TAF\textsubscript{II}105 mediates activation of anti-apoptotic genes by NF-\(\kappa\)B

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The transcription factor NF-\(\kappa\)B is important for expression of genes involved in immune responses, viral infections, cytokine signaling and stress. In addition NF-\(\kappa\)B plays a crucial role in protecting cells from TNF-\(\alpha\)-induced apoptotic stimuli, presumably by activating anti-apoptotic genes. Here we report that the substoichiometric TFIID subunit TAF\textsubscript{II}105 is essential for activation of anti-apoptotic genes in response to TNF-\(\alpha\), serving as a transcriptional coactivator for NF-\(\kappa\)B. The putative coactivator domain of TAF\textsubscript{II}105 interacts with the activation domain of the p65/RelA member of the NF-\(\kappa\)B family, and further stimulates p65-induced transcription in human 293 cells. Moreover, inhibition of TAF\textsubscript{II}105 activity by overexpression of a dominant negative mutant of TAF\textsubscript{II}105 decreased NF-\(\kappa\)B transcriptional activity and severely reduced cell survival in response to TNF-\(\alpha\). Similarly, expression of anti-sense TAF\textsubscript{II}105 RNA sensitized the cells to TNF-\(\alpha\) cytotoxicity. These results suggest that TAF\textsubscript{II}105 is involved in activation of anti-apoptotic genes by NF-\(\kappa\)B.

Keywords: apoptosis/NF-\(\kappa\)B/TAF\textsubscript{II}105/TNF-\(\alpha\)

Introduction

Initiation of transcription is a key regulatory step affecting gene expression in response to a variety of extra- and intracellular signals, during developmental processes and for tissue specificity. The rate of transcription initiation is determined by proximal and distal enhancer elements that are bound by gene-specific transcription factors (activators or repressors); these are modular in nature, typically consisting of a DNA-binding domain and one or more activation (or repression) domains. The transcription-initiation site is determined by a number of general transcription factors (GTFs) that assemble around the core promoter to form the preinitiation complex. The general transcription factor TFIID plays an essential role in transcription initiation as it recognizes and binds the core promoter and nucleates the assembly of the other general initiation factors (TFIIB, TFIIF, TFIH and RNA polymerase II) (for recent reviews see Orphanides et al., 1996; Hoffman et al., 1997).

Studies of transcriptional activation mechanisms revealed that TFIID is also required for mediating transcription-activation signals by gene-specific activators (Horikoshi et al., 1988; Pugh and Tjian, 1990). TFIID is a multisubunit complex that consists of the TATA box-binding protein TBP and a number of TBP-associated factors (TAFs) that are present in all cells (Dynlacht et al., 1991; Tanese et al., 1991; Zhou et al., 1992; Poon and Weil, 1993; Reese et al., 1994). Some of these TAFs have been shown to bind activation domains of activators directly (Goodrich et al., 1993; Hoey et al., 1993; Chen et al., 1994; Jacq et al., 1994; Chiang and Roeder, 1995; Sauer et al., 1995a; Thut et al., 1995). This interaction appears to be essential for activator-dependent transcriptional stimulation in reconstituted transcription reactions \textit{in vitro} (Goodrich et al., 1993; Hoey et al., 1993; Chen et al., 1994; Sauer et al., 1995a; Thut et al., 1995). Several studies suggested that TAFs are also required for transcriptional activation \textit{in vivo} (May et al., 1996; Mengus et al., 1997; Suzuki-Yagawa et al., 1997; Wang et al., 1997). Interestingly, recent genetic studies in yeast suggested that TAF subunits, although essential for viability, are not generally required for transcription activation (Moqtaderi et al., 1996; Walker et al., 1996). Considering the findings that certain classes of activators from metazoans are unable to function in yeast (Attardi et al., 1991; Tansey and Herr, 1997), it is possible that a development of complex regulatory programs in metazoans was accompanied by an increase in the need for TAFs as coactivators.

