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$TAF_{II}105$ mediates activation of anti-apoptotic genes by NF-κB

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

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 (TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) 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 *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 *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 and Tjian, 1993; Künzler *et al*., 1994; Ponticelli *et al*., 1995) and that certain *Drosophila* and human TAFs have no evident homolog in yeast (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, $TAF_{II}105$, that is enriched in TFIID of human B lymphocytes constituting ~10% of TFIID in these cells and ~0.5–1% in non-B cells (Dikstein *et al*., 1996). Therefore, $TAF_{II}105$ 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. $TAF_{II}105$ shares regions of high homology with the core TFIID subunits $TAF_{II}130$ from human and $TAF_{II}110$ 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 $dTAF_{II}110$ and $hTAF_{II}130$ directs interaction with activation domains of activators (Chen *et al*., 1994; Tanese *et al*., 1996). Interestingly, in a TFIID complex containing $TAF_{II}105$, $TAF_{II}130$ is also present in the same complex.

Here we show that $TAF_{II}105$ serves as a coactivator for the transcription factor NF-κB *in vivo*. The NF-κB family plays a fundamental role in immune responses and is essential for cytokine-inducible gene expression, in particular TNF-α- and IL-1-induced genes (for review see Verma *et al*., 1995; Baeuerle and Baltimore, 1996; Baldwin, 1996). Our studies reveal that $TAF_{II}105$ interacts directly with p65/RelA and mediates gene activation by the p65 activation domain. Recent studies suggest that NF-κB 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 $TAF_{II}105$ 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 $TAF_{II}105$-containing TFIID complex and NF-κB transcription factors.

Results

$TAF_{II}105$ interacts with the trans-activation domains of NF-κB members

To identify $TAF_{II}105$ target activators we looked for transcription factors that are capable of interacting with $TAF_{II}105$. Among these we analyzed members of the NF-κB family. For this purpose, recombinant $TAF_{II}105$ 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 $TAF_{II}105$ was incubated with *in vitro*-translated and ^{35}S-labeled p50 and p65 (relA) subunits NF-κB family. As shown in Figure 1A, p65 but not p50 specifically and efficiently interacts with $TAF_{II}105$. To determine whether the interaction between p65 and $TAF_{II}105$ is directed by the putative N-terminal coactivator domain of $TAF_{II}105$, this region was expressed in *Escherichia coli* as a fusion with glutathione *S*-transferase (GST105ΔC), purified and subjected to a binding reaction with ^{35}S-labeled p65. p65 specifically binds the N-terminus of $TAF_{II}105$ (Figure 1B). We also tested other members of the NF-κB family for interaction with $TAF_{II}105$ and found that c-rel (but not RelB) binds to $TAF_{II}105\Delta C$ (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; Jacq *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 $TAF_{II}105$ 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 ^{35}S-labeled $TAF_{II}105$. The specificity of the interaction was determined by a similar binding reaction with ^{35}S-labeled $hTAF_{II}130$, a closely related homolog of $TAF_{II}105$ (Dikstein *et al*., 1996; Mengus *et al*., 1997). The activation domain of p65 specifically binds $TAF_{II}105$ but not $TAF_{II}130$ (Figure 1C). Moreover, $TAF_{II}105$ failed to bind the activation domains of another transcription factor (E2A, Figure 1D), ruling out the possibility that $TAF_{II}105$ is a general activation-domain binding protein.

NF-κB dependent transcription is stimulated by $TAF_{II}105$

To examine the possibility that $TAF_{II}105$ is an activation-domain-specific coactivator of p65/RelA, the effect of

