Review TRENDS in Biochemical Sciences Vol.30 No.1 January 2005

Phosphorylation of NF-κB and IκB proteins: implications in cancer and inflammation

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Nuclear factor-κB (NF-κB) is a transcription factor that has crucial roles in inflammation, immunity, cell proliferation and apoptosis. Activation of NF-κB mainly occurs via IκB kinase (IKK)-mediated phosphorylation of inhibitory molecules, including IκBα. Optimal induction of NF-κB target genes also requires phosphorylation of NF-κB proteins, such as p65, within their transactivation domain by a variety of kinases in response to distinct stimuli. Whether, and how, phosphorylation modulates the function of other NF-κB and IκB proteins, such as B-cell lymphoma 3, remains unclear. The identification and characterization of all the kinases known to phosphorylate NF-κB and IκB proteins are described here. Because deregulation of NF-κB and IκB phosphorylations is a hallmark of chronic inflammatory diseases and cancer, newly designed drugs targeting these constitutively activated signalling pathways represent promising therapeutic tools.

Cellular responses to bacterial or viral infections and to stress require rapid and accurate transmission of signals from cell-surface receptors to the nucleus [1]. These signalling pathways rely on protein phosphorylation and, ultimately, lead to the activation of specific transcription factors that induce the expression of appropriate target genes. Among the activated transcription factors, the nuclear factor-κB (NF-κB) family proteins are essential for inflammation, immunity, cell proliferation and apoptosis. NF-κB exists in a latent state in the cytoplasm and requires a signalling pathway for activation. Such NF-κB-activating pathways are triggered by a variety of extracellular stimuli and lead to the phosphorylation and subsequent proteasome-mediated degradation of inhibitory molecules, the inhibitor of NF-κB (IκB) proteins [2]. Activated NF-κB migrates into the nucleus to regulate the expression of multiple target genes. The NF-κB–IκB complex can also shuttle between the cytoplasm and the nucleus in unstimulated cells, but the nuclear export is more efficient and, therefore, the NF-κB–IκB complex is mainly cytoplasmic in resting cells.

Here, we tentatively demonstrate the key role of protein phosphorylation in NF-κB activation. We summarize the current knowledge in this field, namely all the kinases known to phosphorylate the NF-κB and IκB proteins, including recent data regarding B-cell lymphoma 3 (BCL-3) phosphorylation. In addition, we illustrate how deregulation of NF-κB and IκB phosphorylation is crucial in inflammation and cancer, and suggest potential targets for the design of new and specific therapeutic agents.

Three NF-κB-activating pathways

Three distinct NF-κB-activating pathways have emerged, and all of them rely on sequentially activated kinases (Figure 1). The first pathway –the classical pathway – is triggered by pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and leads to the sequential recruitment of various adaptors including TNF-receptor-associated death domain protein (TRADD), receptor-interacting protein (RIP) and TNF-receptor-associated factor 2 (TRAF2) to the cytoplasmic membrane [3]. This is followed by the recruitment and activation of the classical IκB-kinase (IKK) complex [4], which includes the scaffold protein NF-κB essential modulator (NEMO; also named IKKγ) [5], IKKα and IKKβ kinases [6]. Once activated, the IKK complex phosphorylates IκBα on Ser32 and Ser36, and is subsequently ubiquitinated and degraded via the proteasome pathway. A second pathway –the alternative pathway – is NEMO-independent and is triggered by cytokines such as lymphotoxin β [7], B-cell activating factor (BAFF) [8] or the CD40 ligand [9] (Figure 1) and by viruses such as human T-cell leukaemia virus [10] and the Epstein–Barr virus [11]. This signalling pathway relies on the recruitment of TRAF proteins to the membrane and on the NF-κB-inducing kinase (NIK) [12], which activates an IKKα homodimer – the substrate of which is the ankyrin-containing and inhibitory molecule p100 [13]. Once phosphorylated by IKKα on specific serine residues located in both the N- and C-terminal regions [14], p100 is ubiquitinated and cleaved to generate the NF-κB protein p52, which moves as heterodimer with RelB into the nucleus. In both cases, phosphorylation of the inhibitory molecules is essential for the activation of NF-κB, and this has been demonstrated by the inability of cells expressing an IκBα mutant that does not become phosphorylated to activate NF-κB [15].

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Available online 7 December 2004

 0968-0004/$ - see front matter doi:10.1016/j.tibs.2004.11.009

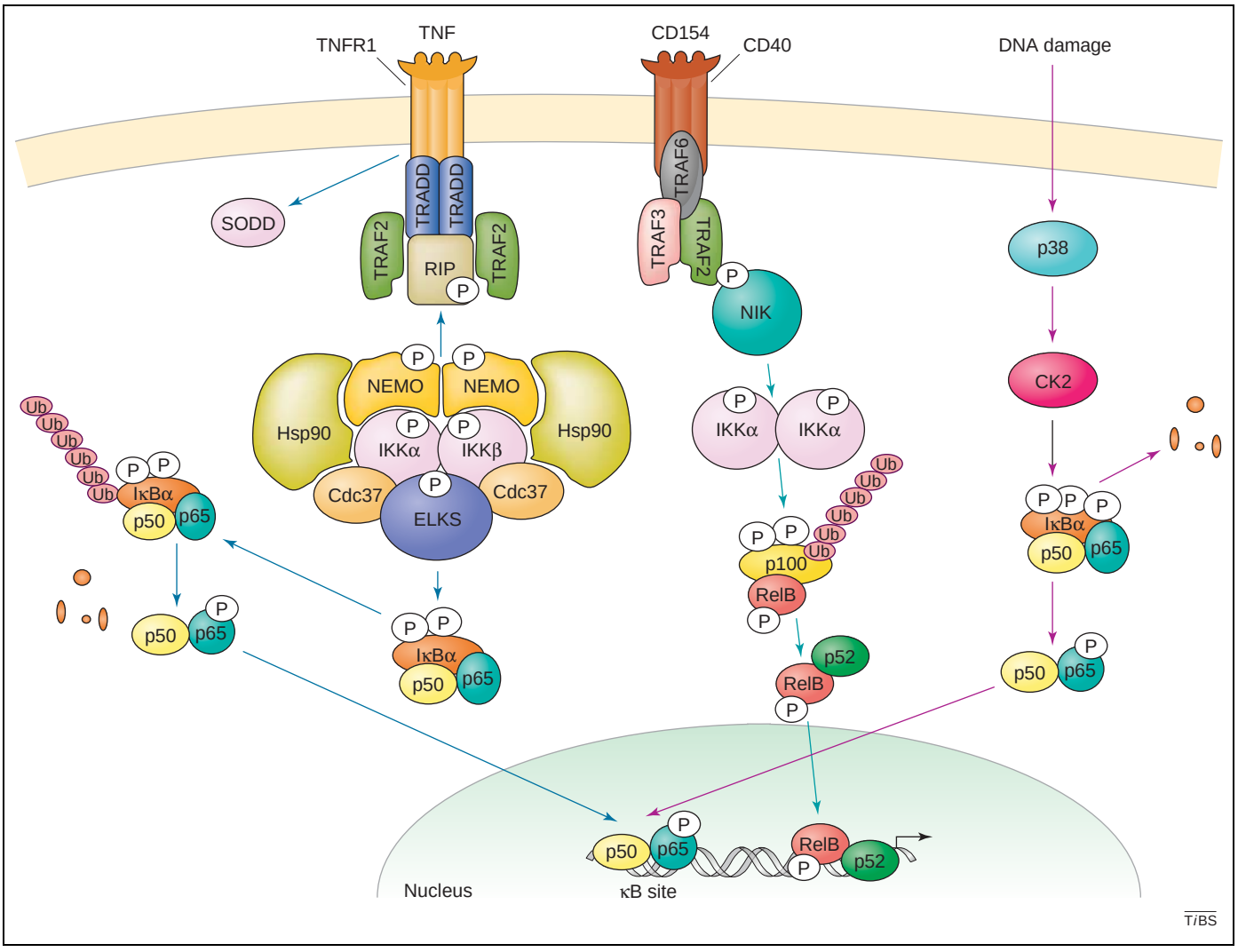

Figure 1. The classical (blue arrows), alternative (green arrows) and atypical (purple arrows) NF-κB-activating pathways as illustrated by the TNF-α-mediated, CD40-mediated and DNA-damage-mediated NF-κB activation pathways, respectively. In the classical NF-κB-activating pathway, upon binding of TNFα to TNFR1, SODD is released from the receptor and triggers the sequential recruitment of the adaptors TRADD, RIP and TRAF2 to the membrane. Then, TRAF2 mediates the recruitment of the IKK complex – composed of IKKα, IKKβ and NEMO – to the TNFR1 signalling complex. Hsp90 and Cdc37 are also part of the IKK complex and are required for the TNFα-induced IKK activation and shuttling of the IKK complex from the cytoplasm to the membrane, and ELKS connects IκBα to the IKK complex [83]. Activation of the IKK complex leads to the phosphorylation of IκBα at specific residues, ubiquitination through binding of ubiquitin proteins and degradation of this inhibitory molecule via the proteasome pathway. Then, the heterodimer p50–p65 is released and migrates to the nucleus where it binds to specific κB sites and activates a variety of NF-κB target genes, including IL-8, IL-6, TNFα and many more. The alternative pathway is triggered by binding of the CD40 ligand to its receptor, leading to recruitment of TRAF proteins and the sequential activation of NIK and IKKα, which then induces the processing of the inhibitory protein p100. p100 proteolysis releases p52 which forms heterodimers with RelB. This pathway is NEMO-independent and relies on IKKα homodimers. The atypical pathway, which is triggered by DNA damage such as UV, relies on sequential p38 and CK2 activations, and involves phosphorylation and subsequent IκBα degradation via an IKK-independent pathway. Subsequently, free NF-κB moves into the nucleus to activate its target genes. Note that the DNA-damaging agent doxorubicin also triggers p65 phosphorylation via a p53- and RSK1-dependent pathway (not shown). Phosphorylation of the signalling molecules in addition to NF-κB and IκB proteins are illustrated. Abbreviations: CK2, casein kinase 2; ELKS, Glu-Leu-Lys-Ser; Hsp90, heat shock protein 90; IκB, inhibitor of NF-κB; IKK, IκB kinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; RIP, receptor-interacting protein; RSK1, ribosomal S6 kinase 1; SODD, silencer of death domains; TNF-α, tumour necrosis factor α; TNFR1, TNF receptor 1; TRADD, TNF-receptor-associated death domain protein; TRAF, TNF-receptor-associated factor; Ub, ubiquitin.

Atypical IκBα phosphorylations for NF-κB activation

Most stimuli activate NF-κB by IKK-mediated IκBα phosphorylation on N-terminal serine residues. The third signalling pathway is classified as atypical because it is independent of IKK, still requires the proteasome and is triggered by DNA damage such as UV [16] or doxorubicin [17] (Figure 1). UV radiation induces IκBα degradation via the proteasome, but the targeted serine residues are located within a C-terminal cluster, which is recognized by the p38-activated casein kinase 2 (CK2) [16]. Oxidative stress also leads to NF-κB activation via IκBα tyrosine phosphorylation [18]. The N-terminal Tyr42 residue is crucial for this pathway [19], and the Syk protein tyrosine kinase seems to be required for H_2O_2-mediated NF-κB activation [20]. All of these phosphorylation events are signal-induced. However, IκBα is also constitutively phosphorylated on Ser293 within its C-terminal Pro-Glu-Ser-Thr sequence by CK2, and this phosphorylation is required for rapid proteolysis of IκBα [21–23].

Phosphorylations of other IκB proteins

All these reports convincingly demonstrate that phosphorylation of IκB proteins such as IκBα and p100 is essential for NF-κB activation. The inhibitory molecule IκBβ is also targeted for phosphorylation on Ser19 and

Ser23 by the IKK complex [24] and this phosphorylation triggers IκBβ degradation – similar to IKK-mediated IκBε phosphorylation of N-terminal serine residues [25].

The ankyrin-containing and inhibitory molecule p105 is also subjected to TNFα and IKK-mediated phosphorylation on Ser927 and Ser932 [26] (Figure 2). This phosphorylation is required for subsequent p105 ubiquitination and processing into the NF-κB protein p50 [26]. In addition, glycogen synthase kinase 3β (GSK3β) phosphorylates p105 on Ser903 and Ser907 and stabilizes p105 by preventing its degradation in unstimulated cells [27]. However, GSK3β-mediated p105 phosphorylation is required to prime IKK-mediated p105 phosphorylation and subsequent degradation upon TNFα treatment [27]. Therefore, GSK3β exerts a dual role towards p105, depending on whether or not the cells are stimulated [27]. The mitogen-activated kinase kinase kinase (MAP3K) Tpl2 (also named Cot) is another kinase that regulates p105 phosphorylation and subsequent degradation [28]. Although Tpl2 fails to phosphorylate p105 *in vitro*, it might affect p105 phosphorylation via a downstream kinase [28]. Interestingly, p105 interacts and governs Tpl2 stability in the MAP kinase signalling pathway [29]. Lipopolysaccharide (LPS)-mediated Tpl2 activation involves the dissociation of Tpl2 from p105, which requires IKKβ but not IKKα [30], and leads to subsequent Tpl2 degradation. Therefore, p105 phosphorylation is a key event in the LPS-stimulated mitogen-activated kinase (MAPK)/extracellular signal-related kinase (ERK) kinase (MEK) and ERK signalling cascade.

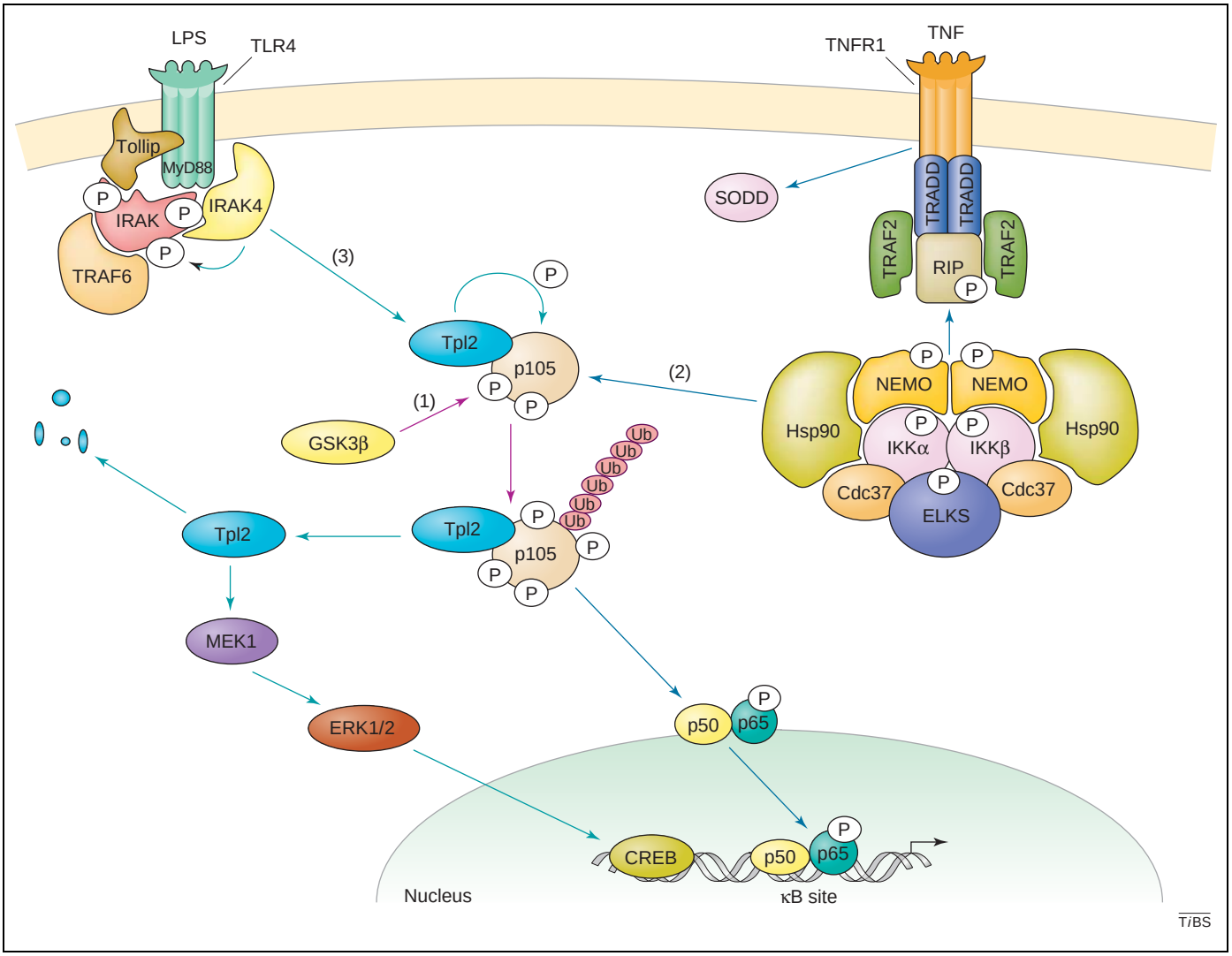

Figure 2. The p105-dependent pathways. The p105 inhibitory molecule is a phosphoprotein involved in three signalling pathways. **(1)** The first (purple arrows) relies on GSK3, which phosphorylates and stabilizes p105 in resting cells. This primo-phosphorylation also triggers p105 processing upon IKK-mediated phosphorylation in stimulated cells. **(2)** The second (blue arrows) occurs upon binding of TNFα to the TNFR1, which activates the IKK complex by the sequential recruitment of TRADD, RIP and TRAF2 to the membrane. IKKβ-mediated phosphorylation of p105 triggers its processing into p50; p50, in turn, moves as a heterodimer with p65 into the nucleus. **(3)** The third pathway (green arrows) is Tpl2-dependent and occurs through the TLR4 in LPS-stimulated cells. This treatment triggers the phosphorylation of the kinase IRAK by IRAK4 through a Tollip and a MyD88-dependent pathway, and leads to the activation of Tpl2, which phosphorylates its interacting partner p105. Activated Tpl2 activates ERK1/2 through a MEK1 pathway, leading to the binding of the transcriptional factor CREB to the regulatory sequences of its target genes. Tpl2 is quickly degraded once activated. Abbreviations: CREB, cAMP response element-binding; ELKS, Glu-Leu-Lys-Ser; ERK, extracellular signal-related kinase; GSK3, glycogen synthase kinase 3; Hsp90, heat shock protein 90; IκB, inhibitor of NF-κB; IKK, I-κB kinase; IRAK, interleukin-1-receptor-associated kinase; LPS, lipopolysaccharide; MEK, mitogen-activated kinase/ERK kinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; RIP, receptor-interacting protein; SODD, silencer of death domains; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR1, TNF receptor 1; Tpl2, tumour progression locus-2; TRADD, TNF-receptor-associated death domain protein; TRAF, TNF-receptor-associated factor; Ub, ubiquitin.

Other IκB proteins include BCL-3 and IκBζ [31], they harbour ankyrin repeats but are constitutively nuclear, and are described as transcription factors. IκBζ is required for the expression of a subset of genes that are activated in Toll-like receptor (TLR) and interleukin (IL)-1 receptor signalling pathways, as judged by the severe impairment of IL-6 production in response to a variety of TLR ligands and to IL-1β in IκBζ-deficient cells [32]. Both BCL-3 and IκBζ proteins are phosphorylated *in vivo*, but potential kinases have not yet been characterized. Moreover, it is not clear whether, and how, these phosphorylation events modulate NF-κB activation. However, recent data demonstrate that BCL-3 is constitutively phosphorylated by GSK3 on two C-terminal residues [33]. GSK3-mediated BCL-3 phosphorylation targets the degradation of BCL-3 via the proteasome pathway and, therefore, limits its expression. This BCL-3 phosphorylation inhibits its oncogenicity and modulates its ability to regulate a subset of target genes [33]. Therefore, phosphorylation is a key mechanism for the regulation of BCL-3 activity.

Optimal NF-κB activation by phosphorylation of p65

Besides the phosphorylation and subsequent degradation of inhibitory molecules, protein kinases are also required for optimal NF-κB activation by targeting functional domains of NF-κB proteins themselves. These additional

