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Regulation at multiple levels of NF-κB-mediated transactivation by protein acetylation

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Abstract

Evidence has accumulated that deacetylation and acetylation events are implicated in the regulation of NF-κB transcriptional activity. Several groups have reported potentiation of NF-κB-mediated gene induction [by specific inducers (such as TNFα)], following deacetylase inhibition by trichostatin A or sodium butyrate. This potentiation reflects a complex acetylation-dependent regulation of NF-κB-dependent transactivation. This acetylation-dependent regulation occurs at multiple levels. First, acetylation of histones regulates the NF-κB-dependent gene accessibility. Second, unidentified acetylation events modulate temporally the IKK activity and subsequently the duration of NF-κB presence and DNA-binding in the nucleus. Third, direct acetylation of the NF-κB subunits p65 and p50 regulates different NF-κB functions, including transcriptional activation, DNA-binding affinity and IκBα assembly. Finally, acetyltransferases and deacetylases interact directly with several proteins involved in the NF-κB signaling pathway, including NF-κB itself, IκBα, IKKα and IKKγ. These interactions probably allow acetylation of NF-κB itself, of other transcription factors and of histones associated with NF-κB-regulated genes. The present review discusses these recent data obtained on the role of protein acetylation in the regulation of the NF-κB cascade.

Keywords: NF-κB; Acetylation; TSA; TNFα; Synergism

1. Introduction

NF-κB is a ubiquitously expressed family of transcription factors controlling the expression of numerous genes involved in inflammatory and immune responses and cellular proliferation (reviewed in [1–4]). In mammals, there are five known members of NF-κB/Rel family: p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel and RelB. The most abundant form of NF-κB is a heterodimer of p50 and p65. In unstimulated cells, NF-κB is sequestered in the cytoplasm in an inactive form through interaction with the IκB inhibitory proteins (including IκBα, IκBβ and IκBε, of which the best studied is IκBα). In the canonical activation pathway (Fig. 1, top panel), upon stimulation of cells by specific inducers [such as the proinflammatory cytokine tumor necrosis factor α (TNFα)], IκBα is phosphorylated on two specific serine residues by a large cytoplasmic IκB kinase (IKK) complex, that consists of the kinase catalytic subunits IKKα and IKKβ and the regulatory subunit NEMO/IKKγ (reviewed in [1,5]). This phosphorylation marks IκBα for polyubiquitination by the E3-SCF$^{\beta\text{-TrCP}}$ ubiquitin ligase complex, a specific ubiquitin ligase belonging to the SCF (Skp-1/Cul/Fbox) family, and for degradation by the 26S proteasome (reviewed in [3]). Degradation of IκBα allows a rapid and transient translocation of NF-κB to the nucleus, where it activates transcription from a wide variety of promoters, including that of its own inhibitor IκBα. The newly synthesized IκBα enters the nucleus and removes NF-κB from its DNA-binding sites and transports it back to the cytoplasm, thereby terminating NF-κB-dependent transcription (reviewed in [1,4]).

Protein acetylation influences a broad set of cellular processes including diverse aspects of transcriptional regulation through the recruitment of enzymes: the deacetylases (HDACs) and the acetyltransferases (HATs). The packaging of eukaryotic DNA into chromatin plays an active role in transcriptional regulation by interfering with