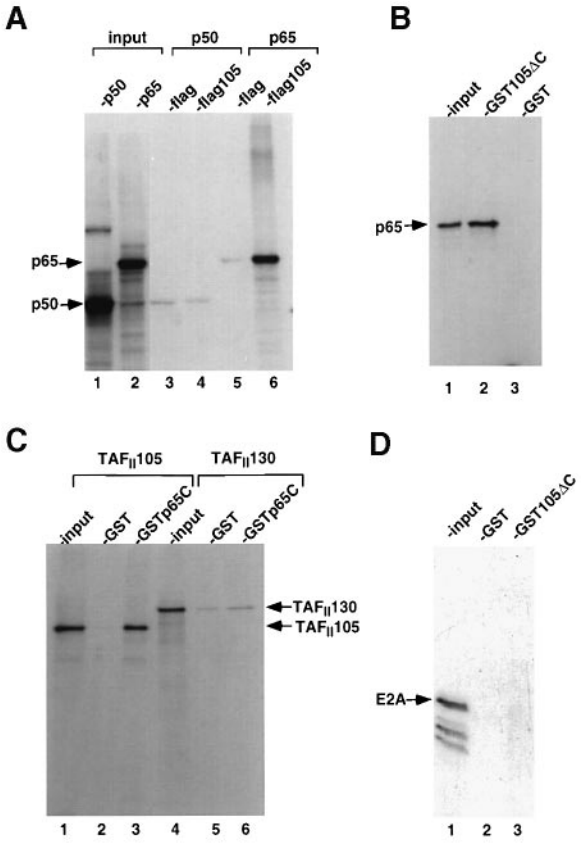

Fig. 1. Interaction of $hTAF_{II}105$ with p65/RelA. (**A**) p65/RelA and p50 proteins were translated *in vitro* and labeled with [^{35}S]methionine using rabbit reticulocyte lysate. These proteins were used for interaction assay with immobilized flag-tagged $TAF_{II}105$ as indicated on top of each lane. As a control, the labeled proteins were incubated with the flag beads. The bound proteins were eluted, resolved on SDS–PAGE and autoradiographed. Input lanes represent 10% of the labeled protein used for the binding reaction. (**B**) Binding reaction between ^{35}S-labeled p65/RelA and the purified N-terminal fragment of $TAF_{II}105$ (amino acid 1–552) fused to glutathione *S*-transferase (GST) and bound to glutathione beads (lane 2). As a control a similar reaction was performed using the same beads bound by GST (lane 3). Input lanes represent 10% of p65 used for the binding assay. (**C**) Interaction between $TAF_{II}105$ and the C-terminal activation domain of p65. The C-terminus of p65 (amino acid 398–551) was expressed as a GST-fusion protein, affinity-purified by glutathione beads and subjected to a binding reaction with *in vitro*-translated and ^{35}S-labeled $TAF_{II}105$ (lanes 1–3) or $TAF_{II}130$ (lanes 4–6). GST-containing beads were used as a control in a similar binding reaction. Input lanes represent ~10% of the labeled proteins used. (**D**) Binding assay as in (B) using ^{35}S-labeled E2A transcription factor.

$TAF_{II}105$ on the p65 activation domain was analyzed by transient transfection experiments. The C-terminal activation domain region of p65 (amino acid residues 398–551) was fused to the yeast Gal4 DNA-binding domain (G4–p65C) and cotransfected into human 293 cells together with Gal4-dependent luciferase reporter plasmid in the presence or absence of $TAF_{II}105$ expression vector. As expected, the Gal4 DNA binding domain (G4–DBD) in the absence of the activation domain lacks transcription activity and is not induced by $TAF_{II}105$ (Figure 2A, columns 3 and 4). G4–p65C strongly stimulated luciferase activity (415-fold; Figure 2A, column 5). This activity is further potentiated by coexpression of $TAF_{II}105$ (4163-fold; Figure 2A, column 6). The specificity of $TAF_{II}105$ action was tested by its coexpression

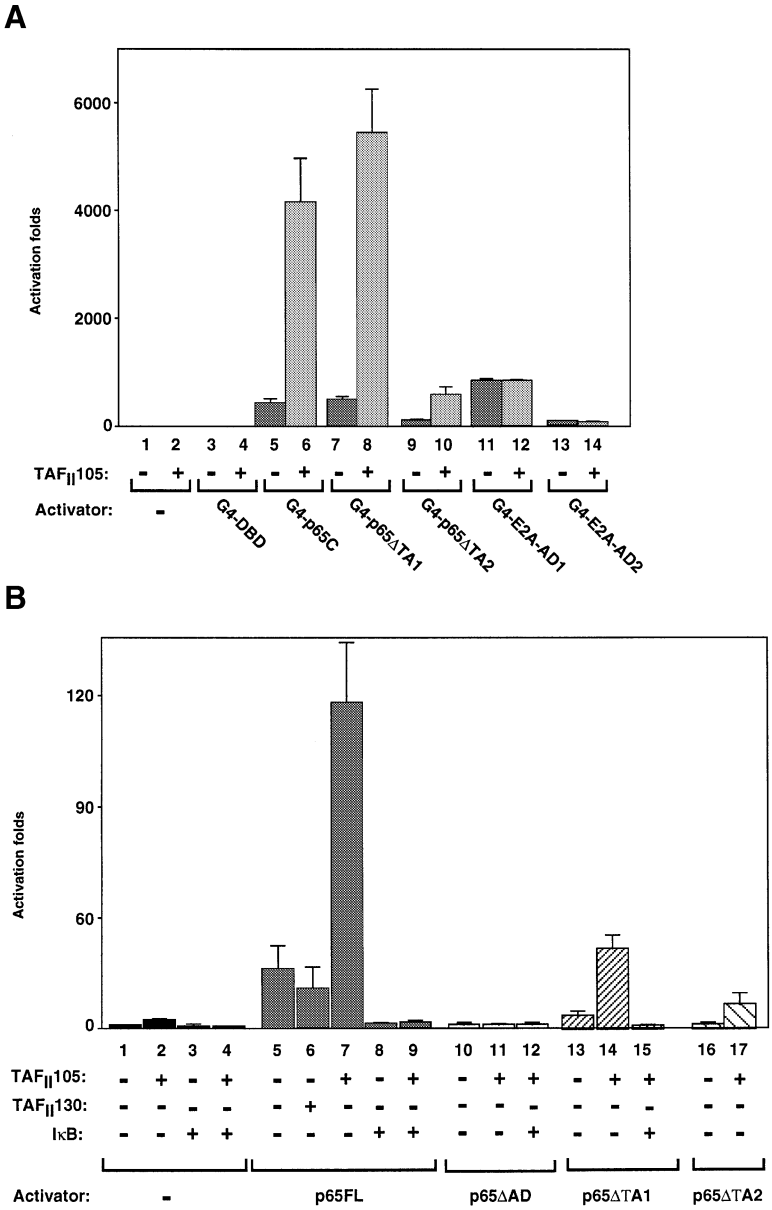

Fig. 2. Stimulation of p65 transcriptional activity by $TAF_{II}105$. (**A**) Human 293 cells were transfected with a luciferase reporter plasmid containing 5 tandem Gal4 binding site together with expression plasmids for either the Gal4 DNA binding domain (G4-DBD, columns 3 and 4) or G4-DBD fused to the indicated activation domains in the absence (odd number columns) or presence (even number columns) of $TAF_{II}105$ 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: $TAF_{II}105$ (columns 2, 4, 7, 9, 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 TA_1 (p65ΔTA1, amino acid 1–521, columns 13–15) and p65 deleted of TA_2 (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 $TAF_{II}105$ and $TAF_{II}130$ were verified by Western blot, and of p65 wild-type and mutants by EMSA (data not shown).

with Gal4 fused to E2A activation domains (G4–E2A–AD1 and G4–E2A–AD2) in which $TAF_{II}105$ has no stimulatory effect (columns 11–14).

To further study the functional significance of the $TAF_{II}105$–NF-κB interaction, we tested the effect of $TAF_{II}105$ 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 $TAF_{II}105$ expression plasmids into 293 cells. As expected, p65 activates NF-κB reporter 24-fold (Figure 2B, column 5). When $TAF_{II}105$ 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, $TAF_{II}105$ potentiates NF-κB-dependent transcription in HeLa cells (data not shown). By contrast, $hTAF_{II}130$ failed to stimulate p65 activity (Figure 2B, column 6). To determine whether the activation by $TAF_{II}105$ 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,

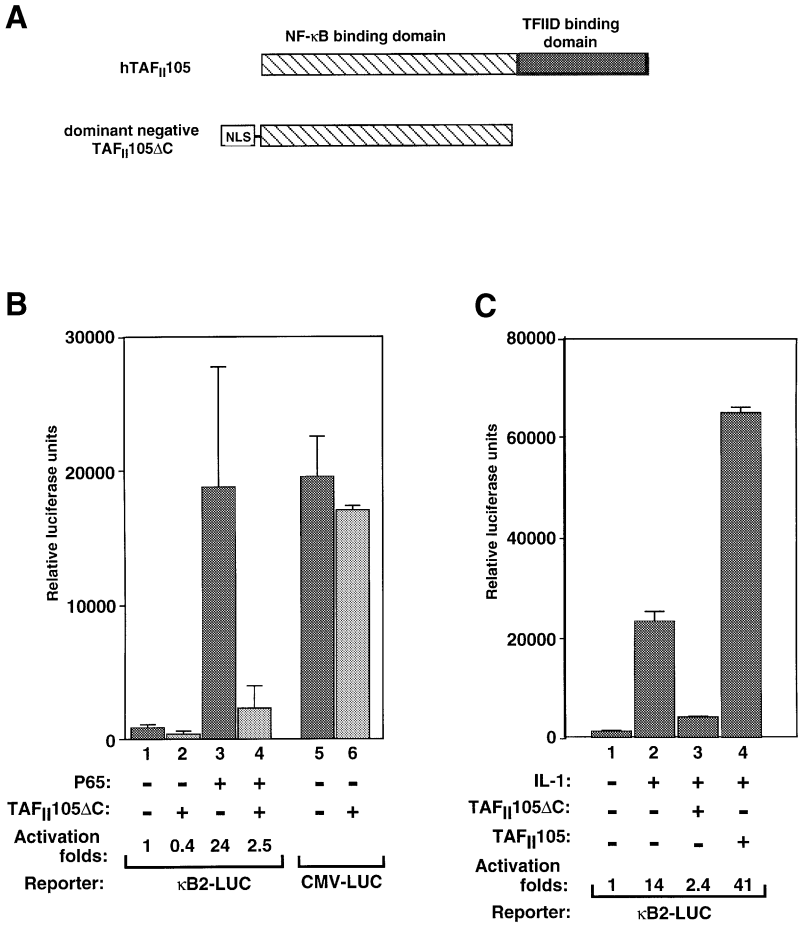

Fig. 3. Interference with TAF$_{II}$105 function inhibits NF-κB transcriptional activity. (**A**) Schematic representation of the TAF$_{II}$105 putative functional domains and the dominant-negative mutant of TAF$_{II}$105 (TAF$_{II}$105ΔC). NLS indicates nuclear localization signal. Expression of mutated TAF$_{II}$105 was confirmed by Western blot (data not shown). (**B**) Inhibition of NF-κB transcription by TAF$_{II}$105ΔC. Luciferase reporter plasmids driven by two tandem NF-κB sites (columns 1–4) or CMV enhancer (columns 5 and 6) were transfected into 293 cells together with the following plasmids: empty expression vector (lanes 1 and 5), p65/RelA (lanes 3 and 4) and TAF$_{II}$105ΔC (columns 2, 4 and 6). These results are the average of five independent transfection assays with similar results. (**C**) Inhibition of IL-1-induced NF-κB activity by dominant negative TAF$_{II}$105. NF-κB luciferase reporter was transfected into 293 cells together with β-actin–β-gal and either an empty expression vector (columns 1, 2 and 3) or TAF$_{II}$105ΔC plasmid (column 3) or TAF$_{II}$105 (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 TAF$_{II}$105 is observed, ruling out the possibility that TAF$_{II}$105 affects core promoter function.

