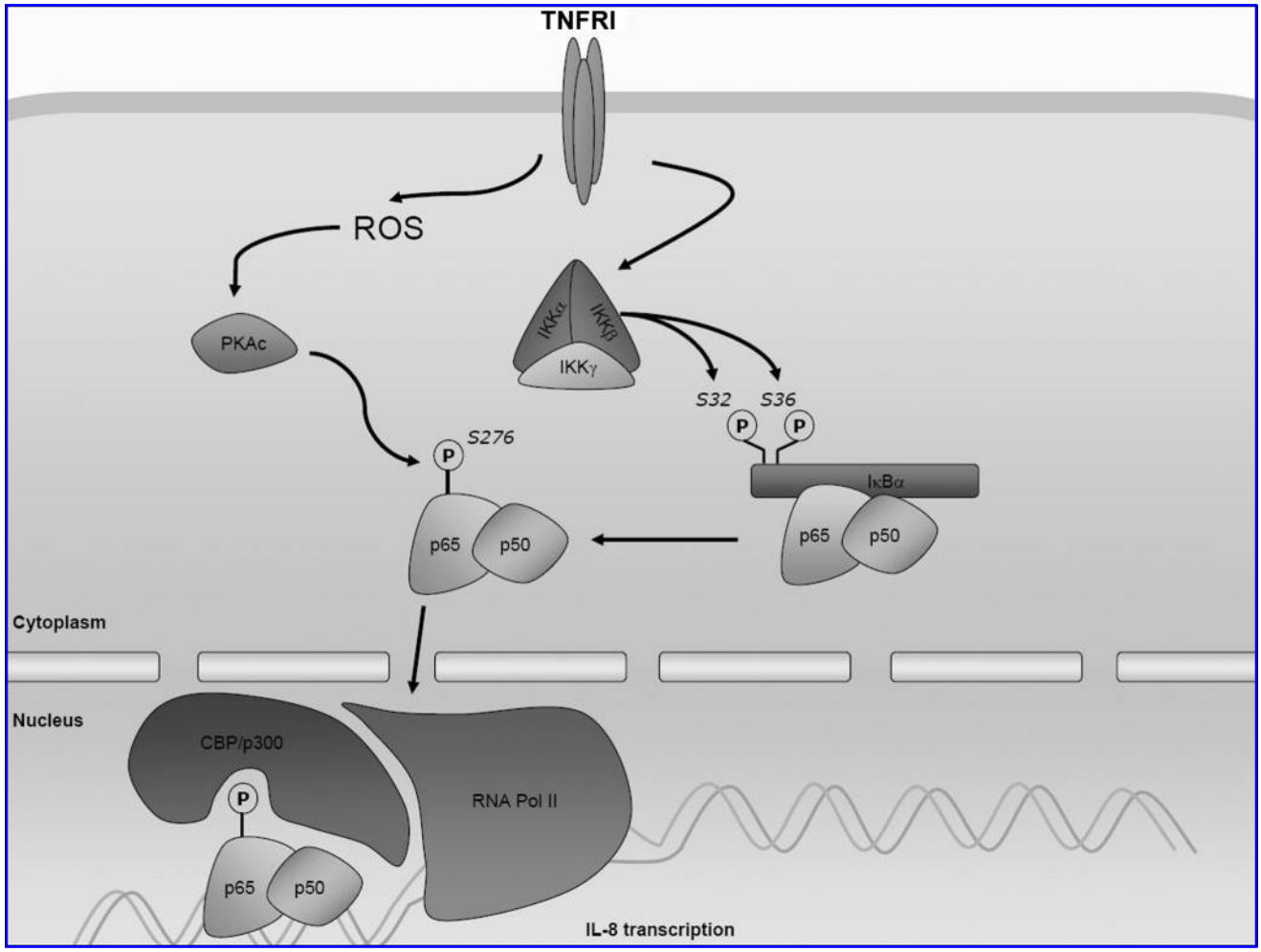

FIG. 6. Redox-dependent phosphorylation of p65 on Ser276 by PKA. Ligation of the TNFRI by TNFα induces the classical pathway of NF-κB activation and ROS production. ROS are required for PKAc-mediated phosphorylation of p65 on Ser276, which allow recruitment of the co-activator CBP/p300 and full transcription of the IL-8 gene by RNA PolII.