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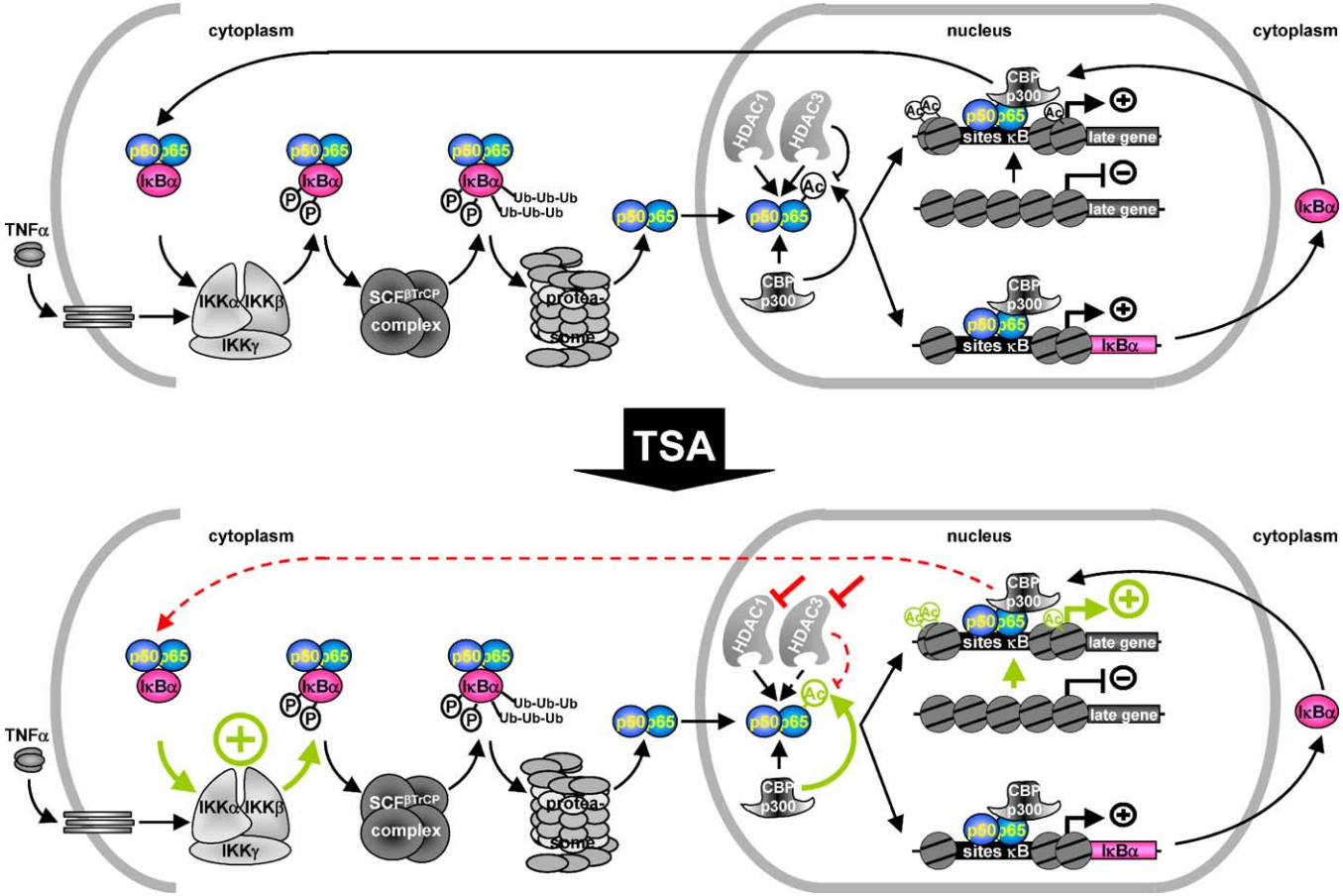

Fig. 1. TSA enhances TNFα-induced activation of the NF-κB signaling pathway at different levels. Top panel: upon stimulation by TNFα, IκBα is phosphorylated by the IκB kinase complex, containing IKKα, IKKβ and IKKγ. Then NF-κB (p50/p65) enters into the nucleus and activates the expression of multiple genes: those that have to be conformationally modified to become accessible to NF-κB (late genes) and those constitutively and immediately accessible to NF-κB (early genes including the IκBα gene). The newly synthesized IκBα protein enters the nucleus, removes NF-κB from its target genes, and takes it back to the cytoplasm to terminate the phase of NF-κB activation. Bottom panel: idem but the TSA-enhanced steps are indicated in green with thick arrows and the TSA-impeded steps are indicated in red with dashed arrows.

the accessibility to the transcription factors. Acetylation of specific lysine residues within the amino-terminal tails of nucleosomal histones is generally linked to chromatin disruption and transcriptional activation of genes. Consistent with their role in altering chromatin structure, many transcriptional coactivators (including hGCN5, CBP/p300, P/CAF, SRC-1) possess intrinsic acetyltransferase activity that is critical for their function [6–8]. Similarly, corepressor complexes include proteins that have deacetylase activity (reviewed in [9–13]). Moreover, reversible acetylation has also been identified as a critical post-translational modification of non-histone proteins, including general and specific transcription factors, non-histone structural chromosomal proteins, HATs themselves, the HIV-1 Tat protein, non-nuclear proteins (α-tubulin) and nuclear import factors (such as human importin-α). 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 (reviewed in [7,14–17]).

It is now well established that NF-κB-dependent transcription requires multiple coactivators possessing HAT activity: CBP and its paralogue p300, p300/CBP-associated factor (P/CAF) and SRC-1/NcoA-1 [18–22]. The interactions between NF-κB and these HATs suggest the existence of a link between acetylation events and NF-κB-mediated transactivation. A role for acetylation in the regulation of NF-κB-mediated transactivation has definitively emerged with the finding by our laboratory and other groups, that deacetylase inhibitors (HDACi) (such as trichostatin A (TSA) or sodium butyrate) enhance NF-κB-dependent gene expression in the presence of TNFα [23–30]. However, the data of these different groups 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 (Fig. 1). First, it has been shown that in addition to its interactions with acetyltransferases, NF-κB also interacts directly with several deacetylases [25–27,31–34]. A subtle competition between HAT and HDAC activities regulates the acetylation rate of histones and non-histone proteins. The use of HDACi causes a global hyperacetylation of all acetylable proteins in the cell. Second, the most studied NF-κB heterodimer is composed of two subunits p50 and p65, which are both acetylated at multiple lysine residues; the HATs p300/CBP play a major role in this latter process in vivo [27,34–39]. 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). Third, we have demonstrated that HDACi enhance the duration of TNFα-induced NF-κB translocation in the nucleus, thereby participating in the strong transcriptional synergism observed between HDACi and TNFα [29,30]. Fourth, two distinct classes of NF-κB-activable 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 class of NF-κB-activable genes are hyperacetylated after stimulation, before NF-κB recruitment [40]. HDACi could thereby increase the accessibility to these latter genes and thus favour their NF-κB-dependent transcription.

In this review, we will describe and discuss these recent data demonstrating the complex involvement of protein acetylation in the regulation of NF-κB-dependent transactivation.

2. Potentiation of TNFα-induced NF-κB activation by HDACi resulting from a delayed cytoplasmic reappearance of IκBα

IκBα plays a pivotal role in the NF-κB signaling pathway by regulating the duration of NF-κB activation. The primary level of regulation of NF-κB activity is through its retention in the cytoplasm via interactions with IκBα in preinduction states (reviewed in [1]). Following stimulation with proinflammatory cytokines such as TNFα, the resynthesis of de novo IκBα leads to the postinduction nuclear accumulation of IκBα, thereby inducing nuclear export of NF-κB. This latter event is part of a negative feedback system ensuring a transient NF-κB transcriptional response and the restoration of the preinduction state of NF-κB:IκB complexes [41]. Our laboratory has provided strong evidence that HDACi affect this negative feedback regulation known as postinduction repression of NF-κB function. Indeed, we have shown that the replenishment of the cytoplasmic pool of IκBα is delayed in cells co-treated with TNFα and TSA compared to cells treated with TNFα alone (Fig. 2B) [29,30]. This delay is due neither to a defect in IκBα mRNA production, nor to a nuclear retention of IκBα, but rather appears to result from a persistent proteasome-mediated degradation of neo-synthesized IκBα [30]. We have observed that TSA prolongs the TNFα-induced IKK activity (Fig. 2A) [30]. The phosphorylation of IκBα by IKK is critical to its proteasome-mediated degradation. Thus, the prolongation of IKK activity could explain the persistent degradation of neo-synthesized IκBα observed in the presence of TNFα + TSA versus TNFα alone. Consequently, IκBα can not accumulate in the nucleus to remove NF-κB from target gene promoters, transport it back to the cytoplasm and terminate the NF-κB response. In conclusion, unidentified acetylation events lead to a prolonged IKK activity, thereby prolonging the presence and DNA-binding activity of NF-κB in the nucleus and enhancing NF-κB-dependent transactivation (Fig. 2C and D). This observation accounts, at least in part, for the transcriptional synergism observed between TNFα and HDACi on NF-κB-regulated promoters.

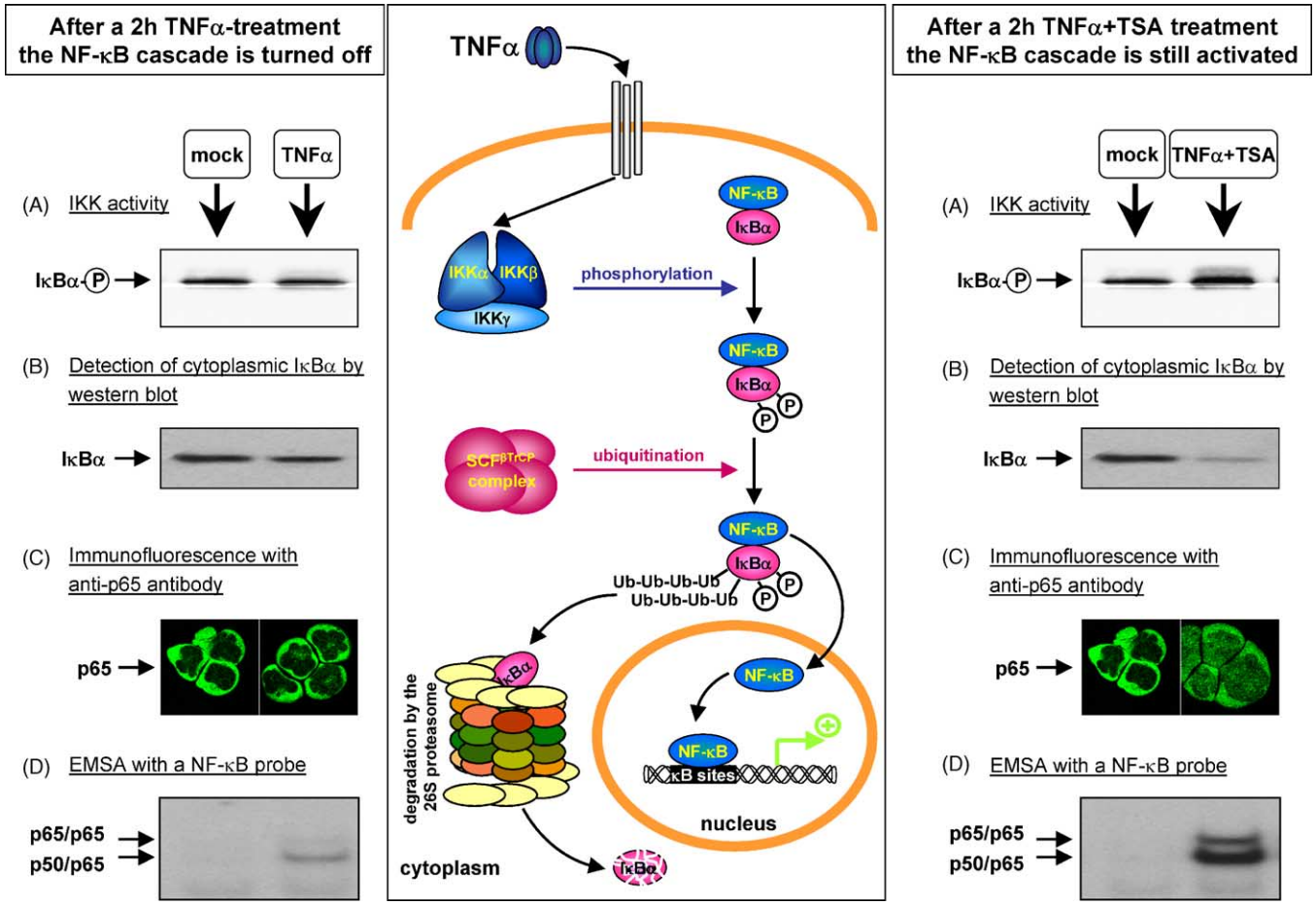

Fig. 2. TSA prolongs TNFα-induced activation of the NF-κB signaling cascade. Left and right panels: (A) TSA prolongs TNFα-induced IKK kinase activity. Endogenous IKK activity was measured in HeLa cells treated with TNFα and/or TSA for 2 h. IKKs were immunoprecipitated with an anti-IKKγ antibody and associated kinase activity was assayed by using purified GST-IκBα$_{1–54}$ fusion protein as a substrate. The phosphorylation rate of this fusion protein was detected by western blot using an anti-phospho IκBα antibody. (B) Delay in the cytoplasmic reappearance of IκBα in response to TNFα + TSA vs. TNFα treatment. Cytoplasmic extracts were prepared from HeLa cells treated or not with TNFα and/or TSA for 2 h and analyzed by western blotting. (C) TSA prolongs the TNFα-induced nuclear presence of p65. Cells were stimulated with TNFα and/or TSA for 2 h. p65 localisation was determined by indirect immunofluorescence using a p65 rabbit polyclonal antibody. (D) TSA prolongs TNFα-induced NF-κB binding to DNA. EMSA were performed using nuclear extracts prepared from cells treated with TNFα and/or TSA for 2 h and an oligonucleotide corresponding to the HIV-1 κB sites as probe. Middle panel: NF-κB activation pathway.

3. Recruitment by p65 of antagonist coregulatory proteins: acetyltransferases and deacetylases

Coregulatory proteins (coactivators and corepressors) have been shown to be required for gene expression regulation by many transcription factors. These coregulatory proteins likely function by facilitating or bridging the transactivators to the basal transcriptional machinery as well as by altering chromatin structure. Consistent with their role in altering chromatin structure, many coactivator proteins possess a HAT domain, which is capable of acetylating specific lysine residues in the amino-terminal tails of the core histones, but also in a wide variety of non-histone proteins. In contrast to coactivators, corepressor complexes include proteins possessing intrinsic HDAC activity.

Activation of transcription by NF-κB has been shown to require a number of coactivators containing HAT activity, including p300/CBP, SRC-1 and P/CAF [18,19,21,22,42,43]. Signal-induced p65 phosphorylation at serine 276 by PKA enhances recruitment of CBP/p300 [42]. Inactivation of either CBP, or P/CAF or SRC-1 by nuclear antibody microinjection prevents NF-κB-dependent transactivation [22]. When p65 is overexpressed, exogenous expression of CBP, P/CAF or SRC-1 enhances p65-dependent transcriptional activation [18,22]. Activation of IL-6 promoter activity by p65 and CBP/p300 has revealed a strong synergism between the two proteins, which is highly dependent on the HAT activity of CBP/p300 [24]. The viral protein E1A, which binds CBP/p300, specifically inhibits p65-dependent transactivation [18]. SRC-1 specifically binds to the NF-κB subunit p50 but not to the transactivating subunit p65 and potentiates NF-κB-mediated transactivation [21]. Coexpression of p300/CBP together with SRC-1 further enhances the SRC-1-potentiated level of NF-κB transactivation, indicating that both coactivators cooperate to regulate NF-κB-dependent activation [21,22]. Moreover, whereas a direct interaction between NF-κB and the acetyltransferase Tip60 has not been demonstrated, Tip60 can also enhance NF-κB transactivation [44,45].

In addition to coactivators, NF-κB has also been shown to recruit directly or indirectly corepressor complexes, which possess HDAC activity and which are believed to repress basal and induced NF-κB-dependent transcription. These corepressor complexes include HDAC-1 [26,31], HDAC-2 [25,26,33,34], HDAC-3 [27,34] and HDAC-6 [32]. The HDAC-3-p65 interaction seems crucial for the deacetylation of p65 (see Section 3) [27,34]. Overexpression of HDAC-1 and HDAC-2 represses NF-κB-dependent gene expression following TNFα induction or p65 overexpression [26,31]. Chromatin immunoprecipitation assays have shown that HDAC inhibition causes the hyperacetylation of a wild-type stably integrated NF-κB-dependent reporter construct but not of a mutant version of the same construct, in which the NF-κB binding sites are mutated. These latter data suggest that HDAC recruitment via NF-κB could maintain chromatin in a hypoacetylated state [26].

Furthermore, HDACs are known to form complexes directly with the mSin3a corepressor and to interact indirectly through mSin3a with both the N-CoR and SMRT corepressor proteins. The nuclear SMRT corepressor interacts with p50, p65 and IκBα [46,47]. Interaction of SMRT with p65 can be inhibited by overexpression of the CBP coactivator or following TSA treatment [47]. Moreover, exogenous expression of SMRT represses transactivation of a Gal4-dependent reporter gene by a Gal4–p65 fusion, but not in the presence of TSA [46]. Coexpression of mSin3a alone or in combination with HDAC-1 also causes repressive effects [26,46]. The N-Cor corepressor protein coimmunoprecipitates with p50; however, no such coimmunoprecipitation is detected with p65 [45]. Expression of N-Cor is able to repress TNFα-induced expression of a NF-κB-dependent reporter gene [26].

The mechanism, through which p300/CBP and other coactivators enhance while HDAC corepressors inhibit NF-κB transcriptional activity, is certainly multifactorial, involving direct effect on NF-κB itself (see Section 3) and on other transcriptional factors, as well as changes in chromatin structure. The use of TSA could potentiate the acetyltransferase activity of p300/CBP and of other coactivators and thereby enhance NF-κB-dependent activation.

It is now well established that NF-κB is able to interact with both deacetylases and acetyltransferases but what is the mechanism by which NF-κB switches between association with deacetylases and acetyltransferases? Ghosh and coworkers have provided the demonstration of one mechanism allowing the switch between HDAC-1 recruitment and p300/CBP recruitment [31,48]. More specifically, they have demonstrated that phosphorylation of p65 at serine 276 enhances CBP recruitment, while this phosphorylation decreases the affinity of HDAC-1 for p65 [31]. Upon induction, IκBα degradation activates the catalytic subunit of PKA, which phosphorylates p65 at serine 276 [42,49]. Their results indicate that signal-induced phosphorylation of p65 can determine whether p65 associates with some corepressors or some coactivators.

In addition to recruitment of acetyltransferases and deacetylases through NF-κB itself, other members of the NF-κB activation cascade have been shown to interact with such coregulators. Two subunits of the IKK complex interact with CBP (IKKα and NEMO/IKKγ). More specifically, the interaction between IKKα, a subunit which is not critical for cytokine-induced IκBα degradation, and CBP has been demonstrated by coimmunoprecipitation on overexpressed proteins and on endogenous proteins [50]. In addition, the mammalian two-hybrid system has revealed that IKKα strongly interacts with the amino-terminal transactivation domain of CBP, which is also its binding site for p65 [50]. Consistent with the two-hybrid approach, IKKα binds to a glutathione S-transferase (GST)–CBP fusion protein containing the N-terminus of

CBP [50]. The potential role of this interaction is discussed in Section 4. NEMO/IKKγ, the regulatory subunit of the IKK complex, has been demonstrated to shuttle between the cytoplasm and the nucleus and to interact directly with the nuclear coactivator CBP [51]. NEMO/IKKγ competes with p65 and IKKα for binding to the N-terminus of CBP, inhibiting CBP-dependent transcriptional activation [51]. Thereby, in addition to the key role of NEMO/IKKγ in regulating cytokine-induced IKK activity, its binding to CBP can lead to transcriptional repression of NF-κB transactivation.

IκBα associates, in a NF-κB-independent manner, with HDAC-1 and HDAC-3, but not with HDAC-2, -4, -5 and -6 and partially sequesters these HDACs in the cytoplasm. IκBα, probably via the cytoplasmic sequestration of HDACs, enhances the transactivation potential of several homeodomain-containing proteins such as HOXB7 and Pit-1 [52]. These results thus suggest that IκBα may have new and NF-κB-independent roles.

4. Regulation of diverse functions of p50 and p65 by direct acetylation

The two subunits of the prototype NF-κB heterodimer p50/p65 have been demonstrated to be acetylated in vitro and in vivo (reviewed in [39]).

The p50 subunit, which does not possess a transactivating domain, was first demonstrated to be acetylated in vitro by p300/CBP only in the presence of the HIV-1 viral protein Tat [36]. However, in vivo, in the absence of Tat, overexpression of p300 augments p50 acetylation, and this increase is attenuated by deletion of the p300 acetyltransferase domain [37,38]. It is possible that under physiological conditions, p50 acetylation by p300 also depends on a Tat-like cofactor. In vitro, acetylated p50 binds with higher affinity to DNA containing NF-κB binding sites than the unacetylated form of p50 [36]. In agreement with these data, in vivo, enhanced p50 acetylation correlates with increased p50 binding to the cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) promoters, two NF-κB-regulated promoters [37,38]. This increased p50 binding to DNA correlates with an increased recruitment of p300 to these promoters [37,38]. Furia and coworkers have shown that three lysines, K431, K440 and K441 in p50 are acetylated in vitro [36]. The specific role of acetylation of these residues was not demonstrated. Indeed, they have failed to obtain relevant p50 mutant data because the K431A mutant was very unstable in cells, and the K440A/K441A double mutant was unable to enter the nucleus [36].