It has been shown that the C-terminus of p65/RelA contains two strong and independent activation domains called TA$_1$ and TA$_2$ (Schmitz and Baeuerle, 1991). To examine whether TAF$_{II}$105 activity requires the presence of p65 activation domains, we have constructed p65 mutants lacking either TA$_1$ or TA$_2$ or both. The p65 mutant lacking the entire C-terminal activation domain failed to stimulate luciferase in the absence or presence of TAF$_{II}$105 (Figure 2B, columns 10 and 11). Interestingly, p65 lacking either TA$_1$ (p65ΔTA1) or TA$_2$ (p65ΔTA2) is a significantly weaker activator then 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 TA$_1$ 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 TAF$_{II}$105 7–10-fold. Taken together these results strongly suggest that TAF$_{II}$105 can serve as a p65/RelA activation-domain-specific transcriptional co-activator.

p65/RelA activity is inhibited by a dominant negative mutant of TAF$_{II}$105

To further confirm that TAF$_{II}$105 is involved in NF-κB activity we have constructed a plasmid for expression of a dominant negative mutant of TAF$_{II}$105 based on its predicted functional domains (Dikstein *et al*., 1996). This mutant is a truncated form of hTAF$_{II}$105 (TAF$_{II}$105ΔC, amino acid 1–552, Figure 3A), containing the NF-κB binding region but not the TFIID interaction region. Therefore, if TAF$_{II}$105ΔC binds NF-κB *in vivo*, it is likely to inhibit NF-κB-dependent transcription. To test the effect of TAF$_{II}$105ΔC on NF-κB activity, 293 cells were cotransfected with NF-κB-dependent reporter plasmid and p65/RelA transcription factor together with TAF$_{II}$105ΔC.

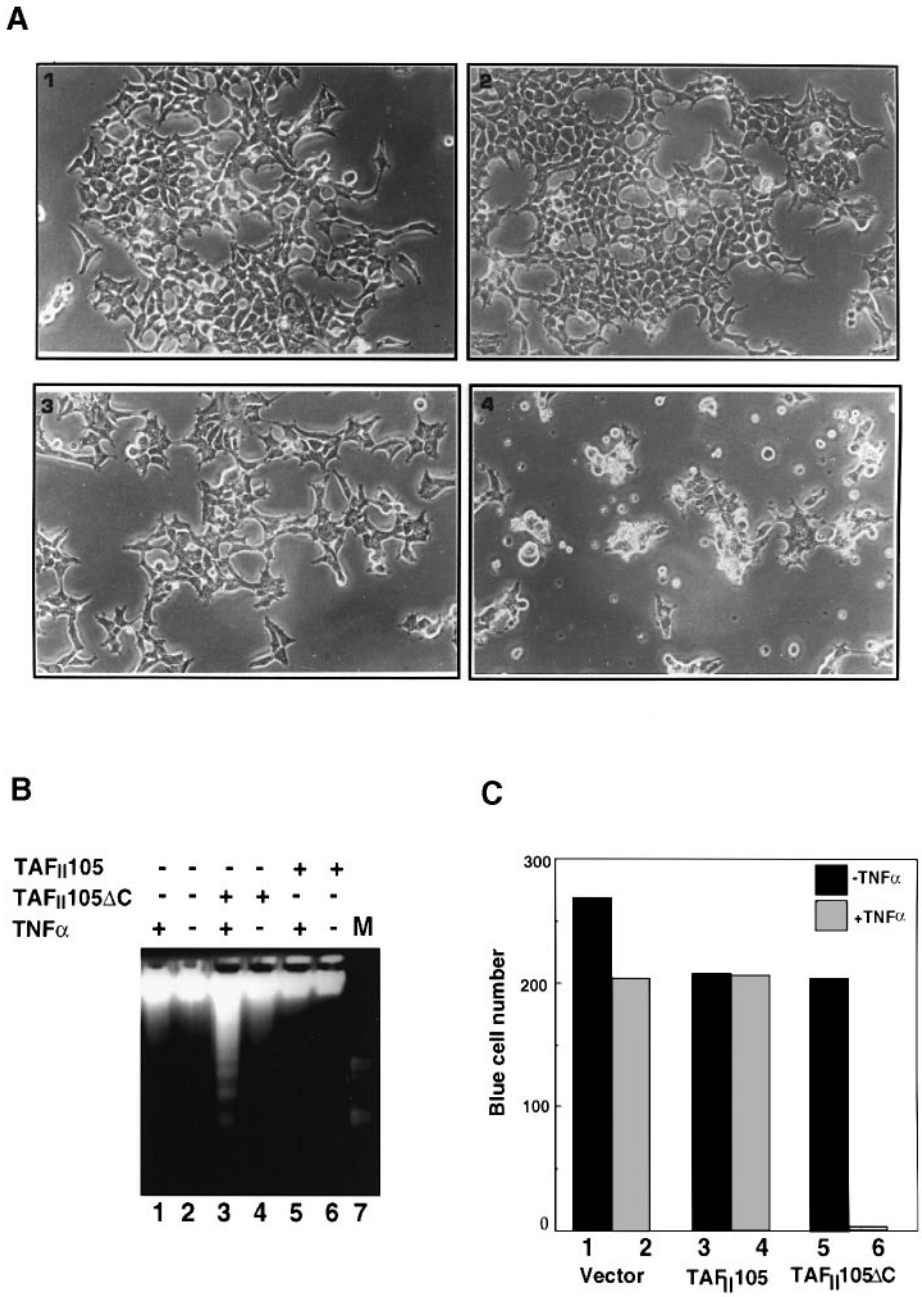

Fig. 4. Dominant negative mutant of TAF$_{II}$105 induces cell death in response to TNF-α. (**A**) 293T cells were transfected with wild-type TAF$_{II}$105 (pictures 1 and 2) or TAF$_{II}$105ΔC (3 and 4) and either untreated (1 and 3) or treated with 15 ng/ml TNF-α (2 and 4). The pictures were taken 24 h after transfection. (**B**) Genomic DNA analysis of 293T cells that were transfected with either empty expression vector (lanes 1 and 2), TAF$_{II}$105ΔC (lanes 3 and 4) or with wild-type TAF$_{II}$105 (lanes 5 and 6). Twelve hours after transfection some of the transfected cells were treated with TNF-α (odd number lanes) and 24 h after transfection DNA was extracted from the transfected cells and analyzed by 1.8% agarose gel. M indicates DNA size marker. (**C**) Cell survival assay. To quantify the apoptosis induction, 293T cells were cotransfected with pCMV*lacZ* reporter plasmid and the indicated expression vectors, and similar to the experiment described in (B), treated with TNF-α (lanes 2, 4 and 6). Twenty-four hours after transfection the cells were stained with X-Gal, and the numbers of blue cells in five randomly chosen fields were determined. These data represent an average of three independent transfection experiments.