NF-κB-dependent gene (45). p65, the binding partner of p50, is also found S-nitrosylated upon cytokine stimulation of cells on Cys38 due to NOS2 (the inducible NO synthase) expression (Figs. 1 and 7). Nuclear SNO-p65 levels are inversely correlated with NF-κB DNA binding and transcriptional activities, suggesting that NO produced by NOS represents a negative feed-back mechanism to avoid excessive inflammation (Fig. 7). In support of this, macrophages extracted from NOS2 KO mice exhibit enhanced p65 DNA binding upon stimulation (45). Several anti-inflammatory and anti-cancer agents target NF-κB cysteine residues. Kamebakaurin and andrographolide, two compounds extracted from Asiatic herbal plants used in traditional medicine, were reported to block NF-κB activation through covalent modification of reduced Cys62 of p50, thereby blocking DNA binding activity of NF-κB (48, 91). Soy isoflavones, a component a soybeans, decreases the amount of APE1/Ref-1 available to reduce NF-κB in prostate cancer cells, which is leading to an increased sensitivity to radiation-induced cell death (24, 64). A promising inhibitor targeting NF-κB DNA binding is E3330, a synthetic quinone derivative (24). E3330 does not suppress IκBα degradation nor NF-κB nuclear translocation but selectively inhibits NF-κB DNA binding activity by targeting p50 Cys62 (33). E3330 acts by binding directly to APE1/Ref-1 and inhibiting its redox function (78). E3330 was reported to inhibit LPS-induced TNFα production, which has therapeutic effects on endotoxin-induced hepatitis in mice (42, 55). Various pharmacological compounds such as sesquipertenes lactones (26, 88) and quinone derivatives epoxyquinone A monomer, thymoquinone and plumbagin (49, 70, 75) also inhibit NF-κB activity by targeting the Cys38 of p65 through various mechanisms, including redox modification or alkylation. Methylglyoxal (MG) is a highly reactive glycolytic metabolite produced *in vivo* upon TNFα stimulation and under certain pathological conditions such as diabetes and neurodegenerative disorders. MG suppresses NF-κB activation by targeting p65 Cys38 (46). This may represent a therapeutically relevant feed-back mechanism decreasing NF-κB activation.

p65 Tyrosine Nitration

Tyrosine nitration is caused by peroxinitrite generated from NO and O_2^{-}. Tyrosine nitration of p65 occurs on Tyr66 and Tyr152 located within the RHD (Fig. 1) and rapidly in-

