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Review

The nuclear factor-kappaB (NF-κB): from a versatile transcription factor to a ubiquitous therapeutic target

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The nuclear factor-κappaB (NF-κB) transcription factors regulate a plethora of cellular pathways and processes including the immune response, inflammation, proliferation, apoptosis and calcium homeostasis. In addition to the complexity of its physiological roles, the composition and function of this family of proteins is very complicated. While the basic understanding of NF-κB signalling is extensive, relatively little is know of the *in vivo* dynamics of this pathway or what controls the balance between various outcomes. Although we know a large number of NF-κB-responsive genes, the contribution of these genes to a specific response is not always clear. Finally, the involvement of NF-κB in pathological processes is only now beginning to be unravelled. In addition to cancer and immunodeficiency disorders, altered regulation of NF-κB has been associated with several inherited diseases. These findings indicate that modulation of the NF-κB pathways may be beneficial. However, our limited knowledge of NF-κB signalling hinders therapeutic approaches: in many situations it is not clear whether the enhancement or inhibition of NF-κB activity would be beneficial or which pathways to interfere with and what the required level of activation is. Further studies of the role of NF-κB are needed as these may result in novel therapeutic strategies for a wide variety of diseases.

Keywords: nuclear factor-kappaB (NF-κB), inhibitory-kappaB (IκB), IκB kinase complex (IKK), transcription factors

INTRODUCTION

Nuclear factor-kappaB (NF-κB) also known as Rel/NF-κB is a family of evolutionarily conserved transcription factors involved in responses to environmental changes (reviewed in Hayden & Ghosh, 2004). Its structure is conserved in species as distinct as sea anemones and humans. The NF-κB family is one of the most-studied eukaryotic transcription factors. Since its discovery in 1986 (Sen & Baltimore, 1986) intense investigations have shown NF-κB to have a role in a variety of biological processes including the immune response, inflammation, proliferation and apoptosis (reviewed in Hayden & Ghosh, 2004; Xiao, 2004; Courtois, 2005). While our understanding of the physiological role of NF-κB is growing, some recent studies have begun associating the NF-κB transcription factors with numerous diseases including inherited syndromes: incontinentia pigmenti, cystic fibrosis and Duchenne muscular dystrophy as well as cancer, arthritis, asthma, neurodegenerative diseases, and immunodeficiency disorders (Aradhya *et al.*, 2001; Kumar & Borick, 2003; Courtois, 2005; Xiao, 2004).

There are five members of the mammalian Rel/NF-κB family (Fig. 1); p65 (RelA), RelB, c-Rel, p50 (NF-κB1), and p52 (NF-κB2), which are characterized by a 300 amino acid, N-terminal sequence, known as the Rel homology domain (RHD) (reviewed in Chen *et al.*, 2001; Hayden & Ghosh, 2004). Human *Rel/NF-κB* gene loci are on different chromosomes: 11q12, 19, 2p13-p12, 4q23 and 10q24 for RelA, RelB, c-Rel, NF-κB1 and NF-κB2 respectively, with at least one locus (11q12) being a site of frequent involvement in aberration in multiple tumour types (Mathew *et al.*, 1993; Deloukas & van Loon, 1993). Rel/NF-κB proteins can be divided into two classes based on sequences C-terminal to their RHD, which mediate subunit dimerisation, inhibitory-kappaB proteins interaction, nuclear localization and

DNA binding (Aradhya & Nelson, 2001; Beinke & Ley, 2004) (see below and Fig. 1). Members of one class (p50, p52) are produced as precursors (105 and 100 kDa respectively) with long C-terminal domains, which inhibit these molecules. Members of this class become active only after proteolytic processing by the proteasome resulting in cleavage of p50 and p52 from their precursors (Lang *et al.*, 2003). The second class includes c-Rel, RelB and p65 (RelA). All members of NF-κB family (except RelB) form a variety of homo- and heterodimers, which are capable of activating a different gene complement (reviewed in Hayden & Ghosh, 2004; Xiao, 2004). It is this combinatorial assembly that allows for the regulation of distinct sets of genes as the specific dimers have distinct DNA-binding site specificities for the related 9-10 base pair DNA sites (called κB sites) (reviewed in Lenardo & Baltimore, 1989).

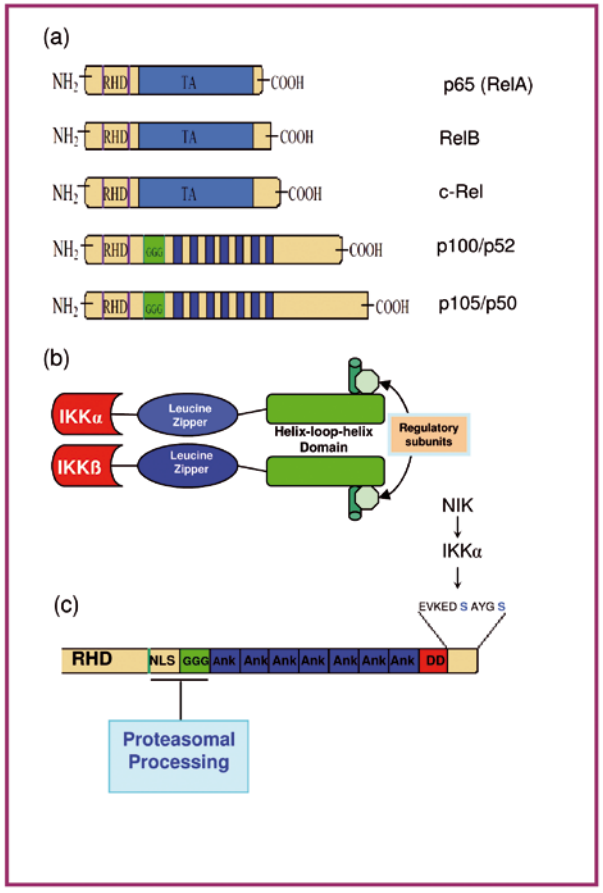

Figure 1. Schematic representation of the Rel/NF-κB proteins family and proteins involved in the NF-κB pathway.
(**a**) Structure of the Rel/NF-κB proteins family. The N-terminal 300 amino-acid RHD domain with the dimerisation domains and NLS. p100 and p105 have a glycine rich hinge region (GGG) between their RHD and C-terminal ankyrin repeats. p65 (RelA), RelB, c-Rel, all contain transactivation domains (TA) as well as RHD. (**b**) IκB kinase (IKK) complex. Leucine zipper mediates interactions between IKK subunits. C-terminal helix-loop-helix domain interacts with both the regulatory subunits and the kinase domain. (**c**) p52 processing occurs *via* NF-κB binding kinase (NIK) stimulation of the IKK complex, followed by IKKα phosphorylating two C-terminal serines on p100. Adjacent to these serine residues is the death domain (DD), of not fully understood function, but which is thought to act as a docking site for IKK, ultimately aiding efficient phosphorylation of the C-terminal serines. The glycine rich hinge region blocks further progression of the proteasome that digests the C-terminal half of p100 after its ubiquitination. Adapted from Bonizzi *et al.*, 2004.

NF-κB ACTIVATION PATHWAYS

NF-κB is maintained in the cytosol bound by Inhibitory-kappaB proteins (IκB). Only when NF-κB has been released it will translocate to the nucleus and bind specific κB sequences in the regulatory regions of target genes (reviewed in Chen *et al.*, 2001; Hayden & Ghosh, 2004; Xiao, 2004; Beinke *et al.*, 2004).

IκBα, IκBβ, BCL-3, IκBε, IκBγ along with the precursor NF-κB proteins p100 and p105 comprise the seven members of the IκB family (Hayden & Ghosh, 2004). IκB proteins retain NF-κB in an inactive form in the cytoplasm by masking nuclear localisation sequences (NLSs) within the RHD. p100 and p105 function as IκBs due to the presence of several (5–7) C-terminal ankyrin repeats responsible for binding the dimerisation domain of NF-κB dimers (Chen *et al.*, 2001). IκBα/NF-κB complexes constantly partake in nuclear–cytoplasmic shuttling as a result of selective NLS masking (Lang *et al.*, 2003; Beinke & Ley, 2004).