Recently, we have identified and cloned a novel TFIID subunit, TAF\textsubscript{II}105, that is enriched in TFIID of human B lymphocytes constituting \(\sim\)10\% of TFIID in these cells and \(\sim0.5–1\%\) in non-B cells (Dikstein et al., 1996). Therefore, TAF\textsubscript{II}105 exists in sub-stoichiometric amounts relative to the core TAFs suggesting that a TFIID complex containing this subunit might be required for transcription of a limited set of genes. TAF\textsubscript{II}105 shares regions of high homology with the core TFIID subunits TAF\textsubscript{II}130 from human and TAF\textsubscript{II}110 from Drosophila (Dikstein et al., 1996; Mengus et al., 1997). The highly conserved C-terminus of these TAFs is implicated in TAF–TAF interaction (Dikstein et al., 1996) and the diverged N-terminus of dTAF\textsubscript{II}110 and hTAF\textsubscript{II}130 directs interaction with activation domains of activators (Chen et al., 1994; Tanese et al., 1996). Interestingly, in a TFIID complex containing TAF\textsubscript{II}105, TAF\textsubscript{II}130 is also present in the same complex.

Here we show that TAF\textsubscript{II}105 serves as a coactivator for the transcription factor NF-\(\kappa\)B \textit{in vivo}. The NF-\(\kappa\)B family plays a fundamental role in immune responses and is essential for cytokine-inducible gene expression, in particular TNF-\(\alpha\)- and IL-1-induced genes (for review see Verna et al., 1995; Baeuerle and Baltimore, 1996; Baldwin, 1996). Our studies reveal that TAF\textsubscript{II}105 interacts directly with p65/RelA and mediates gene activation by the p65 activation domain. Recent studies suggest that NF-\(\kappa\)B has a crucial role in protecting cells from apoptotic
stimuli produced by TNF-α (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996; Wang et al., 1996). We show that interfering with the normal function of TAFI105 either by expression of dominant-negative mutant or reducing its endogenous levels by anti-sense RNA expression, renders the cells sensitive to the killing effect of TNF-α. Our results suggest that activation of anti-apoptotic genes in response to TNF-α is mediated by the TAFI105-containing TFIID complex and NF-κB transcription factors.

Results

**TAFI105 interacts with the trans-activation domains of NF-κB members**

To identify TAFI105 target activators we looked for transcription factors that are capable of interacting with TAFI105. Among these we analyzed members of the NF-κB family. For this purpose, recombinant TAFI105 fused to the flag epitope was produced in Sf9 cells using the Baculovirus expression system and purified by anti-flag antibodies coupled to agarose beads. Immobilized TAFI105 was incubated with *in vitro*-translated and 35S-labeled p50 and p65 (relA) subunits NF-κB family. As shown in Figure 1A, p65 but not p50 specifically and efficiently interacts with TAFI105. To determine whether the interaction between p65 and TAFI105 is directed by the putative N-terminal coactivator domain of TAFI105, this region was expressed in *Escherichia coli* as a fusion with glutathione S-transferase (GST105ΔC), purified and subjected to a binding reaction with 35S-labeled p65. p65 specifically binds the N-terminus of TAFI105 (Figure 1B). We also tested other members of the NF-κB family for interaction with TAFI105 and found that c-rel (but not RelB) binds to TAFI105ΔC (data not shown).

Previous studies of activator–TAF association established that an interaction between coactivator subunits of TFIID and transcriptional activators is directed by the trans-activation domain of the activator (Goodrich et al., 1993; Hoey et al., 1993; Chen et al., 1994; Jaq et al., 1994; Chiang and Roeder, 1995; Sauer et al., 1995a; Thut et al., 1995). Both p65 and c-rel (but not p50) contain a strong activation domain located at the C-terminus (reviewed by Schmitz and Baeuerle, 1995). To determine whether the p65 activation domain is involved in TAFI105 binding, the p65 C-terminus was expressed as a fusion with glutathione S-transferase (GST–p65C), purified, coupled to glutathione–Sepharose and used for binding assay with an *in vitro*-translated and 35S-labeled TAFI105. The specificity of the interaction was determined by a similar binding reaction with 35S-labeled p65 which specifically binds TAFI105 (data not shown). Previous studies of activator–TAF association established that an interaction between coactivator subunits of TFIID and transcriptional activators is directed by the trans-activation domain of the activator (Goodrich et al., 1993; Hoey et al., 1993; Chen et al., 1994; Jaq et al., 1994; Chiang and Roeder, 1995; Sauer et al., 1995a; Thut et al., 1995). Both p65 and c-rel (but not p50) contain a strong activation domain located at the C-terminus (reviewed by Schmitz and Baeuerle, 1995). To determine whether the p65 activation domain is involved in TAFI105 binding, the p65 C-terminus was expressed as a fusion with glutathione S-transferase (GST–p65C), purified, coupled to glutathione–Sepharose and used for binding assay with an *in vitro*-translated and 35S-labeled TAFI105. The specificity of the interaction was determined by a similar binding reaction with 35S-labeled hTAFI105, a closely related homolog of TAFI105 (Dikstein et al., 1996; Mengus et al., 1997). The activation domain of p65 specifically binds TAFI105 but not TAFI130 (Figure 1C). Moreover, TAFI105 failed to bind the activation domains of another transcription factor (E2A, Figure 1D), ruling out the possibility that TAFI105 is a general activation-domain binding protein.