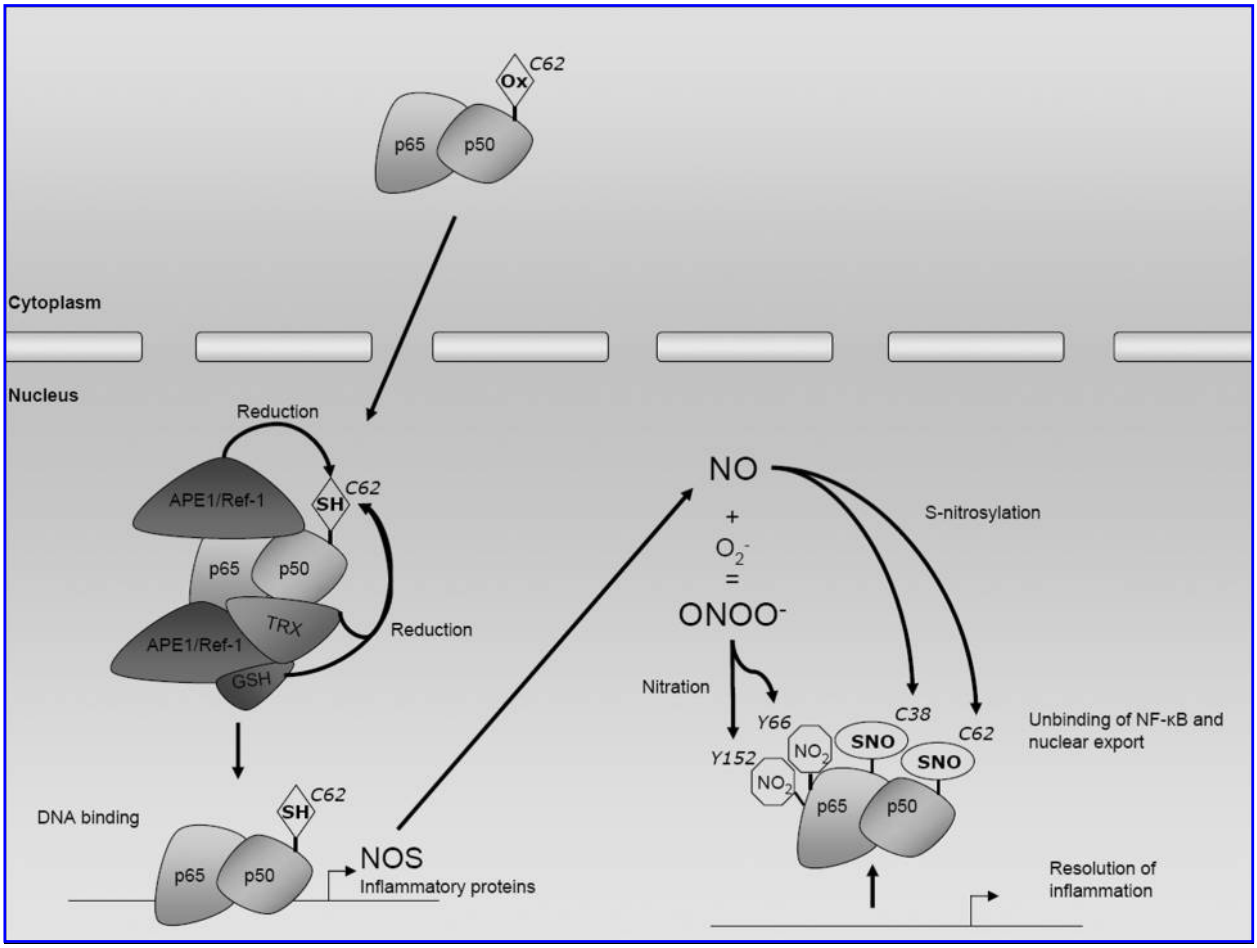

FIG. 7. Redox-regulation of NF-κB DNA-binding. In resting cells, NF-κB is predominantly found in the cytoplasm of the cell, with an oxidized p50 Cys62. Upon activation, NF-κB translocates into the nucleus where p50 Cys62 is reduced by APE1/Ref-1, thereby allowing NF-κB DNA binding. APE1/Ref-1 can both act as a redox factor by directly reducing p50 or as a redox chaperone by promoting the reduction of p50 by TRX or GSH. NF-κB DNA binding leads to NO synthase (NOS) expression, thereby leading to nitric oxide (NO) production. NO can in turn modify p50 C62 and p65 C38 by S-nitrosylation, which unbinds NF-κB from the DNA and contributes to the resolution of inflammation. NO can also react with O_2^- to generate $ONOO^-$. $ONOO^-$ induces tyrosine nitration of p65 on Tyr66 and Tyr152, thereby leading to its association with IκBα for nuclear export.

activates NF-κB via two mechanisms. First, it induces the replacement of p65/p50 with repressive p50/p50 complexes on target promoters. Second, it subsequently induces the association of p65 with IκBα and its export back to the cytoplasm (Fig. 7) (61). NO thus appears to play a key negative role in the termination of NF-κB activation by inducing both cysteine S-nitrosylation and tyrosine nitration. It should be noted that H_2O_2 was reported to have an opposite effect by prolonging TNFα-induced nuclear localization of p65. H_2O_2 promotes Ser32 and Ser36 phosphorylation and degradation of newly synthesized IκBα by inhibiting Cezanne, an anti-inflammatory enzyme negatively regulating TNFRI signaling by targeting RIP1 for deubiquitination (22, 23).

Conclusions

NF-κB proteins play a key role in the regulation of the inflammatory response, but also regulate several important biological processes, such as apoptosis and development. Endogenous or exogenous ROS regulate many steps during NF-κB activation (Fig. 8). ROS not only induce IκB degradation in the cytoplasm, thereby promoting NF-κB nuclear translocation, but also induce several post-translational modifications of p65 that are mandatory for full NF-κB-mediated transcription. It has been shown that TNFα-induced phosphorylation of p65 Ser276, which permits its interaction with the co-activator CBP/p300 and the displacement of HDACs from target promoters, requires endogenous ROS production. ROS produced upon cigarette smoke extracts (CSE) exposure in the mice lung target several regulators of the chromatin unwinding, thereby increasing the accessibility of NF-κB to the promoters of pro-inflammatory genes. Indeed, HDACs (notably HDAC2), are post-translationally modified upon CSE exposure, leading to their ubiquitination and degradation. IKKα, a key chromatin modifier, is found upregulated and bound to many promoters of pro-inflammatory genes upon CSE exposure of lung epithelial cells and monocytes, where it induces chromatin decompaction and increased transcription through acetylation of histone H3 and p65. In contrast, reducing conditions are mandatory for NF-κB DNA binding to

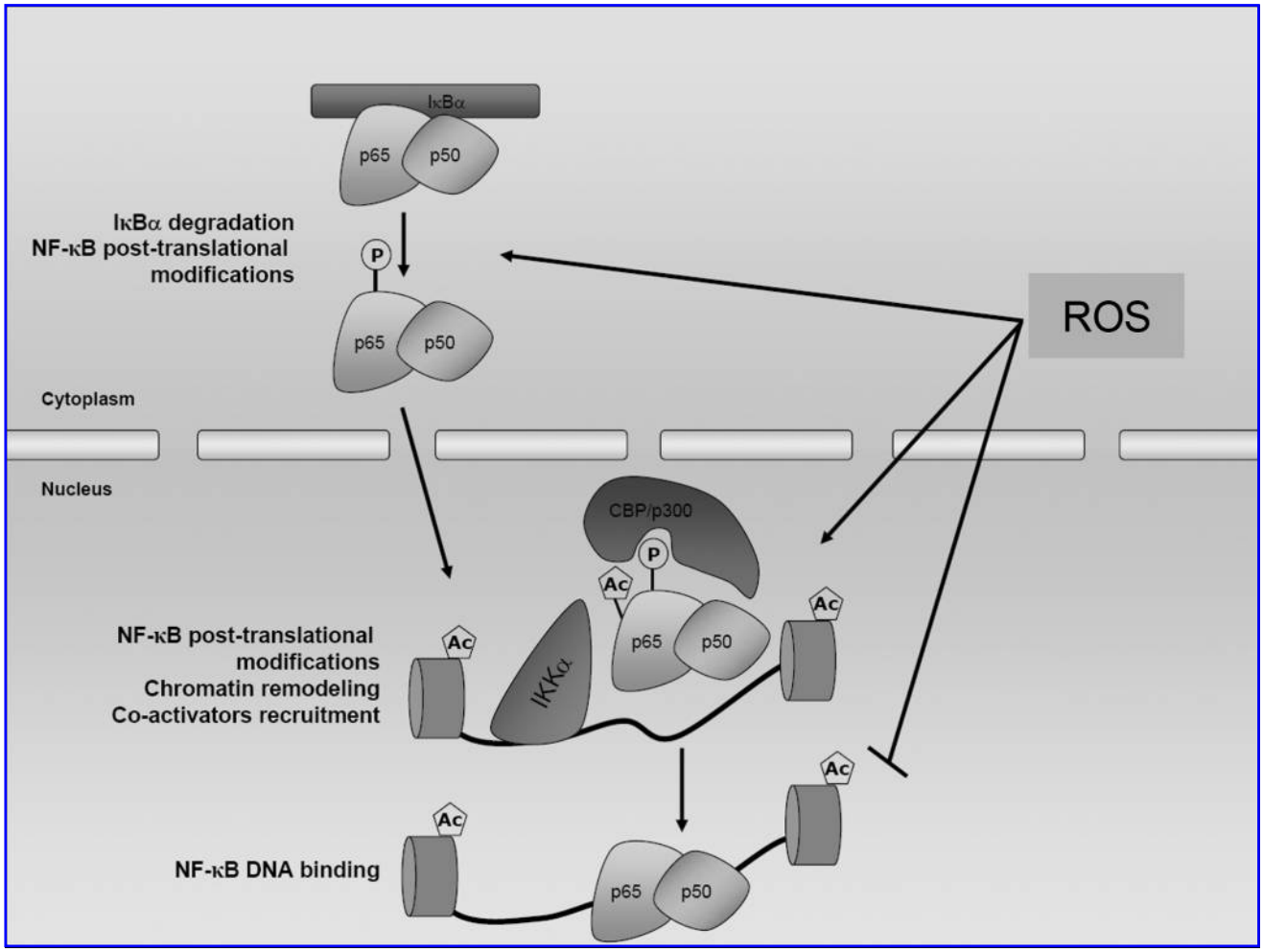

FIG. 8. Redox-regulation of NF-κB activation. ROS enhance the signal transduction pathways leading to NF-κB activation in the cytoplasm and translocation into the nucleus. Post-translational modifications of p65 and chromatin unwinding are also enhanced by ROS. However, NF-κB DNA binding requires reducing conditions. See text for details.

DNA (Fig. 8). Indeed, two redox-sensitive cysteines residues located within the DNA binding region of both p50 and p65 must be reduced for efficient DNA binding. This is achieved by the reducing peptide GSH and enzymes such TRX and the endonuclease APE1/Ref-1. However, the precise molecular basis of the redox-control of NF-κB DNA binding is still poorly understood. Given the importance of NF-κB in promoting several inflammatory diseases, it is tempting to consider antioxidants as important therapeutic tools to dampen inflammation. However, NF-κB plays also important other functions related to apoptosis or development that needs to be taken into consideration during development of new therapeutic strategies. In the same way, the elucidation of the molecular mechanism underlying the reduction of NF-κB to promote DNA binding will aid in developing selective and precisely located agents that would prevent ROS-dependent NF-κB activation in the cytoplasm rather than enhancing its DNA binding capacities in the nucleus.