NF-κB activity is regulated by phosphorylation of the IκB proteins (Lang *et al.*, 2003; Xiao, 2004). External and internal stimuli promote the phosphorylation of IκB proteins *via* the IκB kinase (IKK) complex (Regnier *et al.*, 1997; Mercurio *et al.*, 1997). The IKK complex phosphorylates IκBα on two N-terminal serine residues, creating a binding site for β-transducin repeat-containing protein (βTrCP), and the receptor subunit of a Skp1/Cul1/F-box (SCF) ubiquitin E3 ligase complex (Beinke *et al.*, 2004). Consequently, rapid polyubiquitination of IκBα on two adjacent lysine residues targets it for degradation by the proteosome (Beinke & Ley, 2004; Hayden & Ghosh, 2004; Campbell *et al.*, 2004; Reynaert *et al.*, 2004). When IκBα and IκBβ bind p65/p50 or p65/c-Rel dimers they only mask the NLS on p65, leaving the NLS on p50 and c-Rel accessible. In conjunction with nuclear export sequences (NES) these accessible NLS allow constant shuttling to the nucleus while the inactive proteins have a preferential cytosolic state (Lang *et al.*, 2003; Beinke & Ley, 2004).

Degradation of IκBα exposes the p65 NLS while removing NES, allowing predominant nuclear

localization of NF-κB (Lang *et al.*, 2003). Hypo-phosphorylated IκBβ, by preventing newly formed IκBα from inactivating NF-κB allows for its continual nuclear localisation, prolonging NF-κB signalling and enhanced transactivation (Chen *et al.*, 2001; Hayden & Ghosh, 2004). Increased stimulation of hypophosphorylated IκBβ resulting in prolonged NF-κB signalling can be detrimental and has been associated with several disease states (discussed below).

The IκB kinase (IKK) complex (700–900 kDa) consists of three components, two catalytic subunits IKKα (IKK1) and IKKβ (IKK2) and the regulatory component: NF-κB essential modifier (NEMO) or IKKγ (reviewed in Aradhya & Nelson, 2001; Agou *et al.*, 2002; Beinke & Ley, 2004; Hayden & Ghosh, 2004). The kinase subunits have homology within their functional domains; both have a kinase, leucine zipper and a helix-loop-helix domain (Fig. 1b). In the heterodimeric state (predominant in the cell), the leucine zipper motifs allow formation of both homo- and heterodimers (Agou *et al.*, 2002). Despite their structural homology, recent experiments involving IKKα- and IKKβ-deficient mice revealed phenotypic differences, suggesting a different functional role for each subunit (Agou *et al.*, 2002). These results support existing evidence describing the two pathways in which NF-κB signalling occurs and the independent role of IKKα and IKKβ within them (Hayden & Ghosh, 2004; Bonizzi & Karin, 2004).

Depending on the stimulus, NF-κB signalling is triggered *via* the classical/canonical or *via* the alternative pathway (reviewed in Bonizzi & Karin, 2004). In the classical or canonical pathway IKKβ is activated by cytokines including TNFα and IL-1, which phosphorylate IκBα on two N-terminal serine residues allowing the translocation of NF-κB p105/c-Rel and p50/p65 heterodimers to the nucleus (Fig. 2A). In order to phosphorylate the IKK complex and thus IκBα, TNFα and IL-1 require MAP 3-Ks (MAP kinase kinase kinases), MEKK3 (MAP kinase/ERK (extracellular-signal-regulated kinase) kinase kinase kinase kinase) and TAK1 (transforming-growth-factor-β-activated kinase (Beinke & Ley, 2004). It is suggested, though not confirmed that the role of IKKα within this pathway lies in histone phosphorylation, thus regulating gene expression (Hayden & Ghosh, 2004).

In the alternative pathway IKKα is activated by NF-κB inducers such as lymphotoxin-β (LTβ), B-cell activating factor (BAFF), receptor activator of NF-κB (RANK), or *via* IKK-α interaction with NF-κB binding kinase (NIK) (Bonizzi *et al.*, 2004) (Fig. 2B). With the exception of p100, subsequently phosphorylated IκBs will undergo polyubiquitination and degradation by the proteasome. In the case of p100 processing generates p52/RelB heterodimers which translocate to the nucleus (Bonizzi & Karin, 2004).

While the activation of the IKK complex is thought to occur *via* NEMO, the specific molecular mechanism behind it remains unclear. NEMO primarily associates with IKKβ *via* the first coiled-coil domain (CC1) within its N-terminus while three further domains constitute NEMO's C-terminus: a second coiled-coil (CC2), leucine zipper (LZ) and zinc finger motif (Agou *et al.*, 2002; Agou *et al.*, 2004). The ability of NEMO to self associate and form oligomers (*via* CC2-LZ antiparallel alignment) is significant for IKK activation and possibly for activator recruitment upstream (Poyet *et al.*, 2000; Li *et al.* 2001; Agou *et al.*, 2002). The ability of NEMO to form oligomers has suggested it as a possible therapeutic target in disease states where the inhibition of NF-κB activity is required. Agou *et al.* (2002) induced cell death in human retinoblastoma cells by targeting the oligomerisation of NEMO. Consequently, these experiments have revealed a novel and promising therapeutic strategy where the inhibition of the NF-κB signaling pathway may improve a disease state.

The activation of the NF-κB pathway is generally a transient process, in most cells lasting a short time (30–60 min). To prevent constant NF-κB signalling, down regulation of NF-κB is achieved *via* multiple mechanisms within a feedback loop. In the canonical pathway, one of the target genes activated by NF-κB is that which encodes IκBα. Newly-synthesized IκBα can enter the nucleus, remove NF-κB from DNA, and export the complex back to the cytoplasm, where it remains in an inactive state until stimulated again (Beinke & Ley, 2004).

New evidence suggests the control of the NF-κB pathway is more complex than IKK-mediated regulation only. NF-κB activation described above is termed "typical" and involves inflammatory cytokines (TNF, IL-1) or bacterial products such as lipopolysaccharides (LPS) (Hayden & Ghosh, 2004). "Atypical" activators of the NF-κB signaling pathway include chemotherapeutic compounds and UV-C (Mercurio *et al.*, 1997). These "atypical" activators induce NF-κB signaling differently from the "typical" ones. Several studies have suggested that the "atypical" activators do not phosphorylate serine 32 and 36 of IκBα as they operate in an IKK-independent manner (Bender *et al.*, 1998; Tergaonkar *et al.*, 2003; Kato *et al.*, 2003). In contrast, other studies suggest the IKK complex is required for the atypical activation: the regulatory zinc finger domain within NEMO not involved during typical activation, appears to be required for atypical activation (Huang *et al.*. 2002; Campbell *et al.*, 2004). Furthermore, hypoxic injury and oxidative stress, two other forms of "atypical" stimuli appear to induce NF-κB activity in a process resulting in tyrosine phosphorylation of IκBα (Campbell *et al.*, 2004).

Bearing in mind the complexity of this system, it is not surprising that while the basic understanding of NF-κB signalling is extensive, still little is know of the *in vivo* dynamics of this pathway. The contributions of specific NF-κB dimers to a specific physiological response or what controls the balance between the levels of the various heterodimers are not known. Moreover, although we know a large number of NF-κB-responsive genes, it is often not clear how or which of these induced genes contributes to that response. Finally, only recently we have begun to unravel the involvement of NF-κB in pathological processes. The second part of this review will briefly summarise the major known effects of NF-κB stimulation in human physiology and pathology and present the recently uncovered involvement of NF-κB in pathological processes.

