Post-translational modification of RelA(p65)
NF-κB

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Abstract
Stimulation with diverse agents activates the NF-κB (nuclear factor κB) transcription factor, affecting inflammatory and immune responses, proliferation, differentiation, apoptosis and tumourigenesis. Determining how NF-κB elicits such distinct responses is essential to understanding NF-κB function in diseased tissues. Recent developments illustrating that post-translational modification of NF-κB subunits influences their nuclear role are discussed. These observations suggest that diagnosis and new therapies based on reprogramming NF-κB activity could be more efficient than total NF-κB inhibition.

NF-κB (nuclear factor κB) is a pleiotrophic transcription factor
NF-κB is a sequence-specific transcription factor activated by a bewildering array of stimuli including biological agents such as bacterial lipopolysaccharide and inflammatory cytokines, phorbol esters and cytotoxic stimuli such as chemotherapeutic drugs, UV light and ionizing radiation [1]. There are five members of the mammalian NF-κB family, RelA(p65), RelB, c-Rel, p105/p50 and p100/p52, all of which contain homologous N-terminal RHDs (Rel homology domains). NF-κB functions as a homo- or heterodimer of these subunits, with the RHD mediating dimerization, DNA binding, nuclear localization and interaction with the inhibitor of NF-κB (IκB) proteins. Generally, NF-κB is found in the cytoplasm of resting cells, anchored to IκB. The rate-limiting step of NF-κB activation therefore involves its release from IκB, allowing NF-κB to translocate to the nucleus.

NF-κB is activated in response to hundreds of different stimuli and in turn regulates hundreds of diverse target genes. But how is specificity of NF-κB function achieved? Understanding the complexity of the NF-κB response, and the mechanisms that modify it, is critical if we are to comprehend and ultimately exploit the different roles NF-κB plays in normal tissue and diseases such as cancer. In particular, the ability of anti-cancer therapies (daunorubicin, etoposide, ionizing radiation), tumour initiators (UV, phorbol esters), tumour suppressor proteins (ARF and p53) and oncogenes (Ras and Bcr-Abl) to regulate NF-κB activity, through these modifications, integrates cellular signals into a specific transcriptional response. Some are known to trigger an anti-apoptotic or oncogenic NF-κB, whereas others activate an NF-κB that helps to induce apoptosis, consistent with a tumour-suppressor function [2].

Phosphorylation regulates RelA
These observations suggest greater complexity than that afforded by nuclear translocation of NF-κB after its release from IκB. Admittedly, distinct NF-κB dimer combinations allow some specificity, with differing affinities for binding sites in promoters of NF-κB target genes. However, with most of the responses described above, an important role has been ascribed to the RelA subunit, suggesting that its regulation is critical. Although RelA has been intensively studied in the context of the inflammatory cytokines TNF (tumour necrosis factor) and interleukin-1, recent work from our laboratory has revealed a more dynamic role for nuclear RelA with other stimuli. Interested by the reported ability of NF-κB DNA binding induced by anti-cancer therapies to assist, or prevent apoptosis in different contexts, we investigated the function of RelA in response to cytotoxic stimuli such as daunorubicin and short-wavelength UV (UV-C) or expression of the ARF tumour suppressor. Surprisingly, we found that under these circumstances, RelA bound to the promoters of specific target genes and actively repressed rather than induced transcription. Mechanistically, this resulted from RelA-dependent recruitment of HDACs (histone deacetylases) and subsequent deacetylation of specific promoters [3,4].

Key words: chemotherapy, nuclear factor κB (NF-κB), post-translational modification, RelA, transcription, UV light.

Abbreviations used: HDAC, histone deacetylase; NF-κB, nuclear factor κB; IκB, inhibitor of NF-κB; PK, protein phosphatase, RHD, Rel homology domain; TA1, transactivation domain 1; TNF, tumour necrosis factor α.

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Figure 1 UV-C, daunorubicin and TNF alter the mobility of RelA in one-dimensional SDS/PAGE
U-2 OS cells were stimulated with 40 J/m² UV-C for 3 h, 1 µM daunorubicin for 3 h or 10 ng/ml TNF for 30 min as indicated. Nuclear protein extracts were prepared, analysed by one-dimensional SDS/PAGE and Western-blotted with an antibody to RelA.

(Ser-276 and -311, reviewed in [6]), increased RelA nuclear localization and stability (through Pin-1 isomerization of the bond joining phosphorylated Thr-254 to Pro-255, [7]) or increasing net negative charge of an acidic activation domain shown to bind basal transcription components (Ser-529 and -536, also reviewed in [6]). In contrast, de-phosphorylation of Thr-435 by PP4 (protein phosphatase 4) in response to the chemotherapeutic drug cisplatin increases the transcriptional activity of RelA [8]. We found that the ARF tumour suppressor induced phospho-threonine modification of RelA and repressed RelA transcriptional activity, in a Thr-505-dependent manner [4]. Together, these results suggest that phosphorylation of Thr-435 and -505 inhibits RelA transcriptional activity. The situation is even more complex, with evidence that in an unstimulated cell at least nine residues of RelA are phosphorylated [9]. In addition, more activating phosphorylations have been proposed in the RelA RHD mediated by p21ras and PKCζ [9], in response to the phorbol ester PMA between residues 286 and 470 [10], and in TA1 (transactivation domain 1) or TA2 by CaMKIV [11]. RelA de-phosphorylation has also been implicated in the repression of transcription. PP2A interacts with and de-phosphorylates RelA [12], and SV40 small T antigen inhibition of PP2A activity enhances the activity of RelA [13]. These studies suggest that phosphorylation and de-phosphorylation events control the activity and function of RelA.

Acetylation and ubiquitination provide further mechanisms of control
RelA associates with both histone acetyltransferases and HDACs [6] and reversible acetylation of RelA itself has been described. PMA-inducible acetylation of Lys-122 and -123 by p300 and PCAF reduces RelA binding to DNA, mediating post-activation turn-off of NF-κB [14]. In response...
to TNF, p300 and CREB-binding protein acetylate Lys-218, -221 and -310, conferring transcriptional activation of RelA in a number of ways [6]. Acetylation of Lys-218 weakens RelA interaction with IκBα, modification of Lys-221 also impairs assembly with IκBα and enhances RelA DNA binding. Acetylation of Lys-310 is required for optimum RelA transcriptional activity [6]. Deacetylation of RelA is achieved by HDAC3 or by SIRT1 [6,15]. RelA also undergoes ubiquitination to terminate TNF activation. SOCS-1, a ubiquitin ligase, competes with Pin-1 to bind to the region surrounding Thr-254. SOCS-1 mediates ubiquitination of RelA between residues 220 and 335 and subsequent degradation by the proteosome [7].

These modifications help in explaining the duration and strength of NF-κB response, but they could also hold the key to modulation of transcription at specific promoters. It is probable that concerted modification of RelA by simultaneous signalling pathways targets RelA to activate transcription from some promoters, while actively repressing transcription from others, by interaction with distinct co-factors and transcription factors. Identification of the modifications that specify these functions will allow researchers to assess more accurately NF-κB function in diseased tissues, possibly leading to improved diagnosis and new therapies based on reprogramming RelA activity, rather than its total inhibition.

References

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