As shown in Figure 3B, TAF$_{II}$105ΔC severely inhibited transcriptional activation by p65/RelA. Similarly, cotransfection of the NF-κB reporter with p65/RelA and a dominant negative IκB-α inhibited NF-κB activity, confirming that the observed activity is directed by NF-κB proteins (Figure 2B, columns 3 and 7). TAF$_{II}$105ΔC mutant has no significant effect on the reporter plasmid containing the CMV enhancer (Figure 3B, columns 5 and 6), further supporting the idea that TAF$_{II}$105ΔC is specific to p65/RelA and has no effect on core promoter activity.

Inhibition of TAF$_{II}$105 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

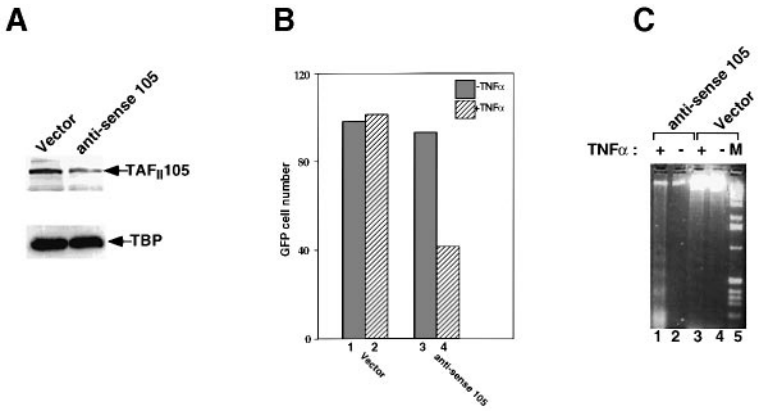

Fig. 5. The effect of $TAF_{II}105$ anti-sense RNA expression on TNF-α treated cells. (**A**) 293T cells were transfected either with empty vector or with expression vector for $TAF_{II}105$ anti-sense RNA. Levels of endogenous $TAF_{II}105$ were analyzed 36 h after transfection by Western blot using affinity-purified anti-$TAF_{II}105$ antibodies (upper panel). As a control the same extracts were used for analysis of TBP levels (lower panel). (**B**) Survival analysis of cells expressing $TAF_{II}105$ anti-sense RNA. 293T cells were cotransfected with CMV-GFP reporter plasmid with either an empty expression vector (lanes 1 and 2) or with expression vector for $TAF_{II}105$ anti-sense RNA (lanes 3 and 4). Twenty-four hours after transfection TNF-α was provided to the cells (lanes 2 and 4) and 24 h later green fluorescent cells were counted in five randomly chosen fields. These data represent an average of four independent transfection experiments. (**C**) DNA fragmentation analysis of cells transfected with either anti-sense $TAF_{II}105$ RNA (lanes 1 and 2) or empty vector (lanes 3 and 4). Twenty-four hours after transfection TNF-α was provided to the cells (lanes 1 and 3) and 16 h later fresh medium containing TNF-α was provided to the cells. Genomic DNA was extracted 48 h after transfection and analyzed by 1.8% agarose gel. M represents a 1 kb DNA molecular size marker.

is enhanced by $TAF_{II}105$ expression (data not shown). Interestingly, when we analyzed the effect of the $TAF_{II}105\Delta C$ 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 $TAF_{II}105$ (Figure 4A). The survival rate after TNF-α stimulation in $TAF_{II}105\Delta C$-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 $TAF_{II}105\Delta C$ 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 $TAF_{II}105\Delta C$ 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 $TAF_{II}105$ (Figure 4B, lanes 5 and 6) or with a similar mutant of $TAF_{II}130$ in the presence or absence of TNF-α stimulus (data not shown).

To further confirm the involvement of $TAF_{II}105$ in activation of anti-apoptotic genes by TNF-α, 293 cells were transiently transfected with a plasmid that directs expression of anti-sense $TAF_{II}105$ RNA. As shown in Figure 5A, transient expression of anti-sense $TAF_{II}105$ reduced the endogenous level of $TAF_{II}105$ 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 $TAF_{II}105$ (~20% as determined by X-gal staining after transfection, data not shown). The reduction in $TAF_{II}105$ 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 $TAF_{II}105$ is likely to be due to inhibition of NF-κB activity. Indeed, we found that TNF-α-induced NF-κB activity is inhibited by $TAF_{II}105\Delta C$ (data not shown). However, since the majority of the cells expressing $TAF_{II}105\Delta C$ 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 $TAF_{II}105\Delta C$ 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, $TAF_{II}105\Delta C$ significantly decreases NF-κB activity induced by IL-1 whereas wild-type $TAF_{II}105$ 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 $TAF_{II}105\Delta C$ is correlated with p65–$TAF_{II}105$ interaction

To further investigate the involvement of $TAF_{II}105$ in NF-κB induction of anti-apoptotic genes we generated deletion mutants of $TAF_{II}105\Delta C$. 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 $TAF_{II}105\Delta C$ 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 $TAF_{II}105$ and p65/RelA plays a role in activation of anti-apoptotic genes.

