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RESEARCH HIGHLIGHT

# Talking to histone: methylated RelA serves as a messenger

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The mammalian NF-κB family of transcription factors functions as a major regulator of innate and adaptive immunity and inflammatory responses, plays critical roles in controlling cell proliferation, differentiation and apoptosis by regulating a wide range of genes that govern these various processes [1]. The prototypical NF-κB (p50 and RelA heterodimer) is sequestered and inactivated in the cytoplasm by a family of inhibitors, called IκBs, in unstimulated cells. NF-κB is activated in response to a variety of stimuli, including inflammatory cytokines and viral and bacterial infections, which lead to the activation of the IκB kinase complex (IKK), phosphorylation and degradation of IκBs, and the nuclear translocation of the NF-κB heterodimer.

In addition to the cytoplasmic events required for the activation of NF-κB, it has been well documented that post-translational modifications of RelA in the nucleus, including phosphorylation and acetylation, also play a crucial role in NF-κB activation by dictating the duration and strength of the nuclear NF-κB action, adding another layer of regulation to NF-κB activation [2]. Recently, lysine (Lys, K) methylation has also emerged as an important posttranslational modification in the regulation of NF-κB function. RelA was first reported to be monomethylated by the SET domain-containing methyltransferase Set9 at Lys314 and Lys315 in response to TNF-α or LPS [3, 4]. Methylation of these two lysines negatively regulates the function of NF-κB by inducing the ubiquitination and degradation of promoter-bound RelA [3]. In response to TNF-α, Set9 can also methylate RelA at Lys37, which stabilizes the binding of NF-κB to its enhancers for the activation of a subset of NF-κB target genes [5]. Other stimulus-dependent methylations of RelA at Lys218 and Lys221 by nuclear receptor-binding SET domain-containing protein 1 (NSD1) were reported to enhance the transcriptional activity of NF-κB and the expression of NF-κB target genes [6]. It appears that like RelA phosphorylation and acetylation, lysine methylation occurs at multiple sites in response to different stimuli, and methylation of these various lysines differentially controls the distinct functions of NF-κB.

In contrast to the above mentioned stimulus-coupled methylation events, a very recent study by Levy and co-workers has shown that under unstimulated conditions a chromatin-bound population of RelA is subject to methylation by the SET domain-containing methyltransferase SETD6. SETD6-mediated methylation attenuates NF-κB gene expression by establishing a G9a-like protein (GLP)-mediated repressed chromatin state within NF-κB target genes [7].

In a screen of over 40 human SET domain containing lysine methyltransferases, Levy *et al.* found that SETD6 specifically monomethylated RelA at Lys310 (RelA-K310me1). Interestingly, RelA-K310me1 was only detected in the chromatin-associated fraction of the nucleus, and it occupied the promoters of a subset of NF-κB target genes under basal conditions. Unlike the methylations of other RelA lysines, which are induced by TNF-α stimulation, TNF-α dramatically decreased the RelA-K310me1 level.

Functional analysis of RelA-K310me1 revealed a repressive role for SETD6-mediated RelA-K310me1 in NF-κB activation, since the depletion of SETD6 by RNAi led to higher levels of both basal and TNF-α-induced expression of NF-κB genes, and the over-expression of SETD6 negatively regulated the RelA-dependent tumorigenic potential of transformed cells.

By screening CADOR protein microarrays, Levy *et al.* identified the ankyrin repeats of the histone methyltransferase GLP as the only protein motif that recognizes RelA-K310me1. The ankyrin repeat is one of the conserved domains that have been identified to specifically recognize methylated

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lysines [8]. In addition to the ankyrin repeats, GLP also contains a C-terminal SET domain that can methylate H3K9 (H3K9me1 and H3K9me2); thus, this protein, usually in a complex with G9a, can both read and write histone methylation marks, characteristic of silent euchromatin [8]. Further experiments demonstrated that GLP bound to chromatin-associated RelA-K310me1 in unstimulated cells in a SETD6-dependent manner, and that TNF-α disassociated the RelA-bound GLP, resulting in decreased H3K9me1 and H3K9me2.

To study the regulation of the RelA-K310me1, Levy *et al.* examined the effect of the PKC-ζ-mediated phosphorylation of Serine (Ser, S) 311, which is located next to Lys310 and has been shown to play an opposing role in NF-κB activation [9]. They found that the ability of GLP to bind to RelA-K310me1 was abolished by Ser311 phosphorylation. Further evidence showed that phosphorylation of Ser311 by overexpressed PKC-ζ prevented the interaction of endogenous RelA and GLP and that SETD6-mediated down-regulation of NF-κB target gene expression could be antagonized by overexpressed PKC-ζ in a GLP-dependent manner. These data indicate that during activation of NF-κB, phosphorylation of Ser311 plays a dominant role over monomethylation of Lys310 by disrupting the association of GLP with methylated RelA.

The study by Levy and co-workers identified a novel crosstalk between NF-κB and chromatin for the regulation of NF-κB target genes. SETD6-methylated RelA at Lys310 serves as the messenger for the establishment of the crosstalk by recruiting GLP, which leads to the methylation of histone H3K9 and the resultant formation of a transcriptionally repressed state within the local chromatin. The coordinated crosstalk be-

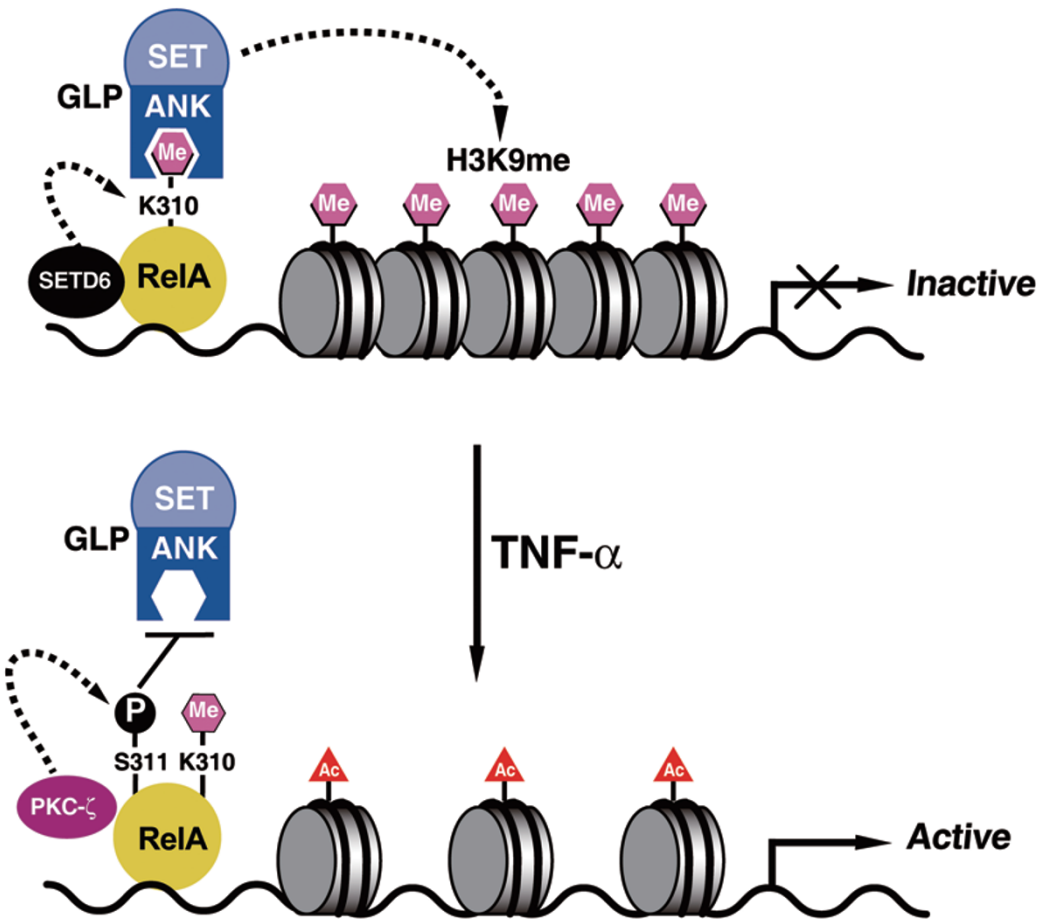


**Figure 1** Regulation of NF-κB by SETD6-mediated methylation of RelA. In resting cells SETD6 binds to and monomethylates chromatin-bound RelA at Lys310 (K310); consequently, the methylation (Me) mark helps recruit ankyrin repeat (ANK)-containing histone methyltransferase GLP, which subsequently methylates histone H3K9 via its SET domain, leading to condensation of local chromatin and repression of NF-κB genes. Upon TNF-α stimulation, PKC-ζ induces phosphorylation (P) of Ser311 (S311), which then prevents the recruitment of GLP, methylation of histone H3K9 and the formation of condensed chromatin. The replacement of the methylated H3K9, likely by acetylation (Ac), allows the expression of NF-κB target genes.

tween methylated RelA and histone H3 maintains the repressed state of NF-κB target genes in unstimulated cells. Upon stimulation, PKC-ζ-mediated RelA phosphorylation at Ser311 likely blocks the methylation at Lys310 and the subsequent binding of GLP, resulting in the recruitment of NF-κB co-activators and the change in the chromatin environment, which readies it for transcription of NF-κB genes (Figure 1).

