Novel sites in the p65 subunit of NF-κB interact with TFIIB to facilitate NF-κB induced transcription

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Abstract Nuclear factor κB (NF-κB) transcription factors regulate a large number of genes in response to inflammation, infection and stressful conditions. In this study, we investigated whether NF-κB p65 regulates the transcription of target genes by interacting with components of the basal transcription machinery. We examined the interaction of p65 with the basal transcription factor IIB (TFIIB). Glutathione *S*-transferase pull down assays showed that the Rel homology domain of p65 is important for binding to TFIIB. Molecular modelling, together with the generation of specific point mutants, revealed that residues 41 R and 42 S in the Rel homology domain of p65 facilitate the interaction with TFIIB. Mutation of these residues showed a decrease in p65 induced transcription, suggesting that they are involved in a functional interaction with TFIIB.

Key words: Protein–protein interaction; Nuclear factor κB; Transcription factor IIB; Transcriptional regulation

1. Introduction

Nuclear factor κB (NF-κB) is the name given to a family of transcription factors that have a common N-terminal Rel homology domain. The Rel homology domain is important for DNA binding, dimerisation, interaction with inhibitory proteins and nuclear localisation [1]. Five members of the NF-κB family (p65 (RelA), c-Rel, RelB, p50/p105 and p52/p100) have been identified in mammals. Three NF-κB proteins (Dorsal, Dif and Relish) have also been found in *Drosophila melanogaster* [2]. The NF-κB proteins can form homodimers or heterodimers. The p65/p50 heterodimer was the first form of NF-κB to be discovered and is the most common dimer in mammals. In most cell types, NF-κB dimers are present in an inactive state in the cytoplasm where they are bound to inhibitor proteins of the IκB family [3]. Many stimuli can activate the dimers by inducing the phosphