Published in final edited form as:

Nat Immunol. 2011 January ; 12(1): 12–14. doi:10.1038/ni0111-12.

Fine tuning NF- κ B: new openings for PKC- ζ

Jorge Moscat and Maria T Diaz-Meco

University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

Jorge Moscat: jorge.moscat@uc.edu

Abstract

NF- κ B is a critical transcription factor that is regulated by several post-transcriptional modifications. The characterization of their roles would help in the design of new therapeutic targets in cancer and inflammation.

A multitude of studies have established the critical role of NF- κ B in the control of key physiological and pathological states, from immunity and inflammation to cancer¹. Regulation of the so-called ‘canonical’ NF- κ B transcription factor by the IKK complex involves its cytosolic-to-nuclear translocation mediated by the phosphorylation and subsequent proteasomal degradation of the inhibitory molecule I κ B α ¹. However, in this issue of *Nature Immunology*, Levy and co-workers describe a previously unknown mechanism in which basal and activated NF- κ B is controlled through the methyltransferase SETD6-mediated methylation of Lys310 of RelA (also called p65), the transactivating subunit of the NF- κ B complex². This event represses chromatin in the region of several NF- κ B-dependent genes and ensures that they are not transcribed unless cells are activated by the appropriate stimuli that trigger phosphorylation of Ser311 by the atypical protein kinase PKC- ζ ². These are very important observations, because they demonstrate a new layer of complexity in the regulation of NF- κ B transcriptional activity through chromatin remodeling and establish PKC- ζ as a critical mediator of the chromatin changes necessary for effective transcription of κ B-dependent genes (Fig. 1).

It is not surprising that the nuclear actions of NF- κ B, a critical transcriptional regulator that controls the expression of thousands of genes, can be modulated and fine-tuned by mechanisms in addition to its all-or-nothing nuclear translocation. It is also known that even in the absence of stimuli, there are NF- κ B subunits in the nucleus that are bound to gene-regulatory elements in the chromatin and that must be kept in check to prevent undesired, uncontrolled activity. Indeed, pioneering experiments established that dimers of the NF- κ B subunit p50 associated with histone deacetylase sit on κ B elements, which serves to deacetylate histones, therefore leading to closed, repressive chromatin that prevents gene expression under basal conditions³. After degradation of I κ B α , p50-RelA heterodimers are released from the inhibitory complex and RelA is phosphorylated at Ser276 by protein kinase A and/or mitogen- and stress-activated protein kinase, promoting interaction of RelA with the transcriptional coactivator CBP³. This interaction results in more CBP-mediated acetylation of Lys310, which is apparently also important for transcriptional activation⁴ and for the acetylation of histones by CBP; this results in the generation of an open, permissive chromatin structure, leading to full transcriptional activity of the NF- κ B complex, which replaces the inactive p50-p50 homodimers in the κ B-regulatory elements³. However,

phosphorylation of RelA at Ser276 is not sufficient to promote binding of CBP, as Ser311 also needs to be phosphorylated in response to tumor necrosis factor (TNF)⁵. This serine residue is specifically targeted by PKC- ζ , which has been shown through genetic manipulations to be required for full NF- κ B transcriptional activity *in vivo* and in cell culture⁶ (Fig. 1). Interestingly, Ser276 and Ser311 both reside in the Rel-homology-dimerization domain. Ser311 is in close proximity to Lys310, and it is unclear whether phosphorylation of Ser311 by PKC- ζ modulates the acetylation of Lys310. Nonetheless, Levy *et al.* show here that it affects Lys310 methylation, which has an effect on the transcriptional activity of many NF- κ B target genes². Their study stems from an unbiased screening of human protein lysine methyltransferases, in which they discover that one of these, SETD6, monomethylates chromatin-associated RelA at Lys310. A series of elegant biochemical experiments shows that methylated RelA resides in a histone H3-rich region near the promoters of several NF- κ B target genes. This finding is important because it suggests that methylated RelA might have a repressive role in the control of gene expression under basal conditions. Indeed, knockdown of SETD6 gives rise to more κ B-dependent transcription under basal conditions and, notably, under stimulated conditions as well. Thus, TNF activation correlates with lower abundance of methylated Lys310, which indicates that demethylation of this residue is a prerequisite for the expression of NF- κ B target genes under activating conditions. The pathophysiological relevance of these findings is confirmed by experiments in which a lower abundance of SETD6 in transformed cells enhances their tumorigenic potential *in vitro* and *in vivo*², consistent with a role for NF- κ B in cancer⁷. Furthermore, Levy *et al.* show that blood mononuclear cells from patients with rheumatoid arthritis or juvenile idiopathic arthritis have much lower expression of *SETD6* mRNA, which suggests the importance of this newly identified pathway in human inflammatory disease².

Once these authors establish the functional relevance of this previously unknown covalent modification of RelA, they go on to determine the precise mechanism whereby RelA methylated at Lys310 controls transcription². They again do an unbiased screen, this time looking for protein motifs that could potentially interact with RelA methylated at Lys310. They find a positive 'hit' in the ankyrin-repeat domain of GLP, which selectively interacts with this modified RelA with an affinity similar to that of the positive control, histone H3 methylated at Lys9 (ref. 2). Interestingly, GLP, along with its partner G9a, are known to methylate histone H3 at Lys9 in chromatin regions with repressed transcription⁸. Consistent with a role for this event in NF- κ B function, chromatin-immunoprecipitation assays show that GLP and methylated histone H3 are lower in abundance at the promoters of canonical κ B-responsive genes after stimulation with TNF and that knockdown of SETD6 results in less interaction of GLP with RelA². Collectively, all these results suggest a model whereby activation of the NF- κ B signaling cascade leads to the release of GLP, which results in the opening of chromatin, allowing efficient activation of κ B-dependent gene transcription.

The next big issue that remains to be addressed is identification of the precise signal that triggers the whole process. Here, PKC- ζ enters the picture; PKC- ζ , along with PKC- λ and PKC- ι , forms the atypical PKC subfamily. In contrast to the classical and novel PKC isoforms, proteins of the atypical PKC subfamily are insensitive to lipids and Ca²⁺ but interact with protein regulators and adaptors through a newly described PB1 protein-protein interaction domain⁹. Levy *et al.* reason that the close proximity of Ser311, a target site for PKC- ζ phosphorylation in response to TNF⁵, to Lys310 might mean that methylation of RelA could be influenced by the PKC- ζ -mediated phosphorylation of Ser311 (ref. 2). Interestingly, through biochemical and cellular studies of PKC- ζ -deficient cells, they find that phosphorylation of Ser311 by PKC- ζ in response to TNF stimulation does in fact lead to displacement of GLP from RelA and therefore to more transcription of κ B-dependent genes. Consistent with published results⁶, Levy *et al.* find that PKC- ζ -deficient cells have lower

production of inflammatory cytokines². Therefore, phosphorylation of RelA at Ser311 by PKC- ζ , in addition to favoring histone acetylation by allowing the recruitment of CBP, also inhibits histone methylation by preventing the interaction of GLP with RelA. This finding highlights the importance of the 'hot spot' at positions 310–311 and the relevance of PKC- ζ in its control (Fig. 1). Interestingly, the regulation of histone methylation by NF- κ B has been shown before to also occur as a consequence of activation of this transcription factor. Studies have shown that JMJD3, which demethylates histone H3 at Lys27, is expressed in macrophages activated by lipopolysaccharide¹⁰. It is believed that this enzyme, by erasing a critical histone mark, controls macrophage differentiation during chronic inflammation¹⁰. Therefore, a model emerges whereby histone-methylation interaction in NF- κ B signaling takes place by two mechanisms: one in which the synthesis of at least one histone demethylase is promoted, and another in which RelA itself is methylated, thus recruiting GLP, which is antagonized by TNF-activated PKC- ζ .

Like every breakthrough, these new findings solve many mysteries but also raise several questions. For example, what is the subunit composition of the NF- κ B complex that contains methylated RelA at the chromatin? Is there a methylated RelA-RelA homodimer or is it in fact a p50-RelA heterodimer? Is this mechanism applicable to all κ B-dependent genes or just those with a more closed promoter conformation? Published results suggest that PKC- ζ directly phosphorylates IKK β *in vitro*¹¹ and that PKC- ζ is required for activation of IKK β *in vivo*⁶. It will be important to define the cell types in which PKC- ζ acts upstream of IKK β and those in which it controls RelA transcriptional activity *in vivo*. Knock-in mouse lines expressing loss-of-function mutations of the sequence encoding Lys310 and Ser311 would help identify the physiological relevance of these modifications, as well as their importance in various diseases to which NF- κ B has been linked. In this context, it will be of great interest to analyze samples from human patients with cancer to determine whether there are alterations in gene expression and/or mutations in the genes encoding SETD6 or GLP and whether there are changes in the acetylation or methylation of Lys310 and/or phosphorylation of Ser311. All these analyses would help establish the pathway identified here as a potential new source of therapeutic targets in the treatment of cancer and inflammatory diseases.

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Figure 1.

PKC- ζ -mediated phosphorylation of RelA regulates transcription. Under basal conditions, RelA (p65) is methylated (Me) at Lys310 by SETD6, promoting recruitment of GLP and methylation of histones, which leads to closed chromatin and inhibited transcription of κ B-dependent genes. After activation of cells by TNF, PKC- ζ phosphorylates Ser311, leading to release of GLP, demethylation of Lys310 and recruitment of CBP to the phosphorylated RelA, which promotes acetylation of Lys310 and that of histones that, together with their diminished methylation, results in enhanced transcription.