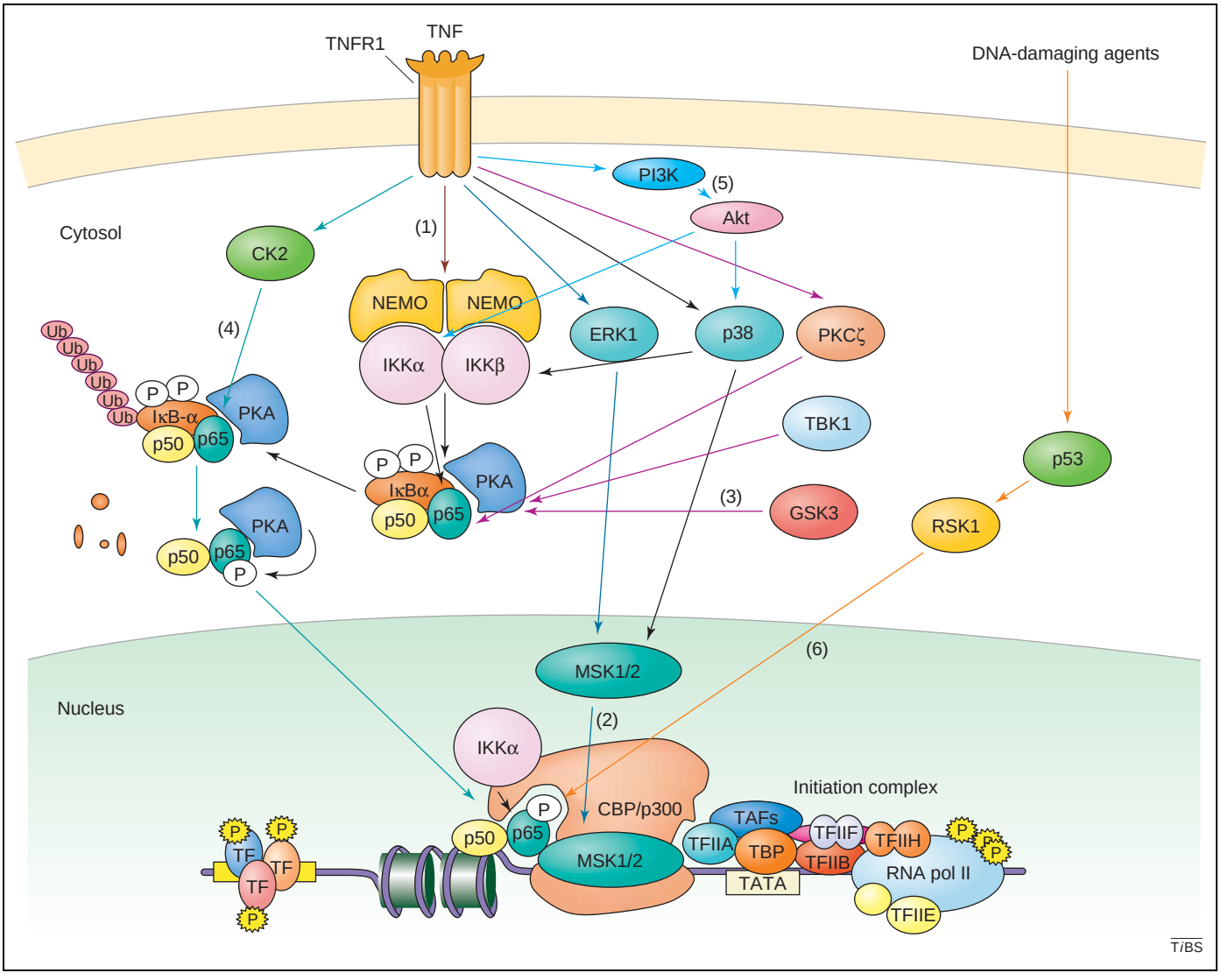

Figure 3. Modulation of the transcriptional activity of p65 by phosphorylation. Upon stimulation by TNFα, a pro-inflammatory cytokine, the IKK complex is activated **(1)** and phosphorylates p65 at Ser536. Because IKKα also moves into the nucleus to modulate NF-κB activity [84,85], it is currently unclear whether IKK-mediated p65 phosphorylation occurs in the cytoplasm and/or in the nucleus. TNFα also triggers MSK1-mediated phosphorylation of p65 a Ser276 in the nucleus via an ERK-dependent pathway **(2)**. The kinases TBK1, GSK3 and PKCζ also phosphorylate p65 **(3)** as shown by the defects of TNFα-mediated NF-κB activation in their respective knock-out mice. CK2-mediated p65 phosphorylation also occurs upon TNFα stimulation **(4)**. Finally, treatment with this cytokine also activates the PI3K–Akt signalling pathway, which phosphorylates p65 through a p38 and IKKβ-dependent, or an IKKα-dependent, mechanism **(5)**. The DNA-damaging drugs trigger RSK1 activation, the substrates of which include p65 **(6)**. All these p65 phosphorylations enhance its transactivation potential by positively regulating p65 interactions with co-activators such as CBP and p300. NF-κB target genes are subsequently induced following the recruitment of the initiation complex that includes the TATA-binding protein TBP, TFIIA, -B,-E,-F,-H, the TAFs in addition to RNA pol II on the promoter. Note that lymphotoxin β also triggers p65 phosphorylation through a NIK- and IKKα-dependent pathway that is not illustrated in this figure. Arrows indicating the pathways are: brown, pathway 1; dark blue, pathway 2; purple, pathway 3; green, pathway 4; light blue, pathway 5; orange, pathway 6. Abbreviations: CK2, casein kinase 2; CBP, CREB-binding protein; CREB, cAMP response element-binding; ERK, extracellular signal-related kinase; GSK3, glycogen synthase kinase 3; IκB, inhibitor of NF-κB; IKK, I-κB kinase; MSK1, mitogen- and stress-activated protein kinase-1; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; RNA pol II, RNA polymerase II; RSK1, ribosomal S6 kinase 1; TAF, TBP-associated factor; TBK1, TRAF-associated NF-κB activator (TANK)-binding kinase 1; TNF, tumour necrosis factor; TF, transcription factor; TNFR1, TNF receptor 1; TRAF, TNF-receptor-associated factor; Ub, ubiquitin.

pathways explain why cells lacking kinases such as GSK3β and TRAF-associated NF-κB activator (TANK)--binding kinase 1 (TBK1; also named T2K or NAK) have defects in NF-κB activation despite an unaltered profile of IκBα phosphorylation and degradation in response to TNFα [34,35]. Therefore, it is assumed that both GSK3β and TBK1 kinases target p65 for phosphorylation and enhance its transactivation potential. Indeed, this hypothesis has been confirmed experimentally, at least, *in vitro* [36,37,38]. In the case of GSK3β, it is worth mentioning that the GSK3β-mediated p65 phosphorylation awaits validation by *in vivo* studies. If such data are confirmed *in vivo*, this observation indicates that GSK3β is important for the modulation of NF-κB and IκB phosphorylations because at least three proteins of this family (p65, p105 and BCL-3) are phosphorylated by this kinase. Therefore, GSK3β inhibits BCL-3 function by targeting its degradation via the proteasome pathway but, by contrast, participates in the functional activation of p65. In other words, GSK3β could favour a rapid NF-κB activation wave by targeting a TNFα- and p65-dependent pathway and limit NF-κB activation in unidentified BCL-3-dependent pathways.

Numerous other studies have reported the ability of various kinases to phosphorylate p65. These p65 phosphorylation events occur in the cytoplasm or in the nucleus and are stimuli-specific and, probably, cell-type-specific (Figure 3). In the cytoplasm, the protein kinase PKAc is maintained in an inactive form by binding to IκBα. After stimulus-induced IκBα-degradation, activated PKAc phosphorylates p65 on Ser276 [39]. This phosphorylation of p65 enhances its ability to recruit histone acetyltransferases such as cAMP response element-binding (CREB)-binding protein (CBP) and p300 [40] and to displace p50–histone deacetylase (HDAC)-1 complexes from DNA [41]. Therefore, PKAc-mediated phosphorylation positively regulates the transactivation potential of p65 [40,41]. Ser276 of p65 is also phosphorylated by the mitogen- and stress-activated protein kinase-1 (MSK1) in the nucleus, and this phosphorylation is required for an optimal TNFα-mediated NF-κB activation [42]. Thus, a single p65 residue is targeted by two kinases in distinct cellular compartments.

Ser311 is another residue within the N-terminal Rel homology domain (RHD) domain that is targeted for phosphorylation by another kinase – protein kinase C (PKC)-ζ – in TNFα-stimulated cells [43]. Similar to the mechanism described for PKAc, PKCζ-mediated phosphorylation of p65 enhances its interaction with CBP and its recruitment with RNA polymerase II on the IL-6 promoter [43]. As a result, this phosphorylation causes optimal p65 transactivation potential as demonstrated by the defect of NF-κB activation in TNFα-stimulated mouse embryonic fibroblast (MEF) PKCζ$^{-/-}$ cells, despite unaltered IKK activation [44].

IL-1β stimulation also triggers p65 phosphorylation within minutes in the cytoplasm and CK2 activity associated with this NF-κB protein has been reported [45]. This cytoplasmic CKII activity is also associated with p65 upon TNFα stimulation and targets Ser529 of this substrate [46]. Moreover, this phosphorylation requires IκBα degradation and is prevented by p65 binding to IκBα in unstimulated cells [46].

In addition, p65 is phosphorylated at Ser536 by a variety of kinases via various signalling pathways. In most cases, these phosphorylations enhance p65 transactivation potential. Upon stimulation by TNFα [47] or the human T-cell lymphotropic virus type 1 Tax protein [48], activation of the IKK complex leads to phosphorylation of p65 at Ser536. Interestingly, whereas TNFα-mediated p65 phosphorylation requires IKKβ, the oncoprotein Tax relies on IKKα [48]. IKKα also phosphorylates p65 at Ser536 in lymphotoxin-β-stimulated cells, and this pathway requires the MAP3K NIK [49]. This mechanism, combined with altered p100 processing into p52, accounts for the defect of lymphotoxin-β receptor-induced NF-κB activation in NIK-deficient mice [50]. The IKKβ-mediated phosphorylation of p65 also occurs at Ser536 upon T-cell co-stimulation by the T-cell receptor. This requires the upstream kinases Tpl2 and PKCθ, but not the phosphatidylinositol 3-kinase (PI3K)–Akt pathway, and regulates p65 nuclear import [51]. The PI3K-dependent pathway, which involves Akt (also known as protein kinase B), also targets the transactivating domain of p65 upon IL-1 stimulation [52–54]. The identity of the downstream effectors of PI3K and Akt required for this pathway is controversial because both IKKβ and p38 have been suggested to be involved [52]. However, the use of IKKα- and/or IKKβ-deficient MEF cells suggests that IKKα is the only kinase required downstream of PI3K and Akt for p65 phosphorylation [54]. Most of these p65 phosphorylations occur upon stimulation by pro-inflammatory cytokines, but other molecules such as DNA-damaging agents, in addition to their ability to target IκBα degradation via an IKK-independent pathway [16], also lead to p65 phosphorylation [55]. Indeed, drugs such as doxorubicin or etoposide activate NF-κB via a p53-dependent pathway that relies on a ribosomal S6 kinase 1-mediated p65 phosphorylation at Ser536 [55]. As a result, the affinity of p65 for IκBα and, consequently, the IκBα-mediated nuclear export of NF-κB are reduced [55].