**NF-κB dependent transcription is stimulated by TAFI105**

To examine the possibility that TAFI105 is an activation-domain-specific coactivator of p65/RelA, the effect of
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Fig. 2. Stimulation of p65 transcriptional activity by TAFII105. (A) Human 293 cells were transfected with a luciferase reporter plasmid containing 5 tandem Gal4 binding site together with expression plasmids for either the Gal4 DNA binding domain (G4-DBD, columns 3 and 4) or G4-DBD fused to the indicated activation domains in the absence (odd number columns) or presence (even number columns) of TAFII105 expression plasmid. (B) Human 293 cells were cotransfected with NF-κB-dependent reporter plasmid together with either empty expression vector or the following expression plasmids: TAFII105 (columns 2, 4, 7, 11, 12, 14, 15, and 17); IkBα (columns 3, 4, 8, 9, 12, and 15); p65 (columns 5–9); p65 mutant lacking activation domains (p65ΔAD, amino acids 1–286, columns 10–12); p65 deleted of TA1 (p65ΔTA1, amino acid 1–521, columns 13–15) and p65 deleted of TA2 (internal deletion of amino acids 366–521, p65ΔTA2, columns 16 and 17). The amount of CMV-derived vector in each transfection assay was kept constant. The results of these transfection experiments are the average of at least three independent transfection experiments with similar results and were normalized with cotransfected β-actin–β-gal that is non-responsive to p65/RelA. Expressions of transfected TAFII105 and TAFII130 were verified by Western blot, and of p65 wild-type and mutants by EMSA (data not shown).

To further study the functional significance of the TAFII105–NF-κB interaction, we tested the effect of TAFII105 on p65/RelA transcriptional activity. For this purpose a reporter plasmid containing two NF-κB elements upstream of a minimal core promoter and a luciferase gene was cotransfected with p65 and TAFII105 expression plasmids into 293 cells. As expected, p65 activates NF-κB reporter 24-fold (Figure 2B, column 5). When TAFII105 was also cotransfected, both the basal NF-κB- and p65-dependent activities were stimulated 3- and 5-fold, respectively (Figure 2B, columns 2 and 7). Likewise, TAFII105 potentiates NF-κB-dependent transcription in HeLa cells (data not shown). By contrast, hTAFII130 failed to stimulate p65 activity (Figure 2B, column 6). To determine whether the activation by TAFII105 requires the presence of NF-κB proteins, a similar experiment was done in the presence of IκBα, a specific inhibitor of NF-κB factors that prevents the translocation of NF-κB into the nucleus (reviewed in Verma et al., 1995; Baeuerle and Baltimore,
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Fig. 3. Interference with TAFII105 function inhibits NF-κB transcriptional activity. (A) Schematic representation of the TAFII105 putative functional domains and the dominant-negative mutant of TAFII105 (TAFII105ΔC). NLS indicates nuclear localization signal. Expression of mutated TAFII105 was confirmed by Western blot (data not shown). (B) Inhibition of NF-κB transcription by TAFII105ΔC. Luciferase reporter plasmids driven by two tandem NF-κB sites (columns 1-4) or CMV enhancer (columns 5 and 6) were transfected into 293 cells together with the following plasmids: empty expression vector (lanes 1 and 5), p65/RelA (lanes 3 and 4) and TAFII105ΔC (columns 2, 4 and 6). These results are the average of five independent transfection assays with similar results. (C) Inhibition of IL-1-induced NF-κB activity by dominant negative TAFII105. NF-κB luciferase reporter was transfected into 293 cells together with β-actin–β-gal and either an empty expression vector (columns 1, 2 and 3) or TAFII105ΔC plasmid (column 3) or TAFII105 (column 4). Twelve hours after transfection IL-1 was added to the cells (columns 2 and 3) and 24 h post-transfection the relative luciferase activity was determined by normalizing with β-gal activity. These results are the average of three independent transfection experiments.

1996; Baldwin, 1996). As expected, IκBα inhibits both the basal and p65-induced NF-κB activity (Figure 2B, columns 3 and 8, respectively). In the presence of IκB (Figure 2B, columns 4 and 9) or a reporter with mutated NF-κB sites (data not shown), no induction of luciferase activity by TAFII105 is observed, ruling out the possibility that TAFII105 affects core promoter function.

It has been shown that the C-terminus of p65/RelA contains two strong and independent activation domains called TA1 and TA2 (Schmitz and Baeuerle, 1991). To examine whether TAFII105 activity requires the presence of p65 activation domains, we have constructed p65 mutants lacking either TA1 or TA2 or both. The p65 mutant lacking the entire C-terminal activation domain failed to stimulate luciferase in the absence or presence of TAFII105 (Figure 2B, columns 10 and 11). Interestingly, p65 lacking either TA1 (p65ΔTA1) or TA2 (p65ΔTA2) is a significantly weaker activator than the wild-type protein, suggesting that both are required for maximal activation potential (Figure 2B, compare columns 13 and 16 with 5). By contrast, in the context of Gal4, deletion of TA1 did not reduce transcriptional activity (Figure 2A, columns 5 and 7). The activities of both activation domains, either in the context of the native protein (Figure 2B, columns 14 and 17) or as Gal4 fusion (Figure 2A, columns 8 and 10) are enhanced by TAFII105 7–10-fold. Taken together these results strongly suggest that TAFII105 can serve as a p65/RelA activation-domain-specific transcriptional co-activator.

p65/RelA activity is inhibited by a dominant negative mutant of TAFII105

To further confirm that TAFII105 is involved in NF-κB activity we have constructed a plasmid for expression of a dominant negative mutant of TAFII105 based on its predicted functional domains (Dikstein et al., 1996). This mutant is a truncated form of hTAFII105 (TAFII105ΔC, amino acid 1–552, Figure 3A), containing the NF-κB binding region but not the TFIID interaction region. Therefore, if TAFII105ΔC binds NF-κB in vivo, it is likely to inhibit NF-κB-dependent transcription. To test the effect of TAFII105ΔC on NF-κB activity, 293 cells were cotransfected with NF-κB-dependent reporter plasmid and p65/RelA transcription factor together with TAFII105ΔC.
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Inhibition of TAFII105 function causes apoptosis in response to TNF-α

NF-κB activity is regulated by a broad range of cytokines and external stimuli. The cytokine TNF-α is among the physiological inducers of NF-κB activity. Recent studies revealed that activation of NF-κB by TNF-α plays an essential role in protecting cells from pro-apoptotic stimuli produced by TNF-α (Beg and Baltimore, 1996; Liu et al., 1996; Wang et al., 1996; Van Antwerp et al., 1996). We found that TNF-α-induced NF-κB transcription activity
is enhanced by TAFII105 expression (data not shown). Interestingly, when we analyzed the effect of the TAFII105ΔC mutant on long-term TNF-α stimulation in 293 cells, we observed dramatic changes in 293 cell morphology that are consistent with cell death (Figure 4A). These morphological changes were not observed in cells transfected with vector alone (data not shown) or with wild-type TAFII105 (Figure 4A). The survival rate after TNF-α stimulation in TAFII105ΔC-expressing cells was <5% as determined by X-gal staining of transfected cells (Figure 4C). To determine whether these changes are related to programmed cell death, 293 cells transiently expressing TAFII105ΔC were induced by TNF-α and assayed for the DNA ladder characteristic to apoptotic response (Figure 4B). As expected, no DNA fragmentation appears in TNF-α-induced 293 cells transfected with empty expression vector (Figure 4B, lane 1). The DNA ladder can be clearly seen only in cells expressing TAFII105ΔC and stimulated with TNF-α (Figure 4B, lane 3). No DNA fragmentation appears in these cells in the absence of TNF-α stimulation (Figure 4B, lane 4), or in cells transfected with wild-type TAFII105 (Figure 4B, lanes 5 and 6) or with a similar mutant of TAFII130 in the presence or absence of TNF-α stimulus (data not shown).

To further confirm the involvement of TAFII105 in activation of anti-apoptotic genes by TNF-α, 293 cells were transiently transfected with a plasmid that directs expression of anti-sense TAFII105 RNA. As shown in Figure 5A, transient expression of anti-sense TAFII105 reduced the endogenous level of TAFII105 protein but not TBP. This reduction is significant considering that although high transfection efficiencies are achieved in these cells, there is still a significant proportion of untransfected or poorly transfected cells that express normal amounts of TAFII105 (~20% as determined by X-gal staining after transfection, data not shown). The reduction in TAFII105 level is correlated with an increase in cell death in response to TNF-α as evident by cell-survival analysis (Figure 5B), DNA fragmentation (Figure 5C) and microscopic examination (data not shown).

The accelerated cell death in response to TNF-α in 293 cells bearing non-functional TAFII105 is likely to be due to inhibition of NF-κB activity. Indeed, we found that TNF-α-induced NF-κB activity is inhibited by TAFII105ΔC (data not shown). However, since the majority of the cells expressing TAFII105ΔC and induced by TNF-α undergo cell death (Figure 4C), one can not rule out the possibility that part of the inhibition observed might be due to the apoptotic effect. Therefore, to test whether TAFII105ΔC can inhibit cytokine-mediated NF-κB activity we have used the cytokine IL-1, which like TNF-α, induces NF-κB activity but does not induce apoptosis. As shown in Figure 3C, TAFII105ΔC significantly decreases NF-κB activity induced by IL-1 whereas wild-type TAFII105 enhances this activity consistent with the effect of these proteins on p65/RelA activity.

Inhibition of p65/RelA activity and TNF-α-induced cell death by TAFII105ΔC is correlated with p65–TAFII105 interaction
To further investigate the involvement of TAFII105 in NF-κB induction of anti-apoptotic genes we generated deletion mutants of TAFII105ΔC. These mutants were analyzed for their ability to bind p65/RelA, for their inhibitory effect on p65/RelA activity and for their effect on the survival of TNF-α-induced cells. As shown in Figure 6, a mutant of TAFII105ΔC deleted of 100 amino acid residues [105ΔC(1–452)] has reduced p65-binding capacity as well as reduced p65-transcription inhibitory activity as compared with 105ΔC(1–552). This partial inhibition of NF-κB activity is insufficient to cause cell death in response to TNF-α (Figure 6C). Similarly, a mutant that does not bind p65 ([105ΔC(1–167)]) is unable to inhibit p65 activity and to affect cell survival in TNF-α-treated cells. These results strongly suggest that the direct interaction between TAFII105 and p65/RelA plays a role in activation of anti-apoptotic genes.
Analysis in cells expressing TAFII105 for transcription activation, or the specific target genes for in vivo and in vitro studies of Drosophila and human TFIID indicated that transcriptional activation by gene-specific activators can be mediated by TAFs through direct interaction with activation domains of transcription activators. However, thus far there is little in vivo evidence documenting the importance of specific interaction between a TAF subunit and the activation domain of an activator for transcription activation, or the specific target genes for individual TAFs. In this study we show that TAFII105 is an activation-domain-specific coactivator of p65/RelA. Moreover, in vivo, TAFII105 is directly involved in activation of TNF-α-induced anti-apoptotic genes by the transcription factor of NF-κB.