FUNCTIONAL SIGNIFICANCE OF NF-κB

Immune responses and inflammation

One of NF-κB most widely studied roles lies with its ability to activate numerous genes involved in a cumulative response to infection. Dendritic cells, macrophages and mucosal epithelial cells express cell surface Toll-like receptors (TLRs) — an important part of the innate immunity, responsible for the detection of microbial molecules (e.g. nucleic acids and bacterial cell wall lipopolysaccharides — LPS). Once stimulated, TLRs activate the NF-κB transcription factor-signaling pathway. Activation of this pathways triggers expression, amongst others, of a number of chemokines such as: RANTES (regulated upon activation, normal T-cell expressed and secretes) and the macrophage inflammatory protein-1α (MIP-1α); pro-inflammatory cytokines: interleukin-1 and -6 (IL-1, IL-6) and the tumor necrosis factor-α (TNFα); adhesion molecules: E-selectin and the vascular cell adhesion molecule-1 (VCAM-1) (reviewed in Liou, 2002; Beinke & Ley, 2004).

Once released, many of these proteins (e.g. TNFα and IL-1) can again activate the NF-κB pathway, leading to amplification of the response. Apart from amplifying and extending innate immunity by inducing effector molecules such as defensins (which possess microbicidal activity) and stimulating production of reactive intermediates such as nitric oxide, NF-κB also provides a molecular link between the innate and adaptive immune systems. It is involved in up-regulation of MHC proteins and CD80/86 on antigen presenting cells resulting in a T-cell mediated response (reviewed in Li & Verma, 2002; Beinke & Ley, 2004). NF-κB role continues through the adaptive immune response, activating both antigen and co-stimulatory receptors on T and B lymphocytes as well as stimulating the B-cell activating factor (BAFF) to induce B-cell differentiation and survival. In addition to its involvement in immune responses, NF-κB activity is required during haemapoiesis and in the regulation of peripheral lymphoid organogenesis (reviewed in Li & Verma, 2002; Weih & Caamona, 2003; Beinke & Ley, 2004; Siebenlist *et al.*, 2005).

Activation of innate immunity, while normally producing self-limited inflammatory responses, can sometimes progress to tissue injury. In lungs it has been associated with sustained NF-κB activation and blockade of the NF-κB pathway can be beneficial (Everhart *et al.*, 2006). Rheumatoid arthritis and Crohn's disease are chronic inflammatory disorders associated with an overproduction of pro-inflammatory cytokines. There is convincing data suggesting that this might be a consequence of NF-κB dysfunction (Burns & Martinon, 2004; Schottelius & Dinter, 2006). Consequently, studies of an effective pharmacological modulation of the NF-κB pathways are underway. Particularly in arthritis both the available therapeutic agents: non-steroidal anti-inflammatory drugs and corticosteroids as well as biologicals, small molecule blockers of the NF-κB pathways, antisense DNA and RNA interference are used as disease-modifying anti-rheumatic drugs with good results (Roman-Blas & Jimenez, 2006). The perpetuated activation of NF-κB in patients with active inflammatory bowel disease is also a very attractive target for therapeutic intervention.

Apoptosis, cell-cycle regulation, oncogenesis and beyond

There is evidence that cytokine-induced activation of NF-κB is an important component of the signal triggering apoptosis in Langerhans β cell. Studies in an inducible transgenic mouse model expressing NF-κB protein inhibitor acting specifically in β cells showed that activation of NF-κB is the main event leading to loss of β cells in diabetes (Eldor *et al.*, 2006). Based on similar observations NF-κB was thought to be a pro-apoptotic molecule.

However, other studies suggest an anti- rather than pro-apoptotic role for NF-κB when activated by an apoptotic stimulus (Chen *et al.*, 2001; Shishodia & Aggarwal, 2002). For example, when activation of TNF receptors triggers NF-κB signalling this promotes cell survival rather than cell death. It is mediated through a suppression of the reactive oxygen species generation and prevention of sustained activation of the Jun-N-terminal kinase (JNK) cascade (Papa *et al.*, 2006). Several murine knockout models (summarized in Table 1) clearly indicated a role for NF-κB in preventing apoptosis linking it with sever-

Table 1. Results of the murine knockout studies where genes encoding specific members of the NF-κB family or the activating kinases were disrupted.

Adapted from Chen *et al.*, 2001.

Geneotype	Phenotype
p65$^{-/-}$ *(ReIA*$^{-/-}$*)*	Apoptosis of hepatocytes, lethal during embryonic development
IKKβ$^{-/-}$	Extensive liver apoptosis, lethal during embryonic development
IKKβ$^{-/-}$/*IKKα*$^{-/-}$ (double knockout)	Extensive liver apoptosis, lethal during embryonic development (similar to above)
IKKα$^{-/-}$	Keratinocyte deficiency: increased thickness of skin, perinatal lethality
Male *NEMO*$^{-/-}$ (X-linked gene)	Extensive apoptosis of cortical and medullar lymphocytes in the thymus, degeneration of liver, lethal by mid-gestation
Female *NEMO*$^{-/-}$	Apoptosis of keratinocytes, abnormal pigmentation, similar to human *Incontinentia pigmenti*
Cross-breed: *p65*$^{-/-}$ or *1KKβ*$^{-/-}$ *TNF-R1*$^{-/-}$ or *TNF-α*	Partial rescue of embryonic lethality. Indicating that NF-κB increases cell vulnerability to TNF-α mediated cytotoxicity

al anti-apoptotic genes: *cyclin D*, *Blf-1* and *Bcl-xl*, *Bcl-2* (the mitochondrial membrane-stabilizing proteins), *TRAF1* and *TRAF2* (TNF receptor-associated factors) and proteins regulating cellular calcium homeostasis (Chen *et al.*, 2001; Torchinsky *et al.*, 2002; Hong & Jung, 2002). This pro-survival activity of NF-κB affects a wide range of biological processes. For example, NF-κB is a major contributing factor in liver regeneration after a partial hepatectomy possibly resulting from NF-κB regulating anti-apoptotic or survival genes that prevent stress-related cell death (Chen *et al.*, 2001; Campbell *et al.*, 2004). The teratogenic effect of cyclophosphamide in the developing brain (foetal anencephaly) is caused by excessive apoptosis due to suppression of NF-κB signalling by the drug (Torchinsky *et al.*, 2002).

Interestingly, both anti-apoptotic and pro-apoptotic effects of NF-κB have been observed in Alzheimer's disease: Activation of NF-κB triggered by secreted β-amyloid precursor protein was neuroprotective (Guo *et al.*, 1998) while β-amyloid 40 (required to form mature plaques) activated the nuclear translocation of p50/p65 dimers and promoted a pro-apoptotic profile of gene expression. Inhibitors of the NF-κB pathway and the NF-κB decoy inhibited this effect suggesting that specific targeting of p50/p65 dimers could be considered for pharmacological intervention in Alzheimer's disease (Valerio *et al.*, 2006). However, this example clearly illustrates how our still incomplete knowledge of NF-κB signalling hinders therapeutic approaches: in many situations it is not clear whether the enhancement or inhibition of NF-κB activity would be beneficial, which pathways to interfere with and what the required level of activation is. Future studies should address the cross-talk between various signalling pathways to gain a clearer understanding of how suggested anti-apoptotic signalling interactions of NF-κB coincide with apoptotic features within a cell.

In response to stress signals, in addition to apoptosis NF-κB can influence the cell-cycle. Human *cyclin D1* and *D3* genes have NF-κB binding sites and, in certain cells, elevated NF-κB activity has been observed between the phases G_0–G_1 of the cell cycle (Hinz *et al.*, 1999). All these suggest involvement of this transcription factor in the regulation of cell cycle (Wang *et al.*, 1996; Guttridge *et al.*, 1999).

