The NF-κB pathway

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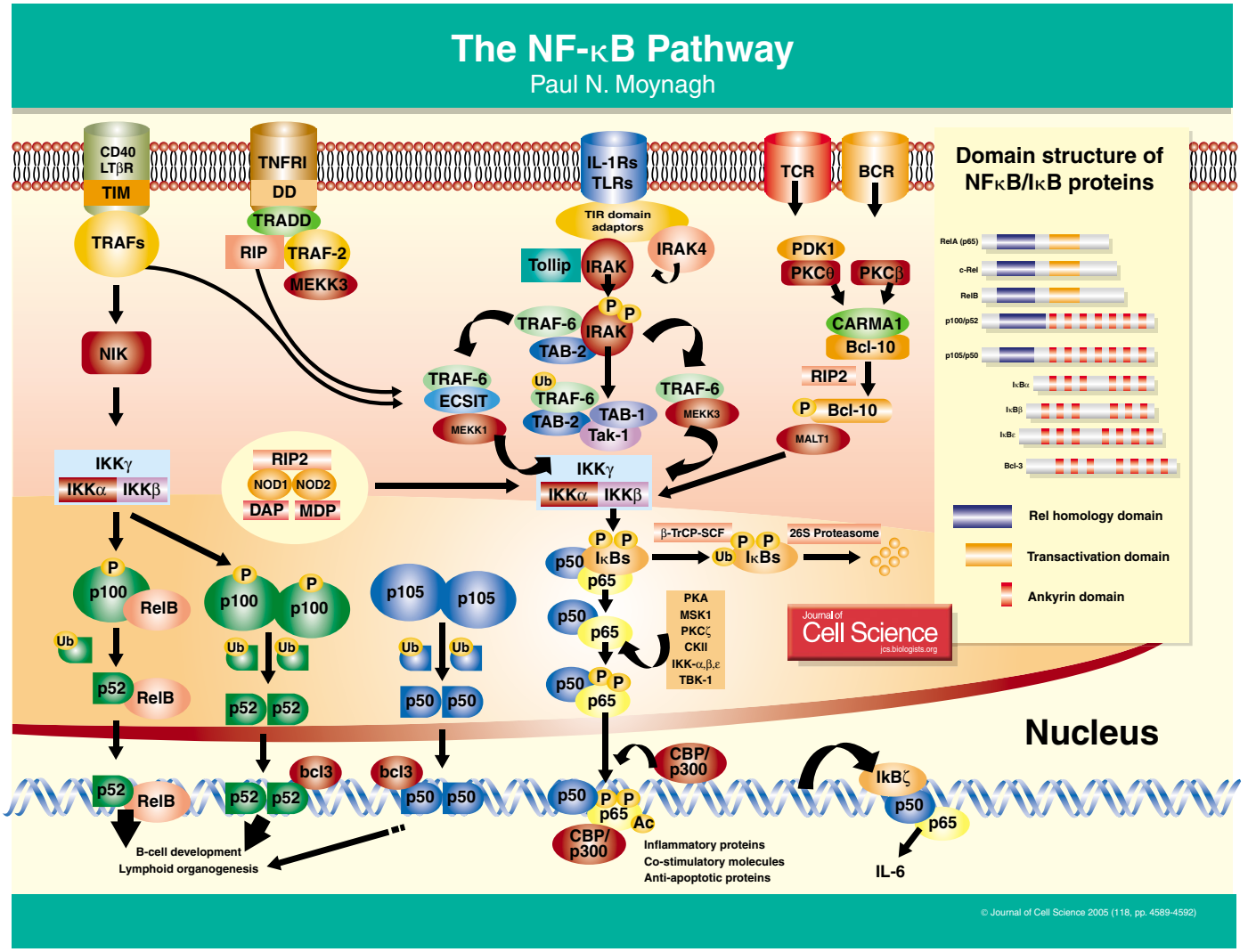
The nuclear factor (NF)-κB transcription factor regulates expression of numerous components of the immune system (Li and Verma, 2002). These include pro-inflammatory cytokines, chemokines, adhesion molecules and inducible enzymes such as cycloxygenase-2 and inducible nitric oxide synthase, which regulate the innate immune response, as well as proteins that regulate the specific immune response, such as major histocompatibility complex and co-stimulatory molecules crucial to the induction phase of specific immunity, and cytokines like interleukin (IL)-2, IL-12 and interferon-γ that control lymphocyte proliferation and differentiation. Dysregulation of this transcription factor can thus lead to inflammatory and autoimmune diseases (Yamamoto and Gaynor, 2001). Since NF-κB also regulates the expression of a variety of proteins that inhibit apoptosis and promote cell survival/proliferation, it is also implicated in carcinogenesis (Karin et al., 2002).