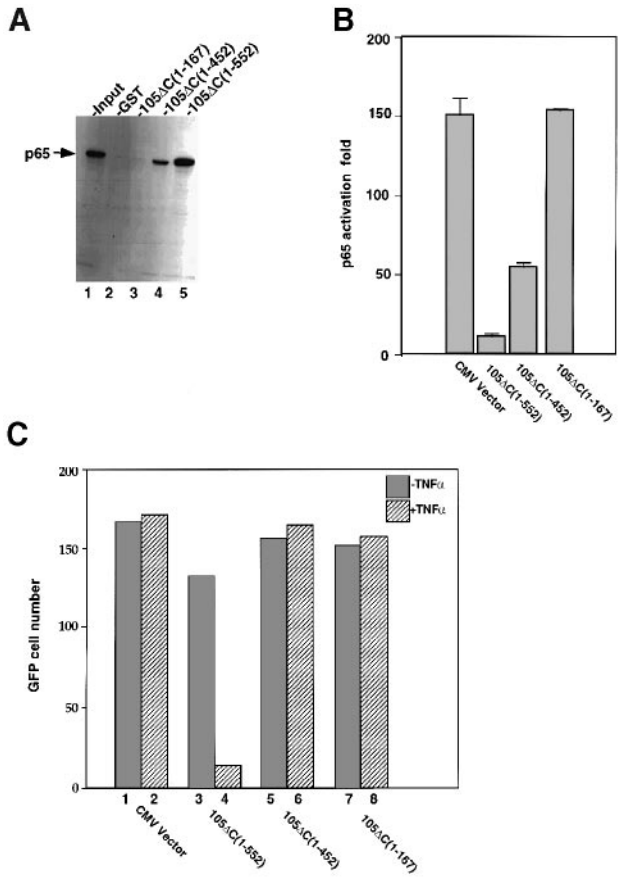

Fig. 6. Analysis of $TAF_{II}105\Delta C$ deletion mutants. (**A**) GST pull-down experiment using ^{35}S-labeled p65/RelA and equivalent amounts of purified N-terminal fragments of $TAF_{II}105$ (indicated by their amino acids residues), fused to GST and bound to glutathione beads (lanes 3–5). As a control a similar reaction was performed using the same beads bound by GST (lane 2). The input lanes represent 10% of p65 used for the binding assay. (**B**) The effect of $TAF_{II}105\Delta C$ mutants on NF-κB transcription activity. Human 293 cells were cotransfected with NF-κB dependent luciferase reporter, p65 expression plasmid and with either empty expression vector or the following expression plasmids: $TAF_{II}105\Delta C$ coding for amino acids 1–552, $TAF_{II}105\Delta C$ coding for amino acids 1–452 or $TAF_{II}105\Delta C$ coding for amino acids 1–167. The results are presented as the ratio between p65 activity relative to the activity of NF-κB reporter without p65 expression. (**C**) Cell-survival analysis in cells expressing $TAF_{II}105\Delta C$ mutants and treated with TNFα. 293T cells were cotransfected with CMV-GFP reporter plasmid either with empty vector or with expression plasmids described in (B). Twelve hours after transfection the cells were untreated or treated with TNF-α and 24 h later the green fluorescent cells in five randomly chosen fields were counted. This data is representative of three independent transfection experiments with similar results. Equivalent expression of $TAF_{II}105\Delta C$ mutants was verified by Western blot analysis.

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 $TAF_{II}105$ is an activation-domain-specific coactivator of p65/RelA. Moreover, *in vivo*, $TAF_{II}105$ 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; Wang *et al*., 1996). Our findings indicate that $TAF_{II}105$ serves as a coactivator for NF-κB-mediated induction of anti-apoptotic genes in response to TNF-α. Blocking of $TAF_{II}105$ function either by expression of $TAF_{II}105$ dominant negative mutant or by reducing the amount of endogenous $TAF_{II}105$ protein by anti-sense RNA, renders the cells sensitive to TNF-α cytotoxicity. Moreover, $TAF_{II}105$ interacts with selected members of the NF-κB family and can potentiate transcriptional activation by NF-κB proteins. Expression of $TAF_{II}105$ 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 $TAF_{II}105$ 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 $TAF_{II}105$ 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 $TAF_{II}105$ 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 $TAF_{II}105$ 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,

1995). 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 $TAF_{II}105$ 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–$TAF_{II}105$–TFIID complex may also regulate the expression of some B-cell-specific genes. Nevertheless it seems that the level of $TAF_{II}105$ in non-B cells is sufficient for the induction of anti-apoptotic genes by NF-κB as TNF-α does not induce $TAF_{II}105$ protein expression (data not shown) and potentiation of NF-κB-dependent transcription by $TAF_{II}105$ 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 complex containing $TAF_{II}105$. A possible mechanism is that enhancer-bound NF-κB interacts with the $TAF_{II}105$–TFIID complex and recruits this complex to the promoter (Sauer *et al*., 1995b). Alternatively, the TFIID–$TAF_{II}105$ complex may pre-occupy the core promoter of these genes and form a complex with NF-κB after it is bound to its target sites (Brunvand *et al*., 1993). A third possibility might be that formation of the NF-κB–$TAF_{II}105$–TFIID complex precedes preferred binding to the promoters of anti-apoptotic genes. The latter two mechanisms suggest selective recognition of promoters by the $TAF_{II}105$-containing TFIID complex. Once a target gene for the NF-κB–$TAF_{II}105$–TFIID complex is identified it will be possible to address the molecular basis of this process.

