Initiation of transcription requires the assembly of a large nucleoprotein complex at the promoter. Transcriptional control can be exerted therefore by regulating the activity of individual components of this complex or by controlling the availability of factors that are required for its assembly. Elements of the promoter that are required for transcriptional activation are bound by combinations of sequence-specific DNA-binding proteins, any one of which can be the limiting factor in a given cell type. By altering the availability or activity of these limiting factors, stimuli from the external environment transmitted by distinct signal transduction pathways can affect specific gene transcription. The nuclear factor (NF)-κB family of transcription factors is controlled at the level of specific nuclear transport and DNA-binding activity by inhibitor proteins of the inhibitor-κB (IκB) family. While NF-κB is exploited by many viruses, including human immunodeficiency virus, for the transcription of viral genes, its primary role appears to be in the rapid induction of cellular genes that are transcribed in the immune and inflammatory responses to challenges such as microbial infection or the presence of inflammatory cytokines.

The induction of nuclear NF-κB DNA-binding activity after treatment of a variety of cell types with agents such as the active phorbol ester phorbol 12-myristate 13-acetate, bacterial lipopolysaccharide or the protein-synthesis inhibitor cycloheximide [1] suggested the existence of an inactive, possibly labile, precursor form of NF-κB. Isolation of an inhibitory protein (IκB) from an inactive cytoplasmic form of NF-κB [2, 3] provided a candidate molecule for the control of NF-κB subcellular localization and DNA-binding activity. Subsequent studies suggested the existence of more than one form of IκB — a 36 kDa form termed IκBα, and a less abundant 43 kDa IκBβ form [4].

As identified originally, NF-κB was composed of two polypeptide species of 50 kDa (p50) and 65 kDa (p65), although the p50 subunit could also be found as a homodimeric protein designated KBFI [5, 6] or EBPI [7]. Purification of the NF-κB subunits and molecular cloning of the corresponding cDNAs identified the p50 and p65 subunits as members of a family of proteins, which includes the c-Rel proto-oncogene product, the avian erythroleukemia virus v-Rel oncoprotein, the Drosophila Dorsal morphogen protein and several species that are more closely related to the NF-κB subunits (reviewed in [8]). All the members of this protein family share a highly conserved N-terminal region responsible for the DNA-binding and dimerization functions of family members. NF-κB/Rel/Dorsal family members also share the property that they can interact with members of the IκB inhibitor protein family. Analysis of cDNAs for the p50 subunit of NF-κB [9, 10] revealed that it was synthesized as a p105 precursor with the C-terminal region of the molecule inhibiting the DNA-binding activity of the N-terminal region. Examination of the predicted amino acid sequence of the C-terminus of p105 revealed eight repeats of a 33 amino acid sequence identified previously in several tissue-differentiation and cell-cycle control proteins and in the erythrocyte ankyrin protein [11].

The subsequent identification of a cDNA (termed MAD3) representing an mRNA that is induced in adhering monocytes that encoded five tandem ankyrin repeats with the ability to inhibit the DNA-binding activity of the p50–p65 NF-κB complex, strengthened the association between ankyrin repeat proteins and IκB activity [12]. Purification of the Rel-associated pp40 1xIκB and isolation of the corresponding cDNA again revealed the presence of five tandem ankyrin repeats that are of similar sequence to those in MAD3 [13]. Furthermore, the product of the human proto-oncogene bcl-3 has been shown to possess seven ankyrin repeat motifs and to act as a specific inhibitor of p50 DNA-binding activity [14], while an IκB-like inhibitor of the Drosophila Dorsal morphogen, cactus, has been shown to inhibit the DNA-binding activity of Dorsal, and to have seven repeats of the ankyrin motif [15]. MAD3 appears to be equivalent to IκBα although IκBβ has yet to be cloned. The C-terminal region of p105 has been shown to be a 121. Purification of the cDNA again revealed the presence of five tandem ankyrin repeats that are of similar sequence to those in MAD3 [13]. Furthermore, the product of the human proto-oncogene bcl-3 has been shown to possess seven ankyrin repeat motifs and to act as a specific inhibitor of p50 DNA-binding activity [14], while an IκB-like inhibitor of the Drosophila Dorsal morphogen, cactus, has been shown to inhibit the DNA-binding activity of Dorsal, and to have seven repeats of the ankyrin motif [15]. MAD3 appears to be equivalent to IκBα although IκBβ has yet to be cloned. The C-terminal region of p105 has been shown to be a.