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Abbreviations Used

APE1/Ref-1 = AP endonuclease 1/redox factor 1
BAFF = B cell activating factor
BCR = B cell receptor
CBP = CREB binding protein
CIA = constitutively and immediately accessible
CK2 = casein kinase 2
CNTF(R) = ciliary neurotrophic factor (receptor)
COPD = chronic obstructive pulmonary disease
COX-2 = cyclooxygenase-2
CS(E) = cigarette smoke (extract)
DMSO = dimethyl sulfoxide
EGF(R) = epidermal growth factor (receptor)
GADD45β = growth arrest and DNA damage-inducing protein 45β
GPX = glutathione peroxidase
GSH = reduced glutathione
GSSG = oxidized glutathione
HAT = histone acetyltransferase
HDAC = histone desacetylase
HER2 = human epidermal growth factor receptor-2
ICAM-1 = intercellular adhesion molecule-1
IFNγ = interferon γ
IκB = inhibitor of κB
IKK = IκB kinase
IL-1β = interleukin-1β
IL-1, -6, -8 = interleukin-1, -6, -8
iNOS = inducible nitric oxide synthase
LPS = lipopolysaccharide
LTβ(R) = lymphotoxin β(receptor)
MCP-1 = monocyte chemoattractant protein-1
MG = methylglyoxal
MHC = major histocompatibility complex
MIP-1α = macrophage inflammatory protein-1α
MnSOD = manganese superoxide dismutase
MSK-1, -2 = mitogen and stress-activated protein kinase-1, -2
NAC = N-acetyl-l-cystein
NEMO/IKKγ = NF-κB essential modulator
NES = nuclear export sequence
NF-κB = nuclear factor-kappa b
NGF(R) = nerve growth factor (receptor)
NIK = NF-κB-inducing kinase
NLS = nuclear localization sequence
NO = nitric oxide
NOS = NO synthase
NOX = NADPH oxidase
PEST = proline-, glutamic acid-, serine- and theronine-rich
PKAc = protein kinase A (catalytic subunit)
RHD = Rel homology domain
RLA = regulated and late accessibility
RNS = reactive nitrogen species
RNA PolII = RNA polymerase II
ROS = reactive oxygen species
SIRT = sirtuin 1
SMRT = silencing mediator for retinoic acid and thyroid hormone receptor
SOD = superoxide dismutase
TAD = transcription activation domain
TCR = T cell receptor
TNFα = tumor necrosis factor α
TNFR1 = tumor necrosis factor receptor 1
TRX = thioredoxin
VCAM-1 = vascular cell adhesion molecule-1
XIAP = X chromosome-linked IAP

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