Materials and methods

In vitro binding experiments

Baculovirus expression of flag-tagged $TAF_{II}105$ was done as described previously (Dikstein *et al*., 1996). ^{35}S-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 $TAF_{II}105$ coupled to flag beads in 0.1 M KCl HEMG buffer (20 mM HEPES pH 7.9, 100 mM KCl, 12.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% NP-40, 1 mM 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 $TAF_{II}105$ 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–$TAF_{II}105$, pCMV–$TAF_{II}105\Delta C$, pCMV–$TAF_{II}130$, GST–$TAF_{II}105\Delta C$, 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 *Hin*dIII site of promoterless pLuc vector (Altschmied and Duschl, 1997). NF-κB oligonucleotides: 5′-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3′; 5′-GATCCGGAAAGTCCCCTCGGAAAGTCCCTA-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 $CaPO_4$ 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 Gal4-dependent reporter were used. The amount of the different activator plasmids was between 5 and 20 ng, and for $TAF_{II}105$- and $TAF_{II}130$-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 glutadialdehyde for 15 min, and then stained with 4 ml of X-Gal solution [2 mg/ml X-Gal in DMF, 3.3 mM $K_3Fe(CN)_6$ and 3.3 mM $K_4Fe(CN)_6$] for 2 h. DNA fragmentation assay was done as follows: 293T cells (2×10^6) were collected 24 h after transfections, resuspended in 400 μl lysis buffer (200 mM Tris pH 8.5, 100 mM EDTA, 1% SDS, and 100 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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References

Altschmied,J. and Duschl,J. (1997) Set of optimized luciferase reporter gene plasmids compatible with widely used CAT vectors. *Biotechniques*, **23**, 436–438.

Attardi,L.D. and Tjian,R. (1993) *Drosophila* tissue-specific transcription factor NTF-1 contains a novel isoleucine-rich activation motif. *Genes Dev.*, **7**, 1341–1353.

Baeuerle,P.A. and Baltimore,D. (1996) NF-κB: ten years after. *Cell*, **87**, 13–20.

Baldwin,A.S. (1996) The NF-κB and IkB proteins: new discoveries and insights. *Annu. Rev. Immunol.*, **14**, 649–681.

Beg,A.A. and Baltimore,D. (1996) An essential role for NF-κB in preventing TNF-α-induced cell death. *Science*, **274**, 782–784.

Brunvand,M.W., Krumm,A. and Groudine,M. (1993) *In vivo* footprinting of the human IL-2 gene reveals a nuclear factor bound to the transcription start site in T cells. *Nucleic Acids Res.*, **21**, 4824–4829.

Chen,J.-L., Attardi,L.D., Verrijzer,C.P., Yokomori,K. and Tjian,R. (1994) Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell*, **79**, 93–105.

Chiang,C.M. and Roeder,R.G. (1995) Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators. *Science*, **267**, 531–536.

Chu,Z.L., McKinsey,T.A., Liu,L., Gentry,J.J., Malim,M.H. and Ballard,D.W. (1997) Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. *Proc. Natl Acad. Sci. USA*, **94**, 10057–10062.

Dikstein,R., Zhou,S. and Tjian,R. (1996) Human $TAF_{II}105$ is a cell type specific TFIID subunit related to $hTAF_{II}130$. *Cell*, **87**, 137–146.

Dynlacht,B.D., Hoey,T. and Tjian,R. (1991) Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell*, **66**, 563–576.

Goodrich,J.A., Hoey,T., Thut,C.J., Admon,A. and Tjian,R. (1993) *Drosophila* TAF$_{II}$40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. *Cell*, **75**, 519–530.

Hoey,T., Weinzierl,R.O.J., Gill,G., Chen,J.-L., Dynlacht,B.D. and Tjian,R. (1993) Molecular cloning and functional analysis of *Drosophila* TAF110 reveal properties expected of coactivators. *Cell*, **72**, 247–260.

Hoffmann,A., Oelgeschlager,T. and Roeder,R.G. (1997) Consideration of transcriptional control mechanisms: do TFIID-core promoter complexes recapitulate nucleosome-like functions? *Proc. Natl Acad. Sci. USA*, **94**, 8928–8935.

Horikoshi,M., Hai,T., Lin,Y.-S., Green,M.R. and Roeder,R.G. (1988) Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell*, **54**, 1033–1042.

Jacq,X., Brou,C., Lutz,Y., Davidson,I., Chambon,P. and Tora,L. (1994) Human TAF$_{II}$30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell*, **79**, 107–117.

Karsan,A., Yee,E. and Harlan,J.M. (1996) Endothelial cell death induced by tumor necrosis factor-alpha is inhibited by the Bcl-2 family member, A1. *J. Biol. Chem.*, **271**, 27201–27204.

Künzler,M., Braus,G.H., Georgiev,O., Seipel,K. and Schaffner,W. (1994) Functional differences between mammalian transcription activation domains at the yeast GAL1 promoter. *EMBO J.*, **13**, 641–645.

Liu,Z-G., Hsu,H., Goeddel,D.V. and Karin,M. (1996) Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell*, **87**, 565–576.

May,M., Mengus,G., Lavigne,A.C., Chambon,P. and Davidson,I. (1996) Human TAF$_{II}$28 promotes transcriptional stimulation by the activation function 2 of the retinoid X receptors. *EMBO J.*, **15**, 3093–3104.