Abbreviations used: IκB, inhibitor-κB; NF-κB, nuclear factor-κB; TNFα, tumour necrosis factor α.
the DNA-binding and nuclear-translocation activities of the p50 NF-κB subunit. While the IκBα species will prevent sequence-specific DNA binding of p50 homodimer, p50–p65 NF-κB heterodimer and c-Rel, it is much more effective at inhibiting DNA binding at p50 homodimers than p50–p65 heterodimers or p65 homodimers [17]. Bcl-3 protein has a similar inhibitory specificity to the C-terminal half of p105, inhibiting the DNA-binding activity of p50 [14, 18], but only inefficiently inhibiting the DNA-binding activity of p65 and c-Rel proteins and forming a ternary complex on DNA with p50B [19]. In contrast, the pp40 inhibitor protein could not inhibit the DNA-binding activity of p50 homodimers, but could inhibit the DNA-binding activity of p65 and c-Rel homodimers, and p59–p65 and p50–c-Rel heterodimers [13]. The closely related MAD3 protein can also inhibit the DNA-binding activity of p65 and c-Rel, but cannot inhibit the DNA-binding activity of p50 homodimers [12, 20].

**Interaction of NF-κB with DNA**

To study the molecular interactions between proteins of the NF-κB family and to generate specific antibodies, we have previously expressed and purified large quantities of active p50, p65, MAD3 (IκBα) and the C-terminal region of p105 (IκBγ) in *Escherichia coli* as thrombin-cleavable fusion proteins with glutathione S-transferase [21, 22]. The subdomain structure of the p50 subunit of NF-κB has been investigated by partial proteolysis [23a] and has revealed that the protein contains a dimeric core that is relatively protease-resistant with N- and C-terminal extensions that are susceptible to proteolysis. At low trypsin concentrations, an initial cleavage takes place after arginine-362 and a second cleavage takes place at higher trypsin concentration after lysine-77. The cleavage after arginine-362 does not alter the DNA-binding characteristics of p50 but removes the nuclear localization signal, indicating that this region occupies a highly exposed position on the surface of the protein. At the N-terminus, cleavage after lysine-77 removes the terminal 43 amino acids leaving the remainder of the protein devoid of DNA-binding activity and suggesting that this region is required for interaction with DNA. This conclusion is strengthened by the observation that the cleavage after lysine-77 did not take place if the protein was first bound to DNA containing its recognition site. This may be due to simple masking of the cleavage site by the bound DNA, or it may be that DNA binding is accompanied by a conformational change in the structure of the protein. Although the sites of cleavage were not determined, a similar conclusion was reached by Fujita et al. [23] who also observed that the sensitivity of the p50 protein to digestion with chymotrypsin was dependent on the precise sequence of the bound DNA. This may be similar to the situation described for the arm of the λ repressor [24, 25] and the β-strand motif of the Arc repressor [26], where the module that contacts DNA is unfolded in solution but adopts a stable conformation when bound to DNA.

A considerable body of evidence suggests that determinants of Rel protein DNA-binding specificity are located between proline-43 at the start of the Rel-homologous region and the trypsin cleavage site at lysine-77. Mutational analysis has indicated that mutations between arginine-57 and leucine-70 modify the DNA-binding properties of the proteins [21, 27, 28], and chemical modification experiments have indicated that bound DNA protects C62 from carboxymethylation by iodoacetate [29]. Although secondary structure prediction programs suggest that this region could form a β-strand and it is known that NF-κB proteins make base and backbone contacts over one complete turn of the DNA double helix [30–32] precise details of the DNA recognition process will only emerge from structural studies.

**NF-κB, IκB interactions**

DNA binding of the homodimeric p50 subunit of NF-κB was inhibited by a bacterially expressed protein containing the ankyrin repeats present in the C-terminus of the p105 precursor, but not by IκBα protein (MAD3). However, p50 was retained on protein affinity matrices containing either the C-terminal ankyrin repeats of p105 or IκBα. To investigate the interaction between p50 and proteins containing ankyrin repeats, we have used a number of approaches to probe the accessibility of the p50 nuclear localization signal in the protein complex [33a]. A monoclonal antibody recognizing a linear epitope including the nuclear localization signal of the p50 protein could immunoprecipitate p50 homodimers, but could not precipitate the protein when it was bound to the C-terminal region of p105. A close association between the nuclear localization signal of p50 and the C-terminal region of p105 was also suggested by proteinase accessibility experiments. While the nuclear localization signal of free p50 is extremely susceptible to cleavage with trypsin, the same site is masked in the presence of the C-terminal ankyrin repeats of p105 and, to a lesser extent IκBα. Removal of the nuclear
localization signal by trypsin digestion generates a protein that is fully competent for DNA binding, but is refractile to inhibition by the C-terminal ankyrin repeats of p105. Although it is now clear that both the C-terminal region of p105 and 1xBa interact with the nuclear localization signal, it is unlikely that this is the only point of contact between the inhibitor protein and the DNA-binding subunits. Indeed, a highly transforming variant of p65 [33], derived by alternative splicing, that contains a nuclear localization signal but has sustained an internal deletion rendering the protein monomeric [34] is unable to interact with 1xBa [20]. An additional indication that sequences other than the nuclear localization signal are required for inhibition by ankyrin repeat proteins come from studies with bcl-3, which can interact with p50 derivatives that lack the nuclear localization signal [18].