It is likely that other, as yet unidentified, p65 kinases are involved, such as one that phosphorylates p65 at Ser468 upon T-cell co-stimulation [56]. Nevertheless, all the reports to date illustrate a crucial role for p65 phosphorylation in NF-κB activation. It is worth mentioning, however, that these p65 phosphorylation data were obtained using different cell lines and it is unclear whether all these p65 phosphorylations occur simultaneously *in vivo* across different cell types. Moreover, it remains to be experimentally demonstrated whether or not p65 phosphorylation on all these sites is required for optimal NF-κB activity. Careful phenotypic analysis of mice deficient for p65 kinases has indicated that there might be several redundancies in kinase action. Reconstitution of p65-deficient cells with various p65 proteins harbouring point mutations at their phosphorylable residues is an elegant way to identify the targeted amino acid that is required for the p65 transactivation potential. This experimental strategy has been conducted, and Ser276 of p65 has been identified as the most important

Table 1. Kinases known to phosphorylate the NF-κB and IκB proteins[a,b]

Substrates	Kinases	Residues	Location	Function	Biological stimuli	Refs
IκBα	IKKβ	Ser32, Ser36	N-terminal domain	Proteasome-mediated degradation	TNFα, IL-1β	[6]
	CK2	Ser293	PEST domain	Destabilization	Constitutive	[21–23]
	Syk	Tyr42	N-terminal domain		H_2O_2	[20]
	CK2	Ser283–Thr299	PEST domain	Degradation	UV light	[16]
IκBβ	IKKβ	Ser19, Ser23	N-terminal domain	Proteasome-mediated degradation	TNFα, IL-1β	[24]
IκBε	IKKβ	Ser18, Ser22	N-terminal domain	Proteasome-mediated degradation	TNFα, IL-1β	[25]
p100	IKKα	Ser108, Ser115, Ser123, Ser872	N- and C-terminal domains	Processing into p52	CD40, BAFF, lymphotoxin β	[7–9]
p105	IKKβ	Ser927, Ser932	PEST domain	Processing into p50	TNFα	[26]
	Tpl2	Indirect phosphorylation		Processing into p50?	Unknown	[28]
	GSK3β	Ser903, Ser907		Stabilization	Constitutive	[27]
BCL-3	GSK3	Ser394, Ser398	C-terminal domain	Proteasome-mediated degradation	Constitutive	[33]
RelA/p65	PKAc	Ser276	Rel homology domain	Regulation of DNA-binding and oligomerization	LPS	[39]
	MSK1/2	Ser276	Rel homology domain	Enhanced transactivation potential	TNFα	[42]
	PKCζ	Ser311	Rel homology domain	Enhanced transactivation potential	TNFα	[43]
	Unknown	Ser468		Unknown	T-cell co-stimulation (CD3/CD28)	[51]
	CK2	Ser529	C-terminal TAD	Enhanced transactivation potential	TNFα, IL-1β	[45.46]
	IKKα	Ser536	C-terminal TAD	Enhanced transactivation potential	HTLV-1 infection, lymphotoxin β	[48.49]
	IKKβ	Ser536	C-terminal TAD	Enhanced transactivation potential	TNFα, T-cell co-stimulation (CD3/CD28)	[47]
	Akt	Ser536	C-terminal TAD	Enhanced transactivation potential	IL-1β	[52.53]
	RSK1	Ser536	C-terminal TAD	Decreased affinity to IκBα	DNA-damaging agents	[55]
	GSK3β	Four sites within amino acids 354–551	C-terminal TAD	Enhanced transactivation potential	Constitutive	[36]
	TBK1	Ser536	C-terminal TAD	Enhanced transactivation potential	IL-1β	[37.38]
	IKKε	Ser536	C-terminal TAD	Enhanced transactivation potential	IL-1β	[38]
RelB	Unknown	Ser368	Rel homology domain	Dimerization and p100 stabilization		[60]
	Unknown	Thr84, Ser552		Degradation	T-cell co-stimulation (CD3/CD28), TPA/ ionomycin	[61]
Crel	PI3K/PKC	Ser471	C-terminal TAD	Enhanced transactivation potential	TNFα	[64]
	PKA-Cβ	Unknown	C-terminal TAD	Enhanced transactivation potential	Unknown	[66]
	Unknown	Unknown tyrosine			G-CSF	[67]
p50	PKAc	S337	Rel homology domain	Enhanced binding to DNA		[69]

[a]Abbreviations: BAFF, B-cell activating factor; CK2, casein kinase 2; G-CSF, granulocyte-colony stimulating factor; GSK3, glycogen synthase kinase 3; HTLV-1, human T-cell leukaemia virus type-1; IκB, inhibitor of NF-κB; IKK, I-κB kinase; IL-1β, interleukin-1β; LPS, lipopolysaccharide; MSK, mitogen- and stress-activated protein kinase; NF-κB, nuclear factor-κB; PEST, Pro-Glu-Ser-Thr; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; RSK1, ribosomal S6 kinase 1; TBK1, TRAF-associated NF-κB activator (TANK)-binding kinase 1; TNFα, tumour necrosis factor α; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; Tpl2, tumour progression locus-2; TRAF, TNF-receptor-associated factor.

[b]The consequences of these phosphorylations on the activity of the NF-κB and IκB proteins are mentioned when experimentally described.

phosphorylated residue for NF-κB-mediated gene activation in TNFα-stimulated MEF cells [57].

Interestingly, although it is now accepted that NF-κB harbours anti-apoptotic properties in most cases, some stimuli such as UV light and chemotherapeutic drugs paradoxically repress NF-κB anti-apoptotic target gene transcription by enhancing the association of p65 with the HDAC proteins [58]. However, this observation cannot be generalized to every cell type and might be cell-type specific. So, how can the same protein (i.e. p65) sometimes activate transcription of a gene but, in other circumstances, repress transcription of the same gene? The explanation might come from differential p65 phosphorylations triggered by chemotherapeutic drugs, as compared with the ones triggered by pro-inflammatory cytokines [58], even if Ser536 is targeted in both pathways (as described). Again, precise mapping of the targeted p65 residues will help to better understand how protein phosphorylation can modulate the ability of p65 to activate or repress anti-apoptotic gene expression by recruiting histone acetylases or deacetylases, respectively. Furthermore, phosphorylation of other NF-κB proteins might affect the ability of NF-κB to activate or repress these genes, as demonstrated by the fact that, similarly to

p65, p52 undergoes the same transcriptional switch from activator to repressor upon induction of endogenous p53 in response to UV light [59].

The crucial role of phosphorylation in the regulation of RelB and c-Rel activity

Multiple residues of the NF-κB protein RelB are phosphorylated. Ser368 seems to be essential for RelB dimerization and p100 stabilization, but not for RelB nuclear import [60]. However, the kinase that targets this site has not been identified. Phosphorylation of RelB at Thr84 and Ser552 in cells stimulated by 12-*O*-tetradecanoylphorbol 13-acetate and ionomycin triggers degradation of RelB via the proteasome pathway but, again, the identity of the kinase that phosphorylates RelB at these residues is unknown at present [61]. What are the consequences of these RelB phosphorylations on NF-κB activation via the IKKα-dependent alternative pathway? Which NF-κB target genes are regulated by RelB phosphorylation? Such issues are currently unclear and certainly deserve further investigation.

The role of protein phosphorylation in the regulation of c-Rel activity has been demonstrated by the inability of a v-Rel protein with Ser→Ala substitutions within its C-terminal domain to transform cells [62]. This mutation also abolishes the transactivating and anti-apoptotic abilities of this protein, suggesting that phosphorylation of these serine residues might be required for v-Rel function [63].

In Jurkat cells, a mutation at Ser471 within the cRel transactivating domain abolishes the ability of these cells to respond to TNFα-mediated NF-κB activation [64]. Ser471 seems to be targeted for phosphorylation by a PI3K- and PKC-dependent pathway [64]. Interestingly, additional downstream residues might also be targeted after phorbol myristate acetate and ionomycin stimulation [65]. Moreover, the kinase PKA-Cβ enhances c-Rel transactivation potential by direct phosphorylation, but the targeted amino acid remains to be identified [66]. Finally, stimulation of neutrophils with granulocyte-colony-stimulating factor (G-CSF) leads to tyrosine phosphorylation of c-Rel and might increase its ability to bind DNA, but both the kinase and the phosphorylated residue are unknown [67]. Taken together, these reports demonstrate that protein phosphorylation is crucial for the regulation of the biological properties of c-Rel, namely its transactivation and oncogenic potential. It is, however, not known which of the anti-apoptotic genes that are induced by c-Rel are sensitive to phosphorylation of this protein.

p50 and p52 NF-κB subunits are phosphoproteins

Although much has been reported regarding the phosphorylation of p65, and to a lesser extend RelB and c-Rel, there is little information about the phosphorylation of other NF-κB proteins, despite the fact that it has been known for many years that members of the NF-κB family (e.g. p50) are phosphorylated upon cell stimulation [68]. Because p50 lacks a transactivating domain, protein phosphorylation regulates its DNA-binding properties. Indeed, PKA-mediated phosphorylation at Ser337, which is located within the Rel homology domain, enhances the p50 DNA-binding abilities [64]. So far, this is the only reporting of a potential p50 kinase, although the sequence surrounding the targeted residue also fits the profile of several other kinases such as calmodulin II, CK2 and protein kinase G [69]. Thus, additional experiments are required to address the physiological relevance of these putative p50 phosphorylations. All the kinases known to phosphorylate the NF-κB and IκB proteins are summarized in Table 1.

Implications of NF-κB and IκB phosphorylation in inflammation and cancer

Because NF-κB is activated by pro-inflammatory cytokines, induces cell proliferation and anti-apoptotic gene expression, and also enhances angiogenesis via vascular endothelial growth factor expression, it is not surprising that aberrant NF-κB activity is a hallmark of cancer and chronic inflammatory diseases. Altered NF-κB activation is caused by deregulated, and often constitutive, NF-κB and IκB phosphorylations, which are major contributors to these diseases. Indeed, constitutive IKK activity and consequently enhanced levels of nuclear p65 have been described in inflammatory diseases [70] and in a variety of solid tumours [71–74]. It is, however, unclear as to whether constitutive p65 phosphorylation is also observed in human cancer cells. Nevertheless, phosphorylation of NF-κB proteins is required for their oncogenicity – as demonstrated for v-Rel [63] – and also for p65 phosphorylation at Ser536 in a model of TNFα-induced transformation of mouse epidermal cells [75]. Recently, the role of NF-κB activation in tumour development has been demonstrated using various animal models, including a mouse model of colitis-associated cancer [76]. Deletion of IKKβ in intestinal epithelial cells leads to a dramatic decrease in tumour incidence in such models, whereas deletion of IKKβ in myeloid cells in these mice results in decreased tumour size by diminished expression of pro-inflammatory cytokines that act as tumour growth factors [76]. These results highlight the ability of IKKβ to link inflammation and cancer [76] and provide additional evidence for specific inactivation of NF-κB as a promising tool to attenuate the formation of inflammation-associated tumours. A similar conclusion was based on a mouse model for hepatitis-associated cancer [77]. *Mdr2*$^{-/-}$ mice, which spontaneously develop hepatitis and subsequently hepatocellular carcinoma, still develop hepatitis but rarely cancer when NF-κB is specifically inactivated using an IκBα super-repressor transgene [77]. Therefore, IKK-dependent constitutive NF-κB activation is required for tumour development. However, phosphorylation can also negatively regulate the oncogenicity of IκB proteins. Indeed, GSK3-mediated BCL-3 phosphorylation attenuates its oncogenic potential by triggering its degradation via the proteasome pathway [33]. Moreover, GSK3 phosphorylation of this oncoprotein affects the ability of BCL-3 to induce a subset of its cancer-relevant target genes such as secretory leucocyte protease inhibitor [33].

Therefore, these aberrant and often constitutive NF-κB and IκB phosphorylations represent promising targets for the treatment of chronic inflammatory diseases and cancer. Selective IKK inhibitors, such as BMS-345541,

have been generated and have shown anti-inflammatory activities *in vivo* that make them, potentially, efficient drugs for rheumatoid arthritis [78]. Regarding the treatment of cancer, effective drugs for acute leukaemia, such as the pyrimidine analogue cytosine arabinoside, induces apoptosis in treated cells, and the underlying mechanism involves the activation of the protein phosphatases 2A and 2B-A and the subsequent p65 dephosphorylation [79,80]. Preventing NF-κB and IκB phosphorylation is also possible with the administration of a cell-permeable peptide that disrupts the interaction between a kinase and its scaffold protein, making the kinase non functional [81]. Such a peptide that targets the interaction between IKKβ and the scaffold protein NEMO inhibits cytokine-induced NF-κB activation and shows promising effects in two models of acute inflammation, namely the phorbol-12-myristate-13-acetate-induced ear edaema and the zymosan-induced peritonitis [81]. Because of the important role of NF-κB in osteoclast differentiation, blocking the activation of this transcription factor is also a good strategy for prevention of inflammatory bone resorption. Therefore, the cell-permeable peptide has also been tested in models of chronic inflammatory diseases involving bone resorption and it does, indeed, inhibit RANKL (receptor activator of NF-κB ligand)-stimulated NF-κB activation and osteoclastogenesis *in vivo* [82]. Moreover, this peptide abrogates joint swelling and reduces destruction of bone and cartilage by lowering levels of TNFα and IL-1β in the same experimental model [82]. Although other strategies such as proteasome inhibition have been developed to block NF-κB activation and have already demonstrated their efficiency in clinical trials, the inhibition of NF-κB-activating kinases might be more specific and, therefore, could generate fewer side effects. However, deciphering the correct biological and pathophysiological roles of each kinase is required to test these novel drugs in the most appropriate settings and, therefore, to reduce the risk of trial failures.

Future directions

Although many phosphorylation sites on NF-κB proteins have been characterized, it is still unclear how these phosphorylations regulate the ability of such proteins to induce or to repress defined target genes. The answers might come from the use of knock-in experiments in which a mouse expressing mutant NF-κB proteins that lack key phosphorylation sites is generated. Phenotypic analysis of these mice would provide a powerful biological model to address the regulation of NF-κB protein activities by phosphorylation *in vivo*.

Acknowledgements

A.C. and M-P.M. are Research Associates at the Belgian National Funds for Research. A.C. is supported by a grant from the Belgian Federation against Cancer and TELEVIE. The members of the laboratory are also supported by grants from the Belgian Funds for Research (FNRS) and the Centre anti-cancéreux. We thank E. Dejardin for helpful discussions.