The cytokine TNF-α is secreted by many cell types upon inflammation, infection, viruses and other external signals. Activation of the TNF receptor stimulates the protein synthesis-independent apoptotic response, and in cells resistant to its cytotoxic effect, it also induces expression of proteins that can block apoptosis. NF-κB has been shown to be a primary mediator of TNF-α responses. Recent studies have indicated that stimulation of NF-κB activity by TNF-α protected cells against cell death, most likely by transcriptional activation of some proteins that restrict apoptosis (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996). Our findings indicate that TAFII105 serves as a coactivator for NF-κB-mediated induction of anti-apoptotic genes in response to TNF-α. Blocking of TAFII105 function either by expression of TAFII105 dominant negative mutant or by reducing the amount of endogenous TAFII105 protein by anti-sense RNA, renders the cells sensitive to TNF-α cytotoxicity. Moreover, TAFII105 interacts with selected members of the NF-κB family and can potentiate transcriptional activation by NF-κB proteins. Expression of TAFII105 mutant, which can interact with NF-κB but is incapable of incorporation into TFIID complex, severely inhibited NF-κB-dependent transcriptional activation whereas similar mutants with impaired p65/RelA binding capacity failed to sensitize cells to TNF-α cytotoxicity and to inhibit NF-κB activity. Taken together, these results suggest that the complex formed between NF-κB- and TFIID-containing TAFII105 after TNF-α stimulation targets promoters of genes whose products provide the cells with protection against cell death.

Recent genetic analysis of yeast TAFs revealed that transcription from many promoters is not affected by TAF inactivation, suggesting that TAFs are not generally required for transcription in yeast (Moqtaderi et al., 1996; Walker et al., 1996). Our study indicates that in human cells TAFII105 functions as an activation-domain-specific mediator of transcription consistent with previous in vitro analyses of TAF function (Verrijzer and Tjian, 1996). It is possible that among the multiple activator targets found associated with the basal transcription machinery, TFIID has evolved to play a more central role in mediating activation signals in metazoans, being able to respond to the highly complex gene-expression programs not found in yeast. Moreover, considering the ability of the p65-activation domain to function in yeast cells (Moore et al., 1993) which are devoid of any member of the TAFII105 family (Tansey and Herr, 1997) and the fact that NF-κB is required for gene expression associated with many distinct cellular processes, it is very likely that TAFII105 is not the only NF-κB transcriptional coactivator and several distinct factors might be involved in the activation of other NF-κB target genes.

In most cells NF-κB transcription factors are localized in the cytoplasm bound by IκB, an inhibitory protein that prevents its nuclear translocation. Signals that activate NF-κB such as cytokines (TNF-α and IL-1) induce phosphorylation of IκB and its subsequent degradation. NF-κB is then translocated into the nucleus and activates its target genes (for review see Miyamoto and Verma,

**Discussion**

Previous in vitro studies of Drosophila and human TFIID indicated that transcriptional activation by gene-specific activators can be mediated by TAFs through direct interaction with activation domains of transcription activators. However, thus far there is little in vivo evidence documenting the importance of specific interaction between a TAF subunit and the activation domain of an activator for transcription activation, or the specific target genes for individual TAFs. In this study we show that TAFII105 is an activation-domain-specific coactivator of p65/RelA. Moreover, in vivo, TAFII105 is directly involved in activation of TNF-α-induced anti-apoptotic genes by the transcription factor of NF-κB.

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In B lymphocytes NF-κB is constitutively nuclear and is important for transcription of some B-cell-specific genes (Baldwin, 1996). Interestingly, a high level of TAFII105 protein is also found in B lymphocytes, constituting ~10% of TFIID in these cells compared with only ~0.5%–1% in non-B cells (Dikstein et al., 1996). It is therefore possible that the NF-κB–TAFII105–TFIID complex may also regulate the expression of some B-cell-specific genes. Nevertheless it seems that the level of TAFII105 in non-B cells is sufficient for the induction of anti-apoptotic genes by NF-κB as TNF-α does not induce TAFII105 protein expression (data not shown) and potentiation of NF-κB-dependent transcription by TAFII105 could be achieved only when suboptimal concentrations of either the NF-κB activator protein or TNF-α were used.

Several TNF-α-induced genes have been implicated as potential candidates for anti-apoptotic function. Overexpression of each provides partial protection against TNF-α cytotoxicity (Wong et al., 1989; Opipari et al., 1992; Karsan et al., 1996; Chu et al., 1997). However, it is likely that other genes, not yet identified, are also required for full protection. The promoters of these genes may be preferred targets for the sub-stoichiometric TFIID required for full protection. The promoters of these genes is likely that other genes, not yet identified, are also possible to address the molecular basis of this process.