As a consequence of NF-κB functioning in apoptosis and in cell cycle regulation this family of transcription factors is of considerable interest when oncogenesis is concerned. The discovery of v-Rel, the oncogenic retroviral c-Rel homologue, responsible for oncogenesis in avian lymphoid cells, was the direct link of NF-κB to oncogenesis (Gilmore *et al.*, 1996). Since then, evidence associating NF-κB with oncogenesis begun to accumulate. However, the role of NF-κB is more complex, as in some cases NF-κB inhibition blocks while in others it promotes tumour development. Enhanced NF-κB activity observed in carcinoma cells is thought to be an important factor in maintaining their survival (Dolcet *et al.*, 2005). This factor has been shown to be elevated in several types of cancer e.g. breast, thyroid, bladder and colon, and several chromosomal alterations involving members of the NF-κB family (c-Rel, p52 and p65) are located within break point regions in diseases such as non-Hodgkin's lymphoma and leukaemia (Chen *et al.*, 2001; Dolcet *et al.*, 2005). Detailed description of this complex role of NF-κB in cancer development, progression and treatment has been discussed in a number of recent reviews (Dolcet *et al.*, 2005; Karin, 2006; Radhakrishnan & Kamalakaran, 2006; Kim *et al.*, 2006; Pikarsky & Ben-Neriah, 2006).

A novel, emerging role of the NF-κB-dependent gene activation associated with regulation of yet another cellular process has been identified in neurones. NF-κB has a role in protecting the auditory neurons from excitotoxic damage and age-related degeneration by helping to maintain calcium homeostasis (Lang *et al.*, 2006). In addition, NF-κB is activated by cerebral ischemia in neurons and astrocytes. In a mouse model of stroke carrying targeted

deletions of IKK proteins in neurons, inhibition of IKK activity reduced lesion size. Likewise, a selective IKK inhibitor mimicked the effect of genetic IKK ablation. This points to NF-κB activation in neurons as an important factor contributing to the ischemic damage in these cells (Zhang *et al.*, 2005; Herrmann *et al.*, 2005). Pharmacological inhibition of this pathway may represent a specific target for stroke treatment.

Unexpectedly, NF-κB has been linked with gene transactivation by synthetic polymers. Pluronic block copolymers, seemingly inert and safe polymer pharmaceuticals used in drug formulations and gene therapy can transactivate endogenous genes *in vitro* and *in vivo*. This activation involves NF-κB pathways (Lavigne *et al.*, 2005; Sriadibhatla *et al.*, 2005). However, the mechanism behind pluronic ability to enhance expression of NF-κB-dependent genes or the compliment of genes that is activated and the biological consequences of such activation remain unclear. Analysis of this system may unearth yet another role for the NF-κB and/or yet another NF-κB signalling pathway with potential toxicological and therapeutic consequences.

NF-κB AND GENETIC DISORDERS

Incontinentia pigmenti and primary immunodeficiencies

Incontinentia pigmenti (IP) was the first genetic disorder to be associated with NF-κB dysfunction. IP is an X-linked dominant disease (Aradhya *et al.*, 2001; Smahi *et al.*, 2002; Gosselin & Abbadie, 2003). While lethal in males, in females due to the randomised mosaic expression, the severity of the disease varies between individuals (Aradhya *et al.*, 2001; Martinez-Pomar *et al.*, 2005). Symptoms include hair loss, nail dystrophy, narrowed teeth, and skin lesions ranging from erythematous skin to a hypopigmented mosaic expression, dysfunctions within central nervous system and retinal detachment resulting in blindness. In 85% of patients IP is caused by a large deletion within the *NEMO* gene (*NEMO*Δ4-10) (Aradhya & Nelson, 2001; Gosselin *et al.*, 2003; Smahi *et al.*, 2002). In the remaining 15% of cases IP arises as a result of small mutations within the *NEMO* gene, including deletions, substitutions and duplications. NEMO, as discussed previously, is a critical component of the NF-κB pathway. In rare cases, where the NEMO mutation does not completely abolish NF-κB activity male, survival is possible. This allelic variant of IP is known as *hypohidrotic ectodermal dysplasia and immunodeficiency* (HED-ID) (Smahi *et al.*, 2002). NEMO knockout experiments showed male lethality at the embryonic day 13 as a result of hepatocyte apoptosis in the absence of NEMO (Aradhya *et al.*, 2001). As expected, *IKKβ* knockout mice display similar phenotypes to the NEMO knockout (as NEMO is required to activate IKKβ), so in the absence of either of these two proteins the subsequent stages within the classical pathway will not occur (Aradhya & Nelson, 2001; Hayden & Ghosh, 2004, Beinke & Ley, 2004). TNF-α is thought to be the causative agent in the development of IP lesions and associated abnormalities (Gosselin & Abbadie, 2003). Signalling *via* NF-κB is abnormal in IP sufferers, leaving cells susceptible to apoptosis induced by TNF-α. p65 (RelA) is specifically associated with the regulation of apoptosis and the mouse p65 knockout revealed embryonic lethality due to hepatocyte apoptosis, presenting similar phenotypes to the murine IP models (Aradhya & Nelson, 2001; Beinke & Ley, 2004). Females (NEMO heterozygotes) were also phenotypically similar to human IP patients, however retinal and central nervous system dysfunctions observed in humans have not been observed in heterozygote mice models. Better understanding of the pathogenesis of IP can be achieved by elucidating the precise time and specific site of NEMO expression along with the NF-κB downstream target genes (Aradhya & Nelson, 2001).

Three primary immunodeficiencies have been associated with mutations in genes involved in NF-κB signalling. X-linked recessive anhidrotic ectodermal dysplasia (EDA) with immunodeficiency is also caused by specific mutations in the gene encoding NEMO that does not completely abolish its function. Autosomal dominant form of this disease results from a mutation in the gene encoding IκBα. Patients with these diseases are at increased risk of severe infections (apart from suffering from defects in morphogenesis of ectodermal structures: e.g. dry skin, conical teeth and thin hair). Finally, autosomal recessive immunodeficiency without EDA is caused by mutations in the gene encoding interleukin-1 receptor-associated kinase-4 (IRAK-4), a protein with a role upstream from the IKK complex (reviewed in Puel *et al.*, 2004).

Paget's disease of bone and familial expansile osteolysis

Ossification and bone resorption are two processes required for adequate bone morphogenesis and regulated by specific signalling pathways. Paget's disease of the bone (PGD) is a common disorder in individuals over 40 that arises as a result of progressive bone resorption by osteoclasts and medullary bone expansion (Rouserie *et al.*, 2003; Michou *et al.*, 2006). Initially, the onset of PGD was associated with a viral infection while current studies are focusing