References

1 Karin, M. and Hunter, T. (1995) Transcripional control by protein phosphorylation: signal transmission from the cell surface to the nucleus. *Curr. Biol.* 5, 747–757
2 Karin, M. and Ben-neriah, Y. (2000) Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annu. Rev. Immunol.* 18, 621–663
3 Hsu, H. *et al*. (1995) The TNF receptor 1-associated protein TRADD signals cell death and NF-κB activation. *Cell* 81, 495–504
4 Devin, A. *et al*. (2000) The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* 12, 419–429
5 Yamaoka, S. *et al*. (1998) Complementation cloning of NEMO, a component of the I κB kinase complex essential for NF-κB activation. *Cell* 93, 1231–1240
6 Zandi, E. *et al*. (1997) The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. *Cell* 91, 243–252
7 Dejardin, E. *et al*. (2002) The lymphotoxin-β receptor induces different patterns of gene expression via two NF-κB pathways. *Immunity* 17, 525–535
8 Claudio, E. *et al*. (2002) BAFF-induced NEMO-independent processing of NF-κB2 in maturing B cells. *Nat. Immunol.* 3, 958–965
9 Coope, H.J. *et al*. (2002) CD40 regulates the processing of NF-κB2 p100 to p52. *EMBO J.* 21, 5375–5385
10 Xiao, G. *et al*. (2001) Retroviral oncoprotein Tax induces processing of NF-κB2/p100 in T cells: evidence for the involvement of IKKα. *EMBO J.* 20, 6805–6815
11 Eliopoulos, A.G. *et al*. (2003) Epstein-Barr virus-encoded latent infection membrane protein 1 regulates the processing of p100 NF-κB2 to p52 via an IKKγ/NEMO-independent signalling pathway. *Oncogene* 22, 7557–7569
12 Xiao, G. *et al*. (2001) NF-κB-inducing kinase regulates the processing of NF-κB2 p100. *Mol. Cell* 7, 401–409
13 Senftleben, U. *et al*. (2001) Activation by IKKα of a second, evolutionary conserved, NF-κB signaling pathway. *Science* 293, 1495–1499
14 Xiao, G. *et al*. (2004) Induction of p100 processing by NF-κB-inducing kinase involves docking IκB kinase α (IKKα) to p100 and IKKα-mediated phosphorylation. *J. Biol. Chem.* 279, 30099–30105
15 Brown, K. *et al*. (1995) Control of IκB-ampha proteolysis by site-specific, signal induced phopshorylation. *Science* 267, 1485–1488
16 Kato, T. *et al*. (2003) CK2 is a C-terminal IκB kinase responsible for NF-κB activation during the UV response. *Mol. Cell* 12, 829–839
17 Tergaonkar, V. *et al*. (2003) IκB kinase-independent IκBα degradation pathway: functional NF-κB activity and implications for cancer therapy. *Mol. Cell. Biol.* 23, 8070–8083
18 Imbert, V. *et al*. (1996) Tyrosine phosphorylation of IκB-α activates NF-κB without proteolytic degradation of IκB-α. *Cell* 86, 787–798
19 Schoonbroodt, S. *et al*. (2000) Crucial role of the amino-terminal tyrosine residue 42 and the carboxyl-terminal PEST domain of IκBα in NF-κB activation by an oxidative stress. *J. Immunol.* 164, 4292–4300
20 Takada, Y. *et al*. (2003) Hydrogen peroxide activates NF-κB through tyrosine phosphorylation of IκBα and serine phosphorylation of p65: evidence for the involvement of IκBα kinase and Syk protein-tyrosine kinase. *J. Biol. Chem.* 278, 24233–24241
21 Lin, R. *et al*. (1996) Phosphorylation of IκBα in the C-terminal PEST domain by casein kinase II affects intrinsic protein stability. *Mol. Cell. Biol.* 16, 1401–1409
22 McElhinny, J.A. *et al*. (1996) Casein kinase II phosphorylates IκBα at S-283, S-289, S-293, and T-291 and is required for its degradation. *Mol. Cell. Biol.* 16, 899–906
23 Schwarz, E.M. *et al*. (1996) Constitutive phosphorylation of IκBα by casein kinase II occurs preferentially at serine 293: requirement for degradation of free IκBα. *Mol. Cell. Biol.* 16, 3554–3559
24 Wu, C. and Ghosh, S. (2003) Differential phosphorylation of the signal-responsive domain of IκBα and IκBβ by IκB kinases. *J. Biol. Chem.* 278, 31980–31987
25 Whiteside, S.T. *et al*. (1997) IκBε, a novel member of the IκB family controls RelA and cRel NF-κB activity. *EMBO J.* 16, 1413–1426
26 Lang, V. *et al*. (2003) βTrCP-mediated proteolysis of NF-κB1 p105 requires phosphorylation of p105 serines 927 and 932. *Mol. Cell. Biol.* 23, 402–413
27 Demarchi, F. *et al*. (2003) Glycogen synthase kinase-3β regulates NF-κB1/p105 stability. *J. Biol. Chem.* 278, 39583–39590
28 Belich, M.P. *et al*. (1999) Tpl-2 kinase regulates the proteolysis of the NF-κB-inhibitory protein NF-κB1 p105. *Nature* 397, 363–368

29 Waterfield, M.R. *et al*. (2003) NF-κB1/p105 regulates lipopolysaccharide-stimulated MAP kinase signaling by governing the stability and function of the Tpl2 kinase. *Mol. Cell* 11, 685–694

30 Waterfield, M. *et al*. (2004) IκB kinase is an essential component of the Tpl2 signaling pathway. *Mol. Cell. Biol.* 24, 6040–6048

31 Yamazaki, S. *et al*. (2001) A novel IκB protein, IκB-ζ, induced by proinflammatory stimuli negatively regulates nuclear factor-κB in the nuclei. *J. Biol. Chem.* 276, 27657–27662

32 Yamamoto, M. *et al*. (2004) Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IκBζ. *Nature* 430, 218–222

33 Viatour, P. *et al*. (2004) GSK3-mediated BCL-3 phosphorylation modulates its degradation and its oncogenicity. *Mol. Cell* 16, 35–45

34 Bonnard, M. *et al*. (2000) Deficiency of T2K leads to apoptotic liver degeneration and impaired NF-κB-dependent gene transcription. *EMBO J.* 19, 4976–4985

35 Hoeflich, K.P. *et al*. (2000) Requirement for glycogen synthase kinase-3β in cell survival and NF-κB activation. *Nature* 406, 86–90

36 Schwabe, R.F. and Brenner, D.A. (2002) Role of glycogen synthase-3 kinase in TNF-α-induced NF-κB activation and apoptosis in hepatocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, G204–G211

37 Fujita, F. *et al*. (2003) Identification of NAP1, a regulatory subunit of IκB kinase-related kinases that potentiates NF-κB signalling. *Mol. Cell. Biol.* 23, 7780–7793

38 Buss, H. *et al.* Constitutive and IL-1-inducible phosphorylation of p65 NF-kB at serine 536 is mediated by multiple protein kinases including IKKα, IKKβ, IKKε, TBK1 and an unknown kinase and couples p65 to TAFII31-mediated IL-8 transcription. *J. Biol. Chem.* (in press)

39 Zhong, H. *et al*. (1997) The transcriptional activity of NF-κB is regulated by the IκB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* 89, 413–424

40 Zhong, H. *et al*. (1998) Phosphorylation of NF-κB p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol. Cell* 1, 661–671

41 Zhong, H. *et al*. (2002) The phosphorylation status of nuclear NF-κB determines its association with CBP/p300 or HDAC-1. *Mol. Cell* 9, 625–636

42 Vermeulen, L. *et al*. (2003) Transcriptional activation of the NF-κB p65 subunit by mitogen- and stress-activated protein kinase-1. *EMBO J.* 22, 1313–1324

43 Duran, A. *et al*. (2003) Essential role of RelA Ser311 phosphorylation by zetaPKC in NF-κB transcriptional activation. *EMBO J.* 22, 3910–3918

44 Leitges, M. *et al*. (2001) Targeted disruption of the PKCζ gene results in the impairment of the NF-κB pathway. *Mol. Cell* 8, 771–780