Mengus,G., May,M., Carre,L., Chambon,P. and Davidson,I. (1997) Human TAF$_{II}$135 potentiates transcriptional activation by the retinoic acid, vitamin D3 and thyroid hormone receptors in mammalian cells. *Genes Dev.*, **11**, 1381–1395.

Miyamoto,S. and Verma,I.M. (1995) Rel/NF-κB/I κB story. *Adv. Cancer Res.*, **66**, 255–292.

Moore,P.A., Ruben,S.M. and Rosen,C.A. (1993) Conservation of transcriptional activation functions of the NF-kappa B p50 and p65 subunits in mammalian cells and *Saccharomyces cerevisiae*. *Mol. Cell. Biol*., **13**, 1666–1674.

Moqtaderi,Z., Bai,Y., Poon,D., Weil,P.A. and Struhl,K. (1996) TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature*, **383**, 188–191.

Opipari,A.W., Hu,J.R., Yabkowitz,R. and Dixit,V.M. (1992) The A20 zinc finger protein protects cells from tumor necrosis factor cytotoxicity. *J. Biol. Chem.*, **267**, 12424–12427.

Orphanides,G., Lagrange,T. and Reinberg,D. (1996) The general transcription factors of RNA polymerase II. *Genes Dev.*, **10**, 2657–2683.

Ponticelli,A.S., Pardee,T.S. and Struhl,K. (1995) The glutamine-rich activation domains of human Sp1 do not stimulate transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*., **15**, 983–988.

Poon,D. and Weil,P.A. (1993) Immunopurification of yeast TATA-binding protein and associated factors. Presence of transcription factor IIIB transcriptional activity. *J. Biol. Chem.*, **268**, 15325–15328.

Pugh,B.F. and Tjian,R. (1990) Mechanism of transcriptional activation by SP1: evidence for coactivators. *Cell*, **61**, 1187–1197.

Reese,J.C., Apone,L., Walker,S.S., Griffin,L.A. and Green,M.R. (1994) Yeast TAFIIS in a multisubunit complex required for activated transcription. *Nature*, **371**, 523–527.

Sauer,F., Hansen,S.K. and Tjian,R. (1995a) Multiple TAFIIs directing synergistic activation of transcription. *Science*, **270**, 1783–1788.

Sauer,F., Hansen,S.K. and Tjian,R. (1995b) DNA template and activator-coactivator requirements for transcriptional synergism by Drosofila bicoid. *Science*, **270**, 1825–1828.

Schmitz,M.L. and Baeuerle,P.A. (1991) The p65 subunit is responsible for the strong transcription activating potential of NF-κB. *EMBO J.*, **10**, 3805–3817.

Schmitz,M.L. and Baeuerle,P.A. (1995) Multi-step activation of NF-κB/Rel transcription factors. *Immunobiology*, **193**, 116–27.

Suzuki-Yagawa,Y., Guermah,M. and Roeder,R.G. (1997) The ts13 mutation in the TAF$_{II}$250 subunit (CCG1) of TFIID directly affects transcription of D-type cyclin genes in cells arrested in G$_1$ at the nonpermissive temprature. *Mol. Cell. Biol.*, **17**, 3284–3294.

Tanese,N., Pugh,B.F. and Tjian,R. (1991) Coactivators for a proline rich activator purified from the multisubunit human TFIID complex. *Genes Dev.*, **5**, 2212–2224.

Tanese,N., Daman,S., Vassallo,M.F., Chen,J.-L. and Admon,A. (1996) Molecular cloning and analysis of two subunits of the human TFIID complex: hTAF$_{II}$130 and hTAF$_{II}$100. *Proc. Natl Acad. Sci. USA*, **93**, 13611–13616.

Tansey,W.P. and Herr,W. (1997) TAFs: guilt by association? *Cell*, **88**, 729–732.

Thut,C.J., Chen,J.-L., Klemm,R. and Tjian,R. (1995) p53 transcriptional activation mediated by coactivators TAF$_{II}$40 and TAF$_{II}$60. *Science*, **267**, 100–104.

Van Antwerp,D.J., Martin,S.J., Kafri,T., Green,D.R. and Verma,I.M. (1996) Suppression of TNF-α-induced apoptosis by NF-κB. *Science*, **274**, 787–789.

Verma,I.M., Stevenson,J.K., Schwarz,E.M. Van Antwerp,D. and Miyamoto,S. (1995) Rel/NF-κB/IκB family: intimate tales of association and dissociation. *Genes Dev.*, **9**, 2723–2735.

Verrijzer,C.P. and Tjian,R. (1996) TAFs mediate transcriptional activation and promoter selectivity. *Trends Biochem. Sci.*, **21**, 338–42.

Walker,S.S., Reese,J.C., Apone,L.M. and Green,M. (1996) Transcription activation in cells lacking TAF$_{II}$s. *Nature*, **383**, 185–188.

Wang,C-Y., Mayo,M.W. and Baldwin,A.S.,Jr (1996) TNF-α and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. *Science*, **274**, 784–787.

Wang,E.H., Zou,S. and Tjian,R. (1997) TAFII250-dependent transcription of cyclin A is directed by ATF activator proteins. *Genes Dev.*, **11**, 2658–2669.

Wong,G.H., Elwell,J.H., Oberley,L.W. and Goeddel,D.V. (1989) Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell*, **58**, 923–31.

Zhou,Q., Lieberman,P.M., Boyer,T.G. and Berk,A.J. (1992) Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. *Genes Dev.*, **6**, 1964–1974.

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