Materials and methods

In vitro binding experiments
Baculovirus expression of flag-tagged TAFII105 was done as described previously (Dikstein et al., 1996). 35S-labeled p65 and p50 and E2A (a gift from Dr Michael Walker) were synthesized in vitro by T7 RNA polymerase and rabbit reticulocyte lysate and incubated with flag beads or with TAFII105 coupled to flag beads in 0.1 M KCl HEMG buffer (20 mM Hepes pH 7.9, 100 mM KCl, 12.5 mM MgCl2, 0.2 mM EDTA, 0.1% NP-40, 1 μM DTT, 0.2 mM PMSF) for 2 h at 4°C. The beads were washed 3 times with the same buffer and twice with 0.2 M NaCl HEMG buffer. The bound proteins were eluted by 5 min boiling in protein sample buffer followed by SDS-PAGE and autoradiography.

Plasmids
Baculovirus expression vector for TAFII105 was previously described (Dikstein et al., 1996). Plasmids for eukaryotic and E.coli expression vectors were constructed according to standard procedures. Details on the construction of pCMV–TAFII105, pCMV–TAFII105AC, pCMV–TAFII130, GST–TAFII105AC, GST–p65C and the various G4–p65 fusions and p65 mutants can be obtained upon request. G4-E2A–AD1 and G4-E2A–AD2 were gifts from Dr Michael Walker. The NF-κB-dependent reporter plasmid was constructed by inserting double-stranded synthetic oligonucleotides (see below) containing two tandem κB sites next to a minimal core promoter from the mouse α-actin promoter (~40 to +80), that was cloned into the HindIII site of promoterless pLuc vector (Altschmied and Duschl, 1997). NF-κB oligonucleotides: 5′-AGCTTTCAGGCTTCAGGAGCTTTCCG-3′; 5′-GATCCGGA-AAGTTCCCCTCGGAAAGTCCCTA-3′.

Propagation and transfection of cell lines
293 cells are embryonic kidney fibroblasts. 293T cells are modified 293 cells constitutively expressing the SV40 large T-antigen and therefore allow replication of plasmids of SV40 origin. 293 and 293T cell lines were maintained in F12 Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Transfections were performed using the standard CaPO4 method.

293 subconfluent cells were transfected in a 24-well multidish using a total of 1 μg plasmid per transfection. Generally, 50 ng of NF-κB-dependent reporter and 10 ng GA4-dependent reporter were used. The amount of the different activator plasmids was between 5 and 20 ng, and for TAFII105- and TAFII130-expressing plasmids it was between 300 and 500 ng. In each transfection the amount of CMV-containing plasmid was kept constant. Luciferase activity was determined as instructed by the manufacturer (Promega). Human recombinant TNF-α was purchased from R & D Systems.

Apoptosis assays
For the survival analysis 293T cells were cotransfected with a pCMV-lacZ or pCMV-GFP reporter plasmid and different expression vectors, and either stained with X-Gal (see below) or directly visualized by microscope for green fluorescent cell detection 48 h after transfection. The number of blue or GFP cells was determined by counting five different randomly chosen fields. For the X-Gal assay the cultures were rinsed with phosphate-buffered saline, fixed with 1% of glutaraldehyde for 15 min, and then stained with 4 ml of X-Gal solution [2 mg/ml X-Gal in DMF, 3.3 mM K3Fe(CN)6 and 3.3 mM K4Fe(CN)6] for 2 h. DNA fragmentation assay was done as follows: 293T cells (2×105) were collected 24 h after transfections, resuspended in 400 μl lysis buffer (1.0% Triton X-100, 0.01 M EDTA, 1% SDS, and 10 μg/ml proteinase K) and incubated overnight at 37°C. DNA was obtained by phenol extraction following ethanol precipitation. The DNA pellet was resuspended in 100 μl TE containing 50 μg/ml RNase A and incubated for 30 min at 37°C. Twenty micrograms of DNA was run onto 1.8% agarose gel in the presence of 0.5 μg/ml ethidium bromide.

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