45 Bird, T.A. *et al*. (1997) Activation of nuclear transcription factor NF-κB by interleukin-1 is accompanied by casein kinase II-mediated phosphorylation of the p65 subunit. *J. Biol. Chem.* 272, 32606–32612

46 Wang, D. *et al*. (2000) Tumor necrosis factor α-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J. Biol. Chem.* 275, 32592–32597

47 Sakurai, H. *et al*. (1999) IκB kinases phosphorylate NF-κB p65 subunit on serine 536 in the transactivation domain. *J. Biol. Chem.* 274, 30353–30356

48 O'Mahony, A.M. *et al*. (2004) Human T-cell lymphotropic virus type 1 tax induction of biologically Active NF-κB requires IκB kinase-1-mediated phosphorylation of RelA/p65. *J. Biol. Chem.* 279, 18137–18145

49 Jiang, X. *et al*. (2003) The NF-κB activation in lymphotoxin β receptor signalling depends on the phosphorylation of p65 at serine 536. *J. Biol. Chem.* 278, 919–926

50 Yin, L. *et al*. (2001) Defective lymphotoxin-β receptor-induced NF-κB transcriptional activity in NIK-deficient mice. *Science* 291, 2162–2165

51 Mattioli, I. *et al*. (2004) Transient and selective NF-κB p65 serine 536 phosphorylation induced by T cell costimulation is mediated by IκB kinase β and controls the kinetics of p65 Nuclear Import. *J. Immunol.* 172, 6336–6344

52 Madrid, L.V. *et al*. (2001) Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-κB through utilization of the IκB kinase and activation of the mitogen-activated protein kinase p38. *J. Biol. Chem.* 276, 18934–18940

53 Sizemore, N. *et al*. (1999) Activation of phosphatidylinositol 3-kinase in response to interleukin-1 leads to phosphorylation and activation of the NF-κB p65/RelA subunit. *Mol. Cell. Biol.* 19, 4798–4805

54 Sizemore, N. *et al*. (2002) Distinct roles of the IκB kinase α and β subunits in liberating nuclear factor κB (NF-κB) from IκB and in phosphorylating the p65 subunit of NF-κB. *J. Biol. Chem.* 277, 3863–3869

55 Bohuslav, J. *et al*. (2004) p53 induces NF-κB activation by an IκB kinase-independent mechanism involving phosphorylation of p65 by ribosomal S6 kinase 1. *J. Biol. Chem.* 279, 26115–26125

56 Schmitz, M.L. *et al*. (2004) A comparative analysis of T cell costimulation and CD43 activation reveals novel signaling pathways and target genes. *Blood* 104, 3302–3304

57 Okazaki, T. *et al*. (2003) Phosphorylation of serine 276 is essential for p65 NF-κB subunit-dependent cellular responses. *Biochem. Biophys. Res. Commun.* 300, 807–812

58 Campbell, K.J. *et al*. (2004) Active repression of anti-apoptotic gene expression by RelA(p65) NF-κB. *Mol. Cell* 13, 853–865

59 Rocha, S. *et al*. (2003) p53 represses Cyclin D1 transcription through down regulation of Bcl-3 and inducing increased association of the p52 NF-κB subunit with histone deacetylase 1. *Mol. Cell. Biol.* 23, 4713–4727

60 Maier, H.J. *et al*. (2003) Critical role of RelB serine 368 for dimerization and p100 stabilization. *J. Biol. Chem.* 278, 39242–39250

61 Marienfeld, R. *et al*. (2001) Signal-specific and phosphorylation-dependent RelB degradation: a potential mechanism of NF-κB control. *Oncogene* 20, 8142–8147

62 Chen, C. *et al*. (1999) Mapping of a serine-rich domain essential for the transcriptional, antiapoptotic, and transforming activities of the v-Rel oncoprotein. *Mol. Cell. Biol.* 19, 307–316

63 Rayet, B. *et al*. (2003) Mutations in the v-Rel transactivation domain indicate altered phosphorylation and identify a subset of NF-κB-regulated cell death inhibitors important for v-Rel transforming activity. *Mol. Cell. Biol.* 23, 1520–1533

64 Martin, A.G. and Fresno, M. (2000) Tumor necrosis factor-α activation of NF-κB requires the phosphorylation of Ser-471 in the transactivation domain of c-Rel. *J. Biol. Chem.* 275, 24383–24391

65 Martin, A.G. *et al*. (2001) Regulation of nuclear factor κB transactivation. Implication of phosphatidylinositol 3-kinase and protein kinase Cζ in c-Rel activation by tumor necrosis factor α. *J. Biol. Chem.* 276, 15840–15849

66 Yu, S.H. *et al*. (2004) Stimulation of c-Rel transcriptional activity by PKA catalytic subunit β. *J. Mol. Med.* 82, 621–628

67 Druker, B.J. *et al*. (1994) Rel is rapidly tyrosine-phosphorylated following granulocyte-colony stimulating factor treatment of human neutrophils. *J. Biol. Chem.* 269, 5387–5390

68 Hayashi, T. *et al*. (1993) Identification of a new serine kinase that activates NF-κB by direct phosphorylation. *J. Biol. Chem.* 268, 26790–26795

69 Hou, S. *et al*. (2003) Phosphorylation of serine 337 of NF-κB p50 is critical for DNA binding. *J. Biol. Chem.* 278, 45994–45998

70 Marok, R. *et al*. (1996) Activation of the transcription factor nuclear factor-κB in human inflamed synovial tissue. *Arthritis Rheum.* 39, 583–591

71 Romieu-Mourez, R. *et al*. (2001) Roles of IKK kinases and protein kinase CK2 in activation of nuclear factor-κB in breast cancer. *Cancer Res.* 61, 3810–3818

72 Biswas, D.K. *et al*. (2004) NF-κB activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10137–10142

73 Gasparian, A.V. *et al*. (2002) The role of IKK in constitutive activation of NF-κB transcription factor in prostate carcinoma cells. *J. Cell Sci.* 115, 141–151

74 Robe, P.A. *et al*. (2004) *In vitro* and *in vivo* activity of the nuclear factor-κB inhibitor sulfasalazine in human glioblastomas. *Clin. Cancer Res.* 10, 5595–5603

75 Hu, J. *et al*. (2004) Insufficient p65 phosphorylation at S536 specifically contributes to the lack of NF-κB activation and transformation in resistant JB6 cells. *Carcinogenesis* 25, 1991–2003

76 Greten, F.R. *et al*. (2004) IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118, 285–296

77 Pikarsky, E. *et al*. (2004) NF-κB functions as a tumor promoter in inflammation-associated cancer. *Nature* 431, 461–466
78 McIntyre, K.W. *et al*. (2003) A highly selective inhibitor of IκB kinase, BMS-345541, blocks both joint inflammation and destruction in collagen-induced arthritis in mice. *Arthritis Rheum.* 48, 2652–2659
79 Sreenivasan, Y. *et al*. (2003) Mechanism of cytosine arabinoside-mediated apoptosis: role of Rel A (p65) dephosphorylation. *Oncogene* 22, 4356–4369
80 Yang, J. *et al*. (2001) Protein phosphatase 2A interacts with and directly dephosphorylates RelA. *J. Biol. Chem.* 276, 47828–47833
81 May, M.J. *et al*. (2000) Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex. *Science* 289, 1550–1553
82 Jimi, E. *et al*. (2004) Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory destruction *in vivo*. *Nat. Med.* 10, 617–624
83 Ducut Sigala, J.L. *et al*. (2004) Activation of transcription factor NF-κB requires ELKS, an IκB kinase regulatory subunit. *Science* 304, 1963–1967
84 Anest, V. *et al*. (2003) A nucleosomal function for IκB kinase-α in NF-κB-dependent gene expression. *Nature* 423, 659–663
85 Yamamoto, Y. *et al*. (2003) Histone H3 phosphorylation by IKK-α is critical for cytokine-induced gene expression. *Nature* 423, 655–659