NF-κB describes various dimeric complexes of members of the Rel protein family, which comprises Rel (c-Rel), Rel A (p65), RelB, NF-κB1 (p50 and its precursor p105) and NF-κB2 (p52 and its precursor p100) (Ghosh et al., 1998). Each possesses an ~300-residue N-terminal Rel-homology-domain, responsible for dimerisation, nuclear translocation and DNA binding. p65, RelB and c-Rel, also contain a C-terminal transactivation domain. Of the various dimeric combinations, p50-p65 is most common. Binding of most NF-κB complexes to motifs in target promoters assists transcription, but homodimeric complexes of p50 or p52 can repress it.

In resting cells, NF-κB proteins are predominantly cytoplasmic, associating with members of the inhibitory IκB family such as IκB-α, IκB-β and IκB-ϵ (Ghosh et al., 1998). These interact with NF-κB through multiple ankyrin repeats and also inhibit its DNA-binding activity. IκB proteins were originally thought to sequester NF-κB in the



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cytoplasm by masking its nuclear localisation sequences (NLSs). However, IκB-α (and probably IκB-ϵ) can only mask one NLS in the dimer; so NF-κB-IκBα complexes undergo constitutive nuclear translocation (Malek et al., 2001). Importantly, a nuclear export signal (NES) in IκB-α precludes high steady-state levels of these complexes in the nucleus (Huang et al., 2000). By contrast, NF-κB–IκBβ complexes fail to undergo such shuttling because IκBβ can mask both NLSs (Malek et al., 2001). This depends on its association with the Ras-like protein, κB-Ras (Chen et al., 2003). κB-Ras also increases the stability of IκBβ (Kanayama et al., 2004).

NF-κB complexes containing the precursor proteins p105 and p100 self-inactivate (Ghosh et al., 1998). Interestingly, other IκB members can form part of active transcriptional complexes at specific promoters. Thus NF-κB-inducible IκBζ (also known as MAIL and INAP) interacts with p50 and promotes transcription at the *IL-6* promoter (Yamamoto et al., 2004). A highly related protein, Bcl-3, interacts with p50 and p52 dimers and forms active transcriptional complexes (Bours et al., 1993; Dechend et al., 1999). However, some studies show it represses transcription (Richard et al., 1999; Wessells et al., 2004).

Activation of NF-κB classically depends on degradation of IκB. A pre-requisite is prior phosphorylation of IκB on two key N-terminal serines by IκB kinases (IKKs) (Yamamoto and Gaynor, 2004). IKK activity resides in a large protein complex comprising two catalytic subunits, IKKα and IKKβ, and a scaffolding subunit, IKKγ/NEMO. The phosphorylation of IκB proteins is followed by the binding of the $E3^{IκB}$ ubiquitin ligase complex β-TrCP-SCF, which polyubiquitinylates IκB and targets it for degradation by the 26S proteasome (Karin and Ben-Neriah, 2000). Activators of the IKK complex include mitogen-activated protein kinase kinase kinases (MAP3Ks) such as MEKK1, MEKK3 and TAK1 and it represents a convergence point for numerous stimuli, including ligands for Toll-like receptors (TLRs), IL-1/IL-18 receptors, the TNF receptor superfamily, and B and T cell receptors.