Since constitutively activated NF-κB is often found in disease conditions such as rheumatoid arthritis and cancers, understanding how NF-κB is inactivated and maintained in the inactive state is as important as understanding how NF-κB is activated. While the study by Levy *et al.* provides a regulatory mechanism for the inactivation of NF-κB target genes under unstimulated conditions, it also raises some intriguing and unanswered questions. For example, what role does p50 play in this methylated RelA-mediated repression of NF-κB target genes? Homodimers of nuclear p50 have long been shown to play an important role in maintaining the repressed state of NF-κB genes by the recruitment of histone deacetylases (HDACs) [10]. Does methylated RelA-mediated repression function as an additional or an alternative context-dependent mechanism? TNF-α treatment decreases RelA-K310me1. Does this apparent reduction in RelA-K310me1 result from an inability of the anti-methylated RelA antibodies to recognize the methylated RelA when Ser311 is phosphorylated, or does it result from a demethylation reaction by an unknown demethylase? Since acetylation of RelA at Lys310 is critical for the transcriptional activation of NF-κB [11], it is reasonable to expect that RelA-K310me1 needs to be removed by a demethylase before p300/CBP could actually access Lys310. However, since this methylated RelA only represents a very small fraction of the total NF-κB within the cell, it is also possible that methylated RelA and acetylated RelA represent different pools and transcriptionally competent states of NF-κB. The role that posttranslational modifications of RelA play in the epigenetic regulation of gene expression is well known from the study of the phosphorylation of Ser276 [12]. In addition to the recruitment of transcriptional machinery to the promoters to activate gene expression, NF-κB, especially when posttranslationally modified, can also create a link to histone tails, whose modifications have emerged as an important regulatory step for the epigenetic control of gene expression and development. Phosphorylation of Ser276 facilitates the recruitment of p300/CBP, which then acetylates RelA and nearby histones, leading to NF-κB gene expression [13]. The current study by Levy *et al.* provides another example of how posttranslationally modified RelA communicates with histones. Since RelA is subject to a variety of posttranslational modifications, it will be of great interest to investigate whether other modifications could similarly establish such a crosstalk with histones for the expression of spatiotemporally regulated NF-κB target genes.

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