The ability of 1xBa to interact with p50 homodimers but not inhibit DNA binding was initially regarded as something of a puzzle, as a trimeric complex containing p50, DNA and 1xBa was not detected in gel electrophoresis DNA-binding assays. However, it was demonstrated that the addition of DNA led to the dissociation of p50, 1xBa complexes [20]. We have extended these observations, and the trypsin protection assay has indicated that the ability to displace not only 1xBa, but also the inhibitory p105 C-terminal region, is dependent on the affinity of the DNA–protein interaction and on the quantity of DNA [33a]. As we have already indicated that p50 undergoes a conformational change when bound to DNA, these data suggest that the dynamic equilibrium shown in Figure 1 is established. In the case outlined in Figure 1 the ternary complex must be rather unstable as it is not detected in gel electrophoresis DNA-binding assays. However, such a ternary complex containing p50B, DNA and bcl-3 has been reported [19]. As bcl-3, 1xBa and the C-terminal region of p105 all contain domains that have the potential to activate transcription [19, 35] it may be that given the appropriate context of DNA-binding heterodimer and DNA recognition sequence both 1xBa and the C-terminal region of p105 (1xBγ) could form stable DNA-binding complexes that can activate transcription.

**Metabolism of NF-κB and 1xB**

To investigate the metabolism of NF-κB and 1xB components in cells exposed to extracellular stimuli, we have made use of antisera that are specific for p50, for p65 and for 1xBα. In these experiments, cells were exposed to stimuli such as tumour necrosis factor α (TNFα) for various lengths of time, and then the cells fractionated into nucleus and cytoplasm, and protein extracts prepared. Samples from these extracts were used to establish NF-κB protein levels by Western blotting, to determine NF-κB activity by gel electrophoresis DNA-binding assays and to investigate protein modification and interactions by immunoprecipitation. As shown in Figure 2, the initial response to TNFα is that levels of 1xBα-immunoreactive material in the cytoplasm fall dramatically, as does the small amount of 1xBα present in the nucleus. We have also shown that TNFα induces an increase in the processing of the p105 precursor to the mature p50 that translocates to the nucleus in association with p65. Phosphate-labelling followed by immunoprecipitation has indicated that both p105 and 1xBα are phosphorylated before degradation. Furthermore, we have demonstrated that the protease inhibitors Tos-Lys-CH₂Cl and Tos-Phe-CH₂Cl block 1xBα degrada-

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**Figure 2**

Metabolism of NF-κB (p50) and 1xBα (MAD3) after exposure of cells to TNFα

Cells were fractionated into nucleus (nucl.) and cytoplasm (cyt.) and protein levels determined by Western blotting using antisera specific for p50 and for MAD3.
Signalling from the Plasma Membrane to the Nucleus

Figure 3
Model for the activation of NF-κB.

- Phosphorylation
- Proteolysis
- Translocation

Activation, p105 processing and the appearance of active NF-κB in the nucleus [36a]. These data suggest a model for NF-κB activation (Figure 3) in which p105 and IκBα become phosphorylated after induction, rendering the proteins susceptible to proteolysis. As the C-terminus of p105 and IκBα mask the nuclear localization signals of the NF-κB subunits, the released p50 and p65 subunits are free to translocate to the nucleus and activate transcription. On the basis that protein synthesis inhibitors could superinduce NF-κB, it was proposed that NF-κB activity was controlled by a labile inhibitor [1]. Recently, the activation-induced degradation of IκBα [36, 37] and p105 turnover [38] has been reported. Our observations that proteinase inhibitors block NF-κB activation indicate that proteolysis is an obligatory step in this pathway.

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Transcription factor phosphorylation by the DNA-dependent protein kinase
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Introduction
An important aspect of cell functioning is the ability to regulate gene expression in response to environmental changes. One of the most common ways of altering gene expression is by modulating the rate of transcriptional initiation. Typically, an environmental signal such as a hormone reaches the cell surface and triggers a series of intracellular signal transduction events that result ultimately in either activation or inactivation of a sequence-specific DNA-binding transcription factor within the nucleus. In many cases, the change in transcription factor activity is achieved via phosphorylation [1, 2]. Important features of phosphorylation are its potential rapidity and reversibility, which allow cells to make speedy responses to environmental stimuli. Another advantage of phosphorylation as a regulatory mechanism is that a single transcription factor can be targeted by more than one kinase, thus providing an effective means of integrating information from a variety of signal transduction pathways.

Although much is known about the structure and function of transcription factors, relatively little is understood about the kinases that affect their activity. In the cell, it is clear that most physiologically relevant transcription factor kinases must reside within the nuclear compartment. Furthermore, for many of these kinases to access their substrates, it is likely that they themselves will have to become associated with the transcriptional machinery assembled on DNA. One kinase that

Abbreviation used: DNA-PK, DNA-dependent protein kinase.

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