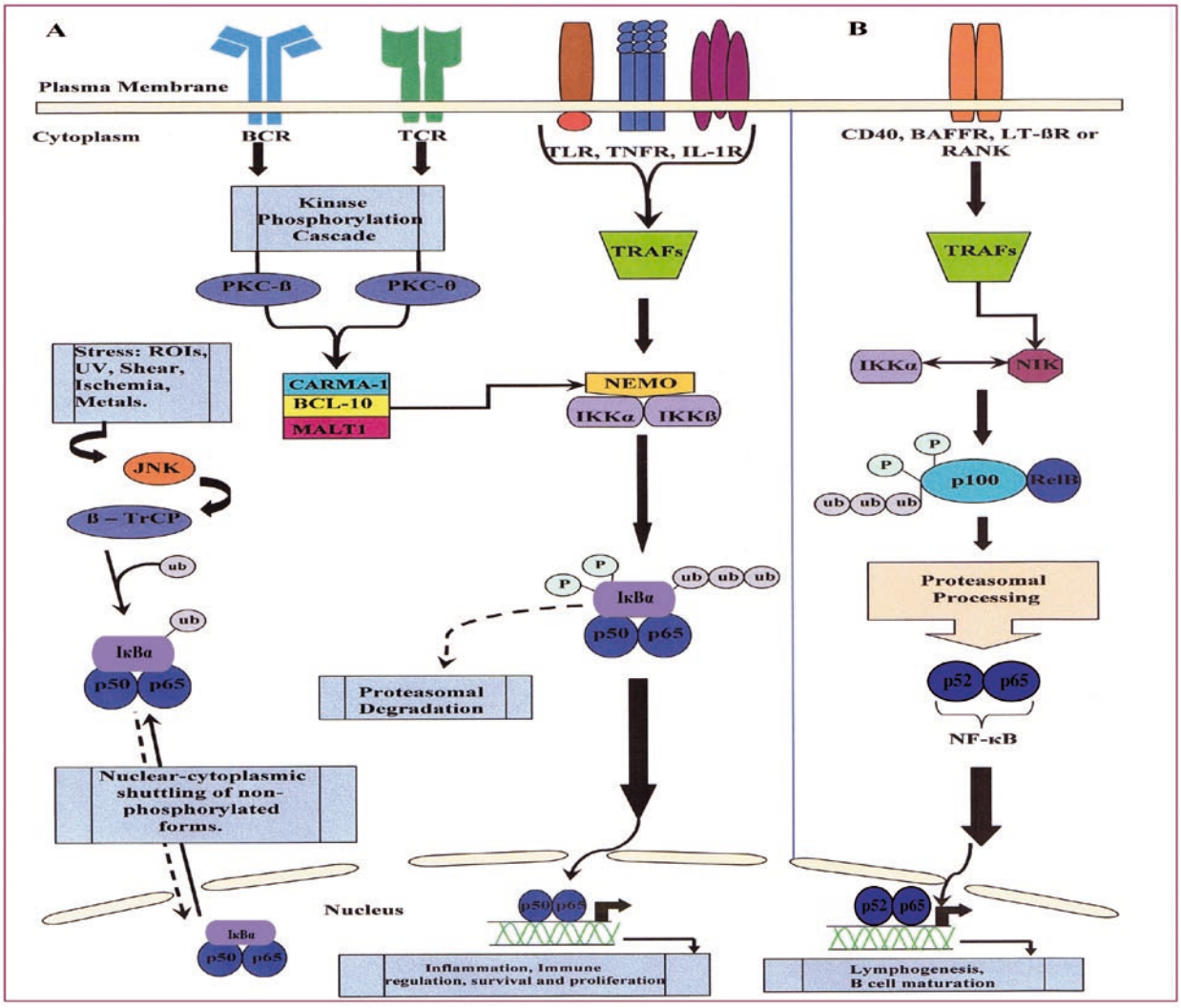

Figure 2. NF-κB signalling pathways.
A. Two classes of receptors are responsible for the activation of NF-κB. Tumour necrosis factor receptor (TNFR), Interleukin-1 receptor (IL-1R) and members of the Toll-like receptor family (TLR) are members of the first class. They signal mainly through the canonical pathway, *via* various kinases and modifiers including members of the TRAF (TNFR-associated factor) family. The T-cell receptor (TCR) and B-cell receptor (BCR) activate NF-κB *via* a phosphorylation cascade involving B-lymphoid kinase (BLK), spleen tyrosine kinase (SYK), Bruton's tyrosine kinase (BTK), src-family kinases p56(lck) (Lck) and p59(fyn) (Fyn) and ξ-chain-associated protein kinase (ZAP70), leading to the activation of protein kinase C-β (PKC-β) and PKC-θ. Following that the IKK complex is activated *via* (CARMA1) the caspase-recruitment domain (CARD) — membrane-associated guanylate kinase (MAGUK) protein1, B-cell lymphoma 10 (BCL-10) and mucosa-associated lymphoid-tissue lymphoma translocation gene 1 (MALT1). **B.** The activation of both pathways can occur *via* the second class of receptors which include: lymphotoxin-β receptor (LT-Rβ), receptor activator of NF-κB (RANK), CD40 and B-cell-activating receptor (BAFFR). In the classical pathway upstream signals activate IKK resulting in the phosphorylation of IκBα leading to its subsequent ubiquitination and degradation by the proteasome. Once released, NF-κB translocates to the nucleus and binds specific sequences in the regulatory regions of target genes. The alternative pathway is regulated by TRAFs, NIK and IKKα. Processing of p100 occurs *via* this pathway. The "atypical" activators of the NF-κB signaling pathway such as stress and UV: the activation of NF-κB by these stimuli is very different to its activation by inflammatory cytokines. It is occurring *via* c-Jun NH_2-terminal kinase (JNK) and β-TrCP E3 ligase signalling. IκBα/NF-κB nuclear-cytoplasmic shuttling is also demonstrated here (Huang *et al.*, 2002; Reynaert *et al.*, 2004). For more details see text.

on genetic predisposition (Rouserie *et al.*, 2003; Michou *et al.*, 2006). An autosomal dominant pattern of inheritance is displayed in familial PGD with incomplete penetrance. Eight potential loci have been identified, providing clues for genetic heterogeneity. Only 5% of patients present with symptoms, which include bone pain, disabling bone deformities and local hypervascularisation (Michou *et al.*, 2006). Nine mutations on chromosome 5p within the Sequestosome (*SQSTM1*) gene are associated with PGD. All these alter the ubiquitination domain resulting in the accumulation of the p62 protein causing the in-

creased activation of NF-κB signalling (Aradhya & Nelson, 2001; Michou *et al.*, 2006). Genetic studies have indicated that several elements of NF-κB pathway: receptor activator of NF-κB (RANK), its ligand RANKL and the decoy receptor OPG are essential regulators of osteoclast development and function (Aradhya & Nelson, 2001; Rousiere *et al.*, 2003). Mutations in RANK are found in familial expansile osteolysis (FEO), which is characterised by damage to the appendicular skeleton and osteolytic lesions. The defective RANK protein is thought to enhance NF-κB activity (Rousiere *et al.*, 2003; Wada *et al.*, 2006). Deregulation of NF-κB in the abnormally dense bone was further confirmed by identification of a mutation in NEMO resulting in osteopetrosis (Schmid *et al.*, 2006). The NF-κB signalling in osteoclast differentiation and bone homeostasis may also represent a therapeutic target in rheumatoid arthritis (reviewed in Jimi & Ghosh, 2005).

Cystic fibrosis

Cystic fibrosis (CF) is the most common autosomal recessive disorder in European Caucasians affecting 1/2500 live births. A systemic disease, with a life expectancy of <30 years, it is more common in the Western world, where 1/25 people are carriers. The defective gene, *Cystic fibrosis transmembrane conductance regulator* (*CFTR*) codes for a chloride ion channel (Hudson, 2001). *CFTR* is involved in the movement of ions and water predominantly in respiratory, gastrointestinal, hepatobiliary and reproductive systems (Minasian *et al.*, 2005). Although the most common mutation in CF is the deletion of phenylalanine 508 (ΔF508), due to a large number of other mutations, sufferers express phenotypes with different severity (Hudson, 2001).

Airway inflammation, a common characteristic of CF was thought to be due to the chronic bacterial infection in the thick and sticky mucus covering the airways (Blackwell *et al.*, 2001; Minasian *et al.*, 2005). Recent evidence however suggested that airway inflammation may also occur in the absence of a bacterial infection and perhaps it is an alteration within the inflammatory pathway itself that leads to the CF phenotype (Blackwell *et al.*, 2001; Minasian *et al.*, 2005). NF-κB initiates the expression of pro-inflammatory cytokines, some of which have been associated with CF pathogenesis (Blackwell *et al.*, 2001). Weber *et al.* (2001) described the enhanced activation of the NF-κB signalling pathway along with increased levels of pro-inflammatory cytokines in lung epithelial cells expressing the mutant *CFTR*. Although the specific dysregulation of the NF-κB pathway within CF epithelial cells has not been delineated, Blackwell *et al.* (2001) have demonstrated the increased production of hypo-phosphorylated IκBβ, resulting in extended and enhanced NF-κB signalling. As the link between NF-κB and the most common *CFTR* mutations emerges, specific targeting of the NF-κB pathway may lead to a reduced inflammatory response and help in slowing down the progression of CF in many patients.

Muscular dystrophies and secondary skeletal muscle loss

The loss of skeletal muscle (muscle wasting, muscle atrophy) occurs through the ubiquitin-proteasome pathway as a result of increased levels of proinflammatory cytokines such as TNF-α (Kumar & Borick, 2003; Glass, 2005). Many diseases (i.e. chronic heart failure, cancer and AIDS) are associated with body wasting and muscle loss (*cachexia*) in particular, which causes profound fragility in the patients that complicate therapy and can accelerate death.

In muscular dystrophy (MD) muscle wasting is a result of primary genetic defects. However, in various types of MD increased levels of circulating TNF-α and IL-1β have been observed (Kumar & Borick, 2003). While this increase of TNF-α and IL-1β has been suggested as a possible trigger event leading to muscle wasting *via* NF-κB signalling, the molecular machinery behind this remains unclear (Langen *et al.*, 2001; Kumar & Borick, 2003). Three mechanism have been proposed: (i) activation of NF-κB results in the enhanced expression of IL-1β, IL-6, TNF-α, MMP-9 and cell adhesion molecules that have the potential to directly promote muscle wasting *via* a positive feedback loop (Hunter *et al.*, 2002; Kumar & Borick, 2003), (ii) NF-κB amplifies the ubiquitin/proteasome pathway (Garcia-Martinez *et al.*, 1995; Reid *et al.*, 2001; Hunter *et al.*, 2002; Kumar & Borick, 2003), or (iii) NF-κB interfering with skeletal muscle differentiation results in the inhibition of tissue repair (Llovera *et al.*, 1997; Kumar & Borick, 2003). Thaloor *et al.* (1999) demonstrated that, in line with a regulatory role for NF-κB in myogenesis, pharmacological inhibition of NF-κB stimulated cell proliferation and differentiation *in vitro* and enhanced muscle regeneration after a trauma *in vivo*.

Duchenne muscular dystrophy (DMD), an X-linked recessive disorder, is the second most common inherited disorder in man (1 in 3500 live male births) (Nowak & Davies, 2004). Mutations in the *DMD* gene result in progressive muscle wasting and are lethal by early adulthood. DMD gene product, dystrophin, is a cytoplasmic protein adjacent to the sarcolemmal membrane in myocytes (Nowak & Davies, 2004; Lovering *et al.*, 2005). It forms an interface between the intracellular contractile apparatus and the extracellular tissue matrix by bridging actin cytoskeleton and the dystrophin associated protein

complex (DAPC) (Nowak & Davies, 2004). Abnormal function of this complex of proteins in transferring the force of contraction to connective tissue and in signal transduction has been proposed to be the cause for myocyte degeneration that occurs in the absence of dystrophin (Nowak & Davies, 2004). Experiments in the *mdx* mouse, a model for DMD, indicated that the lack of dystrophin results in the increased vulnerability of muscle fibres to mechanical stresses, contraction induced damage and hypo-osmotic shock (Kumar & Borick, 2003). In turn, mechanical stretch of skeletal muscle increases IKK activity and subsequent IκB degradation, activating the canonical NF-κB pathway. Interestingly, blockade of NF-κB reduced muscle degeneration and improved function in *mdx* mice (Messina *et al.*, 2006). Acharyya *et al.* (2005) recently demonstrated a regulatory link between tumour cachexia and muscular dystrophy, and specifically demonstrated DAPC inhibiting skeletal muscle atrophy pathways. Yet another muscular dystrophy (limb-girdle muscular dystrophy type 2A, calpain 3 deficiency) is associated with a profound perturbation of the NF-κB pathway and myonuclear apoptosis (Baghdiguian *et al.*, 1999). These discoveries of a role for the NF-κB signalling pathway in inherited and secondary skeletal muscle wasting suggest that NF-κB transactivation might be a potential therapeutic target in some of these diseases (Thaloor *et al.*, 1999; Kumar & Borick, 2003; Nowak & Davies, 2004).

CONCLUSIONS

NF-κB is a pleiotropic transcription factor. It is involved in the transcriptional activation of numerous genes leading to a cumulative immunogenic response, provides a molecular link between the innate and adaptive immune system, whilst playing regulatory roles in haemapoiesis and lymphoid organogenesis (Li *et al.*, 2002; Liou, 2002; Weih & Caamona, 2003; Hayden & Ghosh, 2004; Beinke & Ley, 2004). The over-production of pro-inflammatory cytokines, a possible consequence of NF-κB dysfunction, has been associated with several diseases including rheumatoid arthritis and Crohn's disease (Burns & Martinon, 2004; Schottelius & Dinter, 2006). Furthermore, NF-κB has been linked to the two most common inherited disorders, cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD) (Blackwell *et al.*, 2001; Kumar & Borick, 2003), and is also associated with Paget's disease of bone (PGD). In CF, DMD, PGD and muscle atrophy it is the up-regulation of NF-κB activity that seems to have the detrimental effect (Christman *et al.*, 2000; Blackwell *et al.*, 2001; Reid & Li, 2001; Kumar & Borick, 2003; Rousiere *et al.*, 2003; Minasian *et al.*, 2005; Michou *et al.*, 2006). In contrast, specific primary immunodeficiencies and incontinentia pigmenti arises from the loss of NF-κB function caused by mutations in the genes encoding NEMO, IκBα and IRAK-4 (Aradhya & Nelson, 2001; Smahi *et al.*, 2002). Although initial studies suggested NF-κB to be an apoptotic molecule the results of several murine knockout studies showed that NF-κB may have an anti-apoptotic role, specifically in the protection against stress-related cell death (Chen *et al.*, 2001; Torchinsky *et al.*, 2002; Campbell *et al.*, 2004). In addition, NF-κB seems to have a role as a cell-cycle regulator. Taken together, this versatility provides some explanation for the interplay observed between a number of seemingly unrelated processes, both physiological and pathological, e.g. chronic inflammation and specific forms of cancer or inherited muscular dystrophy and secondary muscle wasting. However, we are often lacking sufficient understanding of the underlying mechanisms. Little is known of the *in vivo* dynamics of this pathway or of the contributions of particular NF-κB elements to specific physiological/pathological processes. It is not clear how or which of a large number of NF-κB-responsive genes contribute to a specific response. Therefore, although the NF-κB transcription factor seems to be a valid therapeutic target in a number of diseases, since it plays such a central role in body homeostasis its long-term general suppression or over-expression would cause severe side effects in non-target tissues and organs. Even within a specific tissue, due to the widespread expression of NF-κB, application of decoys or inhibitors may produce a desired effect in one cell type (e.g. inflammatory response cells) but also an additional, unwanted consequence in other cells in this tissue. In order to progress with therapeutic strategies aimed at NF-κB modulation a better understanding of this pathway in specific diseases is essential. One of the available options could be to link specific inhibitors/activators to a cell-type specific delivery system.

REFERENCES

Acharyya S, Butchbach ME, Sahenk Z, Wang H, Saji M, Carathers M, Ringel MD, Skipworth RJ, Fearon KC, Hollingsworth MA, Muscarella P, Burghes AH, Rafael-Fortney JA, Guttridge DC (2005) Dystrophin glycoprotein complex dysfunction: a regulatory link between muscular dystrophy and cancer cachexia. *Cancer Cell* **8**: 421–432.

Agou F, Ye F, Goffinont S, Courtois G, Yamaoka S, Israel A (2002) NEMO Trimerizes through Its coiled-coil C-terminal domain. *J Biol Chem* **277**: 17464–17475.

Agou F, Courtois G, Chiaravalli J, Baleux F, Coic YM, Traincard F, Israel A, Veron M (2004) Inhibition of NF-kappa B activation by peptides targeting NF-kappa B essential modulator (NEMO) oligomerization. *J Biol Chem* **279**: 54248–54257.

Aradhya S, Nelson DL (2001) NF-κB signaling and human disease. *Curr Opin Genet Dev* **11**: 300–306.

Aradhya S, Woffendin H, Jakins T, Bardaro T, Esposito T, Smahi A (2001) A recurrent deletion in the ubiquitously expressed NEMO (IKK-gamma) gene accounts for the vast majority of incontinentia pigmenti mutations. *Hum Mol Genet* **10**: 2171–2179.

Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LV, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS, Lefranc G (1999) Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkappaB alpha/NF-kappaB pathway in limb-girdle muscular dystrophy type 2A. *Nat Med* **5**: 503–511.