TLRs detect pathogen-associated molecules and induce pro-inflammatory proteins and co-stimulatory molecules that trigger innate and adaptive immunity (O'Neill, 2004). TLRs employ many of the same signalling components as the IL-1 and IL-18 receptors (Martin and Wesche, 2002). These receptors share a conserved Toll/IL-1R (TIR) domain and form dimeric receptor complexes with the same or different TIR-domain-containing proteins. These complexes also recruit intracellular TIR-domain-containing adapter proteins. Four such adapter proteins have been well characterised: Myd88, Mal/TIRAP, TRIF/TICAM-1 and TRAM/TICAM-2 (O'Neill et al., 2003). With the exception of TLR3, Myd88 is universally recruited to all the above receptor complexes (Janssens and Beyaert, 2002). The use of the other adapters is more restricted to specific TLR members. These adapters recruit and activate members of the IRAK family (Martin and Wesche, 2002). IRAK-1 is initially recruited to Myd88 in association with Toll-interacting protein (Tollip). The IRAK-Myd88 association triggers hyperphosphorylation of IRAK by itself and/or by other additional kinases, probably IRAK-4, leading to its dissociation from Myd88 and Tollip and its interaction with the downstream adaptor TRAF-6. The interaction of IRAK with TRAF-6 leads to activation of TAK1 (Ninomiya-Tsuji et al., 1999). IRAK is essential in this activation process, because it promotes the translocation of TAK1-binding protein 2 (TAB2) from the membrane to the cytosol, where TAB2 interacts with TRAF-6 and bridges the association of TRAF-6 with TAK1. The latter, with the help of TAB1, becomes activated and in turn activates the IKK complex. The activation of TAK1 by TRAF-6 depends on the nonclassical polyubiquitinylation (ubiquitin chains linked through Lys63 of ubiquitin) of TRAF-6 (Deng et al., 2000; Wang et al., 2001). Interestingly the tumour suppressor CYLD is a deubiquitinylating enzyme that inhibits ubiquitinylation of TRAF proteins and activation of NF-κB (Brummelkamp et al., 2003; Kovalenko et al., 2003; Trompouki et al., 2003). CYLD is mutated in familial cylindromatosis; this results in loss of its deubiquitinylating activity, increased TRAF-mediated activation of NF-κB and tumorigenesis.

TRAF-6 can activate other MAP3Ks that stimulate the IKK complex. It associates with a novel adaptor protein, ECSIT, that sequentially activates MEKK-1 and IKKs (Kopp et al., 1999). Furthermore, TRAF-6 interacts with MEKK3 and the latter is essential for activation of IKKs by TLR4 and IL-1R (Huang et al., 2004). Finally, TRAF-6 interacts with another adapter, p62, and activates PKCζ, leading to phosphorylation of p65 (see below) (Sanz et al., 2000).

Nod1 and Nod2 are cytoplasmic receptors for microbial ligands that can trigger activation of NF-κB (Athman and Philpott, 2004; Philpott and Girardin, 2004). Both Nod proteins recognise peptidoglycan breakdown products (Athman and Philpott, 2004). Nod1 acts as a receptor for a tripeptide motif containing diaminopimelic acid (DAP) as its terminal amino acid whereas Nod2 recognises a muramyl dipeptide (MDP). The activation of the Nod proteins leads to their oligomerisation and subsequent interaction with and activation of receptor-interacting protein (RIP) 2 (also known as CARDIAK and RICK). RIP2 then associates with IKKγ, leading to activation of the catalytic subunits IKKα and IKKβ.

The TNF receptor superfamily represents another collection of receptors that activate NF-κB (Dempsey et al., 2003; Gaur and Aggarwal, 2003). Some members, including TNFR1, Fas, TRAILR-1 and TRAILR-2, contain a death domain (DD) in their cytoplasmic regions, whereas others, such as TNFR2, lymphotoxin (LT)-βR and CD40 lack a DD. However, both receptor types can activate NF-κB (Dempsey et al., 2003). The engagement of TNFR1, for example, by TNF leads to the recruitment of TNF-receptor-associated death domain (TRADD). TRADD then associates with TRAF2 and RIP. TRAF-2 subsequently recruits the IKK complex to the TNFR-1 complex, where RIP activates the catalytic IKK subunits via

MEKK3 (Yang et al., 2001). Members of the TNF receptor superfamily that lack a DD contain TRAF-interacting motifs in their cytoplasmic regions (Dempsey et al., 2003). Such receptors directly recruit TRAF proteins and activate NF-κB as described above. However, these receptors can also activate NF-κB by a non-classical pathway that is independent of the degradation of IκB.

As stated above, the precursor proteins p105 and p100 have IκB domains in their C-terminal regions. Whereas the processing of p105 to p50 is predominantly constitutive (with IKK-dependent phosphorylation of p105 tending to promote its complete degradation), the processing of p100 to p52 is tightly regulated and signal dependent (Beinke and Ley, 2004). The precursor p100 is normally found as a complex with RelB, and the C-terminal region of p100 represses RelB-mediated transcriptional activity (Solan et al., 2002). The processing of p100 and release of RelB-p52 is triggered by at least three members of the TNF receptor superfamily, namely CD40, LTβR and B-cell-activating-factor receptor (BAFF-R) (Yamamoto and Gaynor, 2004). These receptors cause the sequential activation of NF-κB-inducing kinase (NIK) and IKKα (Xiao et al., 2001). The latter phosphorylates p100, resulting in its polyubiquitinylation and processing to p52 (Senftleben et al., 2001). This allows nuclear translocation of RelB-p52 dimers, which induce genes that are essential for B-cell development and lymphoid organogenesis. IKKα is the specific catalytic subunit of the IKK complex that mediates this non-classical activation of NF-κB. Interestingly, IKKα has an additional nuclear role in that it catalyses the phosphorylation of histone H3 at NF-κB-regulated promoters (Anest et al., 2003; Yamamoto et al., 2003). Such phosphorylation is a necessary pre-requisite for CBP-mediated acetylation of H3 and subsequent enhancement of transcription.

The sensing of antigens by specific T-cell receptors (TCRs) and B-cell receptors (BCRs) on T and B lymphocytes also leads to activation of NF-κB. The engagement of TCRs leads to the immediate activation of a number of protein tyrosine kinases and formation of very large multi-component receptor complexes (Weil and Israel, 2004). This leads to activation of PKCθ and its association with Akt, a kinase activated by the co-stimulatory CD28 pathway (Schmitz et al., 2003). Although the immediate substrates for PKCθ are unknown, a number of downstream effectors leading to NF-κB have been identified. Carma 1 (caspase-recruitment-domain-containing membrane-associated guanylate kinase) links PKCθ to Bcl10, a protein first identified through analysis of chromosomal translocations in mucosa-associated lymphoid tissue (MALT) lymphomas (Bunnell, 2002). Bcl10 is phosphorylated by RIP2 and interacts with a caspase-related protein termed MALT1. This interaction leads to recruitment and synergistic stimulation of the IKK complex. The activation appears to depend on the non-classical polyubiquitinylation of IKKγ that is induced by Bcl10 in a MALT1- and Ubc13-dependent manner. This happens as part of a supermolecular membrane complex at the contact site between the T cell and antigen-presenting cell. Recently 3-phosphoinositide-dependent kinase 1 (PDK1) has been shown to have a key role in assembling this complex of proteins, co-ordinating the recruitment of both the PKCθ and MALT1 complexes (Lee et al., 2005). The activation of NF-κB by BCR receptors shows some similarities to TCR signalling (Weil and Israel, 2004).

The engagement of BCRs leads to a torrent of tyrosine kinase activity and eventual activation of a PKC isoform termed PKCβ that promotes activation of NF-κB. Although the Carma1/Bcl10/MALT1 is known to play a key role in activating NF-κB in B cells, it is unclear whether these proteins functionally link PKCβ to the IKK complex.

The regulation of the transactivation of NF-κB represents another level of control for this transcription factor. Most work has focused on p65 and the mapping of its multiple phosphorylation sites (Schmitz et al., 2004). Thus, Ser276 is phosphorylated by protein kinase A in response to LPS and by mitogen- and stress-activated protein kinase 1 (MSK1) in response to TNF. PKCζ phosphorylates Ser311 in response to TNF. Ser529 is phosphorylated by casein kinase II (CKII) and the IKK complex. Finally, Ser536 is phosphorylated by the catalytic subunits of IKK and the IKK-related kinases IKKε and TRAF-family-member-associated (TANK)-binding kinase 1 (TBK1). Other phosphorylation sites include Ser468 and Thr505 but the responsible kinase(s) awaits identification. The phosphorylation of many of these sites is associated with an increase in the transcriptional activity of p65, and is accompanied by enhanced binding of p65 to coactivating acetylases such as CBP/p300. Interestingly, the latter can acetylate p65 at multiple lysine residues and this is associated with increased transactivation (Chen and Greene, 2003). By contrast, histone deacetylases (HDACs), such as HDAC-1, HDAC-2 and HDAC-3, deacetylate p65, leading to repression of transactivation and also termination of NF-κB activation by increasing the affinity of NF-κB for IκBα.

In summary, NF-κB acts at the crossroads of many signalling pathways. Inappropriate or excessive activation of NF-κB can lead to inflammatory diseases and cancers. The continuing efforts to increase our molecular appreciation of the regulation of NF-κB will be of great value in learning to fully exploit this transcription factor as a therapeutic target.

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