In parallel, two independent groups have demonstrated the direct acetylation of the p65 transactivating NF-κB subunit [27,34,35]. Acetylation of p65 was first demonstrated in vivo by [^{3}H]-acetate radiolabeling, which showed that overexpressed p65 is acetylated by endogenous acetyltransferases [27]. Additional studies have revealed that endogenous p65 is also acetylated following stimulation of cells with TNFα or PMA [27,34]. These two groups have demonstrated that p65 is acetylated following overexpression of p300/CBP and that p65 is deacetylated through a specific interaction with HDAC-3 [27,34,35]. In contrast, although p300 acetylates p65 in vivo, recombinant p300 alone fails to acetylate p65 in vitro despite the use of conditions where the acetylation of p53 or of histones is easily detectable [22,31,42,53,54]. This discrepancy could be explained by the absence in vitro of a unknown cofactor required for efficient acetylation. More astonishingly, our laboratory and Wu's laboratory, who have tried to compare the acetylation level of p65 to the acetylation level of other proteins (p53 or p50), have observed in vivo only traces of acetylated p65 [30,37]. These discrepancies could be due to differences in the technical approaches or suggest that the entire complement of nuclear p65 might not be acetylated.

Benkirane's group has demonstrated that acetylation of p65 reduces its ability to bind to DNA, thus facilitates its removal from DNA and consequently its IκBα-mediated export from the nucleus. They have demonstrated that acetylation of p65 does not affect its interaction with IκBα. They have also shown that lysine residues K122 and K123 are the only acetylated residues and that substitution of these lysines with alanines reduces p65-mediated transactivation [34].

The Greene group has demonstrated the acetylation of p65 on three lysine residues: K218, K221 and K310. Acetylation on residue K221 enhances the DNA-binding activity of p65 and impairs its assembly with IκBα and, therefore, impedes IκBα-dependent nuclear export of the NF-κB complex, thereby allowing the prolongation of the NF-κB response [27,35]. Acetylation at lysine K310 in p65 is required for its full transcriptional activity [35].

Greene laboratory and Benkirane laboratory did not at all identify the same lysine residues as mediating the in vivo acetylation of p65 [34,35]. This is surprising because, according to Benkirane, mutations of lysines K122 and K123 are sufficient to abrogate p65 acetylation [34]. Moreover, the functions assigned to p65 acetylation are in complete opposition in these two studies. The observations by Chen et al. [27] can explain partially the transcriptional synergism observed following deacetylase inhibition and NF-κB-induction. Conversely, the data from Kiernan et al. [34] are not in agreement with this synergism. Indeed, their data indicate that the acetylation of p65 does not enhance NF-κB-dependent activation, but that in contrast, direct acetylation of p65 negatively regulates NF-κB transcriptional activity by lowering binding of NF-κB to DNA and by facilitating its removal and export by IκBα [34].

The reasons for these discrepancies are not clear at this time. It should be noted that many of the experiments performed to study the acetylation of p65 involve the

overexpression of proteins and the use of NF-κB mutant proteins [27,34,35]. Some results obtained following overexpression of mutants has to be interpreted carefully. Lysines K122 and K123 are the only p65 residues that contact the DNA in the minor groove. Therefore, the use of p65 mutants to study the role of acetylation of lysines K122 and K123 in terms of binding to DNA might be difficult to interpret. In conclusion, the role of p65 acetylation should be clarified in the future by further in vivo approaches using endogenous proteins.

5. Co-existence of two distinct classes of NF-κB-dependent genes: those constitutively accessible by NF-κB and those requiring previous chromatin modifications

Natoli's laboratory has demonstrated by Chromatin immunoprecipitation assay that recruitment of NF-κB to target genes occurs in two temporally distinct phases [40]. A subset of target genes, whose promoter is already heavily acetylated before stimulation, is constitutively and immediately accessible to NF-κB and is transcribed immediately after NF-κB recruitment. In contrast, other target genes are not immediately accessible to NF-κB and require stimulus-dependent modifications in their chromatin structure to render NF-κB sites accessible [40]. This late NF-κB recruitment to target promoters is preceded by stimulus-induced histone H4 hyperacetylation [40]. This suggests that a complex containing no NF-κB initially forms on these promoters and directs hyperacetylation, thereby making the chromatin subsequently accessible to NF-κB. Since TSA provokes a global histone hyperacetylation, these observations are consistent with a potentiation of NF-κB-dependent transactivation in the presence of TSA.

Promoters, that require active chromatin modifications to efficiently recruit NF-κB, will be activated if two conditions are fulfilled: first, stimulation has to activate in parallel a pathway required to induce promoter modification and second, NF-κB has to persist in the nucleus long enough to come into contact with the late available binding sites. The second condition can be regulated through IKK persistent activation via unknown acetylation events (see Section 1) or by direct p65 acetylation (see Section 3). In agreement with the first condition, different reports have demonstrated the activation of parallel pathways provoking phosphorylation and acetylation at specific residues of histones locally associated with NF-κB-dependent promoters [50,55,56].

Upon cellular stimulation, the IKK complex is activated. Both IKKβ and IKKα possess a kinase activity but only IKKβ is critical for the classical cytokine-induced IκBα degradation. Nevertheless, mouse embryo fibroblasts deficient in IKKα are defective in the induction of NF-κB-dependent transcription, indicating a role for IKKα in NF-κB-dependent transactivation [57–59]. Two groups have demonstrated that upon stimulation, IKKα is recruited to NF-κB-responsive promoters and mediates the cytokine-induced phosphorylation at serine 10 of histone H3, which is subsequently acetylated at lysine 14 [50,56]. IKKα is able to directly phosphorylate histone H3 [50,56] and to interact with CBP, which could promote the subsequent acetylation of H3 lysine 14 [50].

Because, in response to cytokines, the p38 MAP kinase is activated in parallel with IKK, and because p38 and IKK are required for the induction of a subset of NF-κB-dependent genes, it is possible that these two kinases may deliver signals that modulate the accessibility to selected NF-κB sites in the genome. In this regard, Natoli's laboratory has demonstrated that p38 is able to enhance the accessibility to the NF-κB binding sites found in a subset of NF-κB-dependent promoters [55]. Promoters that recruit NF-κB in a p38-regulated manner undergo a rapid and transient p38-dependent chromatin modification: histone H3 phosphorylation at serine 10 [55]. Serine 10 phosphorylation promotes the subsequent acetylation reaction at H3 lysine 14 and this correlates with transcriptional activation [60,61].

6. Conclusion

Following treatment with cytokines such as TNFα, NF-κB migrates transiently into the nucleus where it activates a wide variety of NF-κB-regulated genes. Two distinct classes of NF-κB-activable genes coexist, the constitutively accessible NF-κB-dependent genes, associated with hyperacetylated histones, and rapidly activated, and those requiring previous chromatin modifications (such as acetylation) and induced later [40]. Thereby, the regulation of NF-κB duration in the nucleus is a major point. The intranuclear duration of NF-κB is of course important for the amplitude of the NF-κB-dependent activation, but above all this duration has to be long enough to allow activation of NF-κB-dependent genes requiring different chromatin modification steps before NF-κB binding itself. The duration of NF-κB in the nucleus can be modulated by undefined acetylation events prolonging IKK activity, and thereby leading to a prolonged NF-κB presence and DNA-binding in the nucleus [30] or, by direct p65 acetylation impeding [34] or facilitating [27] (depending on the report, see Section 3) the post-induction turn-off of NF-κB-mediated transcription. In addition, the association of NF-κB with both HATs and HDACs is also an important level of regulation of NF-κB-dependent transactivation. The exact role of these associations is not completely defined yet, but these associations allow probably the regulation of the acetylation level of NF-κB itself, of other transcriptional factors bound to the NF-κB-dependent promoters, and of the local nucleosomal structure. Finally, the NF-κB subunits p50 and p65 are subjected to direct acetylation. Acetylation of p50 increases its binding to

DNA [36–38], whereas acetylation of p65 regulates different functions of NF-κB, including transcriptional activation, DNA-binding affinity and IκBα assembly [27,34,35,39]. Deacetylase inhibition following TSA treatment favours global hyperacetylation of all cellular acetylable proteins. The transcriptional synergism observed following cotreatment with NF-κB inducers (such as TNFα) and deacetylase inhibitors (such as TSA) reflects these multiple levels of acetylation-dependent regulation of NF-κB-mediated transactivation.

From the studies reviewed here, it is clear that there is a lot that remains to be understood about acetylation-dependent transcriptional regulation of NF-κB. How the different HATs and HDACs are specifically recruited to the NF-κB pathway? Among NF-κB-dependent genes, does it exist a gene specificity and if so, how is it accomplished? Is such a complexity of acetylation-dependent regulation a particularity of the NF-κB pathway or is it a more general regulation mechanism of a broader set of transcription factor pathways? HDACi are emerging as a new class of anticancer agents for the treatment of solid and hematological malignancies but little is known about the molecular events that control their effectiveness [62–64]. Thereby, it will be interesting to investigate the role of acetylation-dependent regulation of the NF-κB cascade in this context. It is hoped that, in the coming years, answers to these and other questions will provide many more exciting insights into the mechanisms of acetylation-dependent transcriptional regulation.

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