Beinke S, Belich MP, Ley SC (2002) The death domain of NF-κB1 p105 is essential for signal-induced p105 proteolysis *J Biol Chem* **277**: 24162–24168.

Beinke S, Ley SC (2004) Functions of NF-κB1 and NF-κB2 in immune cell biology. *Biochem J* **382**: 393–409.

Bender K, Gottlicher M, Whiteside S, Rahmsdorf HJ, Herrlich P (1998) Sequential DNA damage-independent and -dependent activation of NF-kappaB by UV. *EMBO J* **17**: 5170–5181.

Blackwell TS, Stecenko AA, Christman JW (2001) Dysregulated NF-κB activation in cystic fibrosis: evidence for a primary inflammatory disorder. *Am J Physiol Lung Cell Mol Physiol* **281**: 69–70.

Bonizzi G, Karin M (2004) The two NF-κB pathways and their role in innate and adaptive immunity. *Trends Immuno* **25**: 280–288.

Bonizzi G, Bebien M, Otero DC, Johnson-Vroom KE, Cao Y, Vu D, Jegga AG, Aronow BJ, Ghosh G, Rickert RC, Karin M (2004) Activation of IKKalpha target genes depends on recognition of specific kappaB binding sites by RelB:p52 dimers. *EMBO J* **23**: 4202–4210.

Burns KA, Martinon F (2004) Inflammatory diseases: is ubiquitinated NEMO at the Hub? *Curr Biol* **14**: 1040–1042.

Campbell KJ, Rocha S, Perkins ND (2004) Active repression of antiapoptotic gene expression by RelA(p65) NF-κB. *Mol Cell* **13**: 763–764.

Chen F, Castranova V, Shi X (2001) New insights into the role of nuclear factor-κB in cell growth regulation. *Am J Pathol* **159**: 387–397.

Christman JW, Sadikot RT, Blackwell TS (2000) The role of nuclear factor-κB in pulmonary diseases. *Chest* **117**: 1482–1487.

Courtois G (2005) The NF-kappaB signalling pathway in human genetic diseases. *Cell Mol Life Sci* **62**: 1682–1691.

Deloukas P, van Loon AP (1993) Genomic organization of the gene encoding the p65 subunit of NF-kappa-B: multiple variants of the p65 protein may be generated by alternative splicing. *Hum Mol Genet* **2**: 1895–1900.

Devin A, Lin Y, Yamaoka S, Li ZW, Karin M, Liu ZG (2001) The α and β subunits of IκB kinase (IKK) mediate TRAF2-dependent IKK recruitment to tumour necrosis factor (TNF) receptor 1 in response to TNF. *Mol Cell Biol* **21**: 3986–3994.

Dolcet X, Llobet D, Pallares J, Matias-Guiu X (2005) NF-kB in development and progression of human cancer. *Virchows Arch* **446**: 475–482.

Eldor R, Yeffet A, Baum K, Doviner V, Amar D, Ben-Neriah Y, Christofori G, Peled A, Carel JC, Boitard C, Klein T, Serup P, Eizirik DL, Melloul D (2006) Conditional and specific NF-kappaB blockade protects pancreatic beta cells from diabetogenic agents. *Proc Natl Acad Sci USA* **103**: 5072–5077.

Everhart MB, Han W, Sherrill TP, Arutiunov M, Polosukhin VV, Burke JR, Sadikot RT, Christman JW, Yull FE, Blackwell TS (2006) Duration and intensity of NF-kappaB activity determine the severity of endotoxin-induced acute lung injury. *J Immunol* **176**: 4995–5005.

Garcia-Martinez C, Llovera M, Agell N, Lopez-Soriano FJ, Argiles JM (1995) Ubiquitin gene expression in skeletal muscle is increased during sepsis: involvement of TNF-alpha but not IL-1. *Biochem Biophys Res Commun* **217**: 839–844.

Gilmore TD, Koedood M, Piffat KA, White DW (1996) Rel/NF-κB/IκB proteins and cancer. *Oncogene* **13**: 1367–1378.

Glass DJ (2005) Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* **37**: 1974–1984.

Gosselin K, Abbadie C (2003) Involvement of Rel/NF-κB transcription factors in senescence. *Exp Gerontol* **38**: 1271–1283.

Guo Q, Robinson N, Mattson MP (1998) Secreted β-amyloid precursor protein counteracts the proapoptotic action of mutant presenilin-1 by activation of NF-κB and stabilization of calcium homeostasis. *J Biol Chem* **273**: 12341–12351.

Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr (1999) NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol* **19**: 5785–5799.

Hayden MS, Ghosh S (2004) Signalling to NF-κB. *Genes Dev* **18**: 2195–2224.

Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, Malfertheiner M, Kohrmann M, Potrovita I, Maegele I, Beyer C, Burke JR, Hasan MT, Bujard H, Wirth T, Pasparakis M, Schwaninger M (2005) IKK mediates ischemia-induced neuronal death. *Nat Med* **11**: 1322–1329.

Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M (1999) NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol* **19**: 2690–2698.

Hong GS, Jung YK (2002) Caspase recruitment domain (CARD) as a bi-functional switch of caspase regulation and NF-κB signals. *J Biochem Mol Biol* **35**: 19–23.

Huang TT, Feinberg SL, Suryanarayanan S, Miyamoto S (2002) The zinc finger domain of NEMO is selectively required for NF-κB activation by UV radiation and topoisomerase inhibitors. *Mol Cell Biol* **22**: 5813–5825.

Hudson VM (2001) Rethinking cystic fibrosis pathology: the critical role of abnormal reduced glutathione (GSH) transport caused by CFTR mutation. *Free Radic Biol Med* **30**: 1440–1461.

Hunter RB, Stevenson EJ, Koncarevic A, Mitchell-Felton H, Essig DA, Kandarian SC (2002) Activation of an alternative NF-κB pathway in skeletal muscle during disuse atrophy. *FASEB J* **16**: 529–538.

Jimi E, Ghosh S (2005) Role of nuclear factor-kappaB in the immune system and bone. *Immunol Rev* **208**: 80–87.

Karin M (2006) Nuclear factor-kappaB in cancer development and progression. *Nature* **441**: 31–36.

Kato T Jr, Delhase M, Hoffmann A, Karin M (2003) CK2 Is a C-terminal iκb kinase responsible for NF-κB activation during the UV response. *Mol Cell* **12**: 829–839.

Kim HJ, Hawke N, Baldwin AS (2006) NF-kappaB and IKK as therapeutic targets in cancer. *Cell Death Differ* **13**: 738–747.

Kumar A, Boriek AM (2003) Mechanical stress activates the nuclear factor-kappaB pathway in skeletal muscle fibres: a possible role in Duchenne muscular dystrophy. *FASEB J* **17**: 386–396.

Lang V, Janzen J, Fischer GZ, Soneji Y, Bainke S, Salmeron A (2003) βTrCP-mediated proteolysis of NF-κB1 p105 requires phosphorylation of p105 serines 927 and 932. *Mol Cell Biol* **23**: 402–413.

Lang H, Schulte BA, Zhou D, Smythe N, Spicer SS, Schmiedt RA (2006) Nuclear factor κB deficiency is associated with auditory nerve degeneration and increased noise-induced hearing loss. *J Neurosci* **26**: 3541–3550.

Langen RCJ, Schols AMWJ, Kelders MCJM, Wouters EFM, Janssen-Heininger YMW (2001) Inflammatory cytokines inhibit myogenic differentiation through activation of nuclear factor-κB. *FASEB J* **15**: 1169–1180.

Lavigne MD, Pohlschmidt M, Novo JF, Higgins B, Alakhov V, Lochmuller H, Sakuraba H, Goldspink G, MacDermot K, Gorecki DC (2005) Promoter dependence of plasmid-pluronics targeted α galactosidase a expression in skeletal muscle of fabry mice. *Mol Ther* **5**: 12.

Lee NK, Lee SY (2002) Modulation of life and death by the tumor necrosis factor receptor-associated factors (TRAFs). *J Biochem Mol Biol* **35**: 61–66.

Lenardo MJ, Baltimore D (1989) NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. *Cell* **58**: 227–229.

Li Q, Verma IM (2002) NF-κB regulation in the immune system. *Nat Rev Immunol* **2**: 725–734.

Li XH, Fang XQ, Gaynor RB (2001) Role of IKKγ/NEMO in assembly of the IκB kinase complex. *J Biol Chem* **276**: 4494–4500.

Liou HC (2002) Regulation of the immune system by NF-κB and IkB. *J Biol Chem* **35**: 537–546.

Llovera M, Garcia-Martinez C, Agell N, Lopez-Soriano FJ, Argiles JM (1997) TNF can directly induce the expression of ubiquitin-dependent proteolytic system in rat soleus muscles. *Biochem Biophys Res Commun* **230**: 238–241.

Lovering RM, Porter NC, Bloch RJ (2005) The muscular dystrophies: from genes to therapies. *Phys Ther* **85**: 1372–1388.

Martinez-Pomar N, Munoz-Saa I, Heine-Suner D, Martin A, Smahi A, Matamoros N (2005) A new mutation in exon 7 of NEMO gene: late skewed X-chromosome inactivation in an incontinentia pigmenti female patient with immunodeficiency. *Hum Genet* **118**: 458–465.

Mathew S, Murty VV, Dalla-Favera R, Chaganti RS (1993) Chromosomal localization of genes encoding the transcription factors, c-rel, NF-kappa-Bp50, NF-kappa-Bp65, and lyt-10 by fluorescence in situ hybridization. *Oncogene* **8**: 191–193.

Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J (1997) IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. *Science* **278**: 818–819.

Messina S, Bitto A, Aguennouz M, Minutoli L, Monici MC, Altavilla D, Squadrito F, Vita G (2006) Nuclear factor kappa-B blockade reduces skeletal muscle degeneration and enhances muscle function in Mdx mice. *Exp Neurol* **198**: 234–241.

Michou L, Collet C, Laplanche JL, Orcel P, Cornelis F (2006) Genetics of Paget's disease of bone. *Joint Bone Spine* **73**: 243–248.

Minasian C, McCullagh A, Bush A (2005) Cystic fibrosis in neonates and infants. *Early Hum Dev* **81**: 997–1004.

Nowak KJ, Davies KE (2004) Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment. *EMBO* **5**: 872–876.

Papa S, Bubici C, Zazzeroni F, Pham CG, Kuntzen C, Knabb JR, Dean K, Franzoso G (2006) The NF-κB-mediated control of the JNK cascade in the antagonism of programmed cell death in health and disease. *Cell Death Differ* **13:** 712–729.

Pikarsky E, Ben-Neriah Y (2006) NF-kappaB inhibition: a double-edged sword in cancer? *Eur J Cancer* **42**: 779–784.

Poyet JL, Srinivasula SM, Lin JH, Fernades-Alnemri T, Yamaoka S, Tsichilis PN, Alnemri ES (2000) Activation of the IκB kinases by RIP *via* IKKγ/NEMO-mediated oligomerization. *J Biol Chem* **275**: 37966–37977.

Puel A, Picard C, Ku CL, Smahi A, Casanova JL (2004) Inherited disorders of NF-kappaB-mediated immunity in man. *Curr Opin Immunol* **16**: 34–41.

Radhakrishnan SK, Kamalakaran S (2006) Pro-apoptotic role of NF-κB: implications for cancer therapy. *Biochim Biophys Acta* **1766**: 53–62.

Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M (1997) Identification and characterization of an IκB Kinase. *Cell* **90**: 373–383.

Reid MB, Li YP (2001) Tumor necrosis factor-α and muscle wasting: a cellular perspective. *Respir Res* **2**: 269–272.

Reynaert NL, Ckless K, Korn SH, Vos N, Guala AS, Emiel FM, Wouters EFM, van der Vliet A, Janssen-Heininger YMW (2004) Nitric oxide represses inhibitory κB kinase through S-nitrosylation. *Proc Natl Acad Sci USA* **101**: 8945–8950.

Rice NR, MacKichan ML, Israel A (1992) The precursor of NF-κB p50 has IκB-like functions. *Cell* **71**: 243–253.

Roman-Blas JA, Jimenez SA (2006) NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthr Carllage* **14**: 839–848.

Rousiere M, Michou LT, Cornelis F, Orcel P (2003) Pagets's disease of bone. *Best Pract Res Clin Rheum* **17**: 1019–1041.

Schmid JM, Junge SA, Hossle JP, Schneider EM, Roosnek E, Seger RA, Gungor T (2006) Transient hemophagocytosis with deficient cellular cytotoxicity, monoclonal immunoglobulin M gammopathy, increased T-cell numbers, and hypomorphic NEMO mutation. *Pediatrics* **117**: e1049–1056.

Schottelius AJ, Dinter H (2006) Cytokines, NF-kappaB, microenvironment, intestinal inflammation and cancer. *Cancer Treat Res* **130**: 67–87.

Shishodia S, Aggarwal BB (2002) Nuclear factor-κB activation: a question of life or death. *J Biochem Mol Biol* **35**: 28–40.

Siebenlist U, Brown K, Claudio E (2005) Control of Lymphocyte development by nuclear factor κ-B. *Nature* **5**: 435–445.

Smahi A, Courtois G, Rabia SH, Doffinger R, Bodemer C, Munnich A (2002) The NF-κB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. *Hum Mol Genet* **11**: 2371–2375.

Sriadibhatla S, Yang Z, Gebhart C, Alakhov VY, Kabanov A (2005) Transcriptional activation of gene expression by pluronic block copolymers in stably and transiently transfected cells. *Mol Ther* **13**: 804–813.

Tergaonkar V, Bottero V, Ikawa M, Li Q, Verma IM (2003) IkappaB kinase-independent IkappaBalpha degradation pathway: functional NF-κB activity and implications for cancer therapy. *Mol Cell Biol* **23**: 8070–8083.

Thaloor D, Miller KJ, Gephart J, Mitchell PO, Pavlath GK (1999) Systemic administration of the NF-κB inhibitor curcumin stimulates muscle regeneration after traumatic injury. *Am J Physiol* **277**: 320–329.

Torchinsky A, Lishanski L, Wolstein O, Shepshelovich J, Orenstein H, Savion S (2002) NF-κB DNA-binding activity in embryos responding to a teratogen, cyclophosphamide. *BMC Dev Biol* **2**: 2.

Valerio A, Boroni F, Benarese M, Sarnico I, Ghisi V, Bresciani LG, Ferrario M, Borsani G, Spano P, Pizzi M (2006) NF-kappaB pathway: a target for preventing beta-amyloid (Abeta)-induced neuronal damage and Abeta42 production. *Eur J Neurosci* **23**: 1711–1720.

Wada T, Nakashim T, Hiroshi N, Penninger JM (2006) RANKL–RANK signaling in osteoclastogenesis and bone disease. *Trends Mol Med* **12**: 17–25.

Wang Z, Sicinski P, Weinberg RA, Zhang Y, David K (1996) Characterization of the mouse cyclin D3 gene: exon/intron organization and promoter activity. *Genomics* **35**: 156–163.

Weber AJ, Soong G, Bryan R, Saba S, Prince A (2001) Activation of NF-κB in airway epithelial cells is dependent on CFTR trafficking and Cl^- channel function. *Am J Physiol Lung Cell Mol Physiol* **1**: 71–78.

Weih F, Caamona J (2003) Regulation of secondary lymphoid organ development by the nuclear factor-κB signal transduction pathway. *Immunol Rev* **195**: 91–105.

Xiao W (2004) Advances in NF-kappaB signaling transduction and transcription. *Cell Mol Immunol* **1**: 425–435.

Zhang W, Potrovita I, Tarabin V, Herrmann O, Beer V, Weih F, Schneider A, Schwaninger M (2005) Neuronal activation of NF-κB contributes to cell death in cerebral ischemia. *J Cereb Blood Flow Metab* **25**: 30–40.