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Review

Posttranslational modifications of NF-κB: Another layer of regulation for NF-κB signaling pathway

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ABSTRACT

The eukaryotic transcription factor NF-κB regulates a wide range of host genes that control the inflammatory and immune responses, programmed cell death, cell proliferation and differentiation. The activation of NF-κB is tightly controlled both in the cytoplasm and in the nucleus. While the upstream cytoplasmic regulatory events for the activation of NF-κB are well studied, much less is known about the nuclear regulation of NF-κB. Emerging evidence suggests that NF-κB undergoes a variety of posttranslational modifications, and that these modifications play a key role in determining the duration and strength of NF-κB nuclear activity as well as its transcriptional output. Here we summarize the recent advances in our understanding of the posttranslational modifications of NF-κB, the interplay between the various modifications, and the physiological relevance of these modifications.

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1. Introduction

The eukaryotic transcription factor NF-κB/Rel family proteins regulate a wide range of host genes that govern the inflammatory and immune responses in mammals and play a critical role in controlling programmed cell death, cell proliferation and differentiation. In mammals, the NF-κB/Rel family consists of seven proteins, including RelA/p65, c-Rel, RelB, p100, p52, p105 and p50 [1,2]. Each protein contains a Rel homology domain (RHD) within the N-terminus and can form homo- or heterodimers through the RHD [1,2].

The prototypical NF-κB is a heterodimer of p50 and RelA. In unstimulated cells, NF-κB is sequestered in the cytoplasm by its association with an inhibitor protein, IκBα [1,2]. NF-κB is activated by a variety of stimuli, including various proinflammatory cytokines, T- and B-cell receptor signals, and viral and bacterial products. Stimulation of the cells by these agonists leads to the activation of an IκB kinase complex of IκB kinases 1 and 2 (IKK1 and 2, also known as IKKα and IKKβ, respectively) and the non-catalytic NEMO subunit [3]. Activated IKKs then phosphorylate IκBα at serines-32 and -36, inducing its rapid ubiquitination and its degradation in the 26S proteasome [4]. The free NF-κB heterodimer rapidly translocates to the nucleus where it binds to the κB enhancer and stimulates gene expression through the transcriptional activation domain (TAD) of RelA [5]. NF-κB activates hundreds of genes involved in different biological processes including inflammation, proliferation and cell survival.

Many factors have been discovered to contribute to the transcriptional activation of NF-κB target genes, including the binding of different homo- or heterodimers of NF-κB to the cognate κB sites, the recruitment of various basal transcriptional factors and coactivators to the promoters, and the modifications of the histone tails around the promoters of NF-κB target genes [6]. Recent studies indicate that posttranslational modifications of NF-κB, especially of the RelA subunit, play a critical role in fine-tuning the transcriptional activity of NF-κB, adding another important layer of complexity to the transcriptional regulation of NF-κB. In the present review, we will focus on the posttranslational modifications of the RelA subunit of NF-κB, the regulation of these modifications, and the functions of these modifications in the NF-κB-mediated inflammatory response and cancer. Posttranslational modifications of other NF-κB members may be found in other recent reviews [6–8].

2. Posttranslational modifications of NF-κB

2.1. Phosphorylation of RelA

A role for phosphorylation of RelA in the regulation of NF-κB activity has long been suggested [9]. Accordingly, many kinases and phosphorylation sites including seven serines and three threonines have been identified (Fig. 1A). RelA can be phosphorylated both in the cytoplasm and in the nucleus in response to a variety of stimuli. Most of the phosphorylation sites are within the N-terminal RHD and the C-terminal transcriptional activation domains. Phosphorylation of these sites results in either increased or decreased levels of transcription, depending on the sites of phosphorylation, the target genes, and the stimuli.

2.1.1. Phosphorylation of serine 276

Phosphorylation of serine (S) 276 within the RHD was first identified to be mediated by the catalytic subunit of protein kinase A (PKAc) which is activated by lipopolysaccharide (LPS). LPS-induced degradation of IκBα releases the constitutively active PKAc from the constraints of IκBα [10]. Later on, several stimuli, including TNF-α- and TGF-β, were also shown to activate PKAc and induce PKAc-mediated phosphorylation of S276 [11,12]. Phosphorylation of S276 in response to these various stimuli enhances the overall transcriptional activity of NF-κB. S276 is also targeted by mitogen- and stress-activated protein kinase-1 (MSK1) in response to TNF-α, IL-1β, respiratory syncytial virus (RSV) and *Helicobacter pylori* infection [12–15]. Different from PKAc, MSK1 phosphorylates RelA in the nucleus [13]. Phosphorylation of S276 by MSK1 enhances the transcriptional activity of NF-κB and the NF-κB-dependent expression of cytokines including IL-6 and IL-8 [12–15].

The enhanced transcriptional activity of RelA via S276 phosphorylation likely reflects the phosphorylation-induced RelA conformational change, leading to the increased or decreased binding of RelA with NF-κB cofactors. For example, phosphorylation of S276 by PKAc has been shown to weaken the intramolecular interaction between the N- and C-terminal portions of RelA, which in turn allows the efficient binding of p300/CBP to the open RelA [16]. In another study, phosphorylation of S276 by PKAc has been shown to enhance the interaction between RelA and cyclin-dependent kinase 9/Cyclin T1 complexes [17]. Similarly, phosphorylation at S276 by MSK1 facilitates the recruitment of CBP to the promoters of NF-κB target genes [14]. Therefore, the phosphorylation-mediated conformational change appears to enhance the binding of RelA to its cofactors, and these cofactors might form a bridge between NF-κB and the components of the cellular transcriptional machinery. In contrast, the unphosphorylated RelA conformation might facilitate its association with co-repressors, leading to the decreased transcriptional activity of NF-κB. In a recent study with RelA "knock-in" mice expressing RelA-S276A, Dong et al. demonstrate that the unphosphorylated form of RelA associates with HDAC3, which suppresses not only a subset of NF-κB target genes but also represses non-NF-κB-regulated genes through an epigenetic mechanism [18]. In addition to the phosphorylation-dependent recruitment of coactivators or disengagement of co-repressors, it is possible that phosphorylation of S276 regulates the function of NF-κB via other mechanisms. A recent report shows that phosphorylation of S276 by kinase Pim-1 in response to TNF-α enhances the transcriptional activation of NF-κB target genes by stabilizing RelA [19], but the detailed mechanism remains unclear.

2.1.2. Phosphorylation of serine 536

Another well-studied phosphorylation site of RelA is serine 536 within the TAD. S536 is targeted for phosphorylation under various conditions by different kinases including IKKs, ribosomal subunit kinase-1 (RSK1), and TANK binding kinase (TBK1) [20–24] with different functional consequences (Fig. 1A). The IKKs-mediated phosphorylation of S536 is induced by TNF-α, LPS, *H. pylori* and human T lymphotrophic virus-1 (HTLV1)-encoded TAX protein [20–22,24,25]. The enhanced transcriptional activity of NF-κB after phosphorylation of S536 might also result from the conformational change of RelA affecting RelA's interaction with other proteins. Supporting this idea, phosphorylation of S536 by IKKs has been shown to increase RelA's binding with p300 but decrease its binding with co-repressor SMRT (silencing mediator for retinoic acid receptor and thyroid hormone receptor) [26,27]. In addition, RSK1- or TBK1-mediated phosphorylation of S536 lowers RelA's affinity for IκBα and decreases IκBα-mediated nuclear export of NF-κB [22,23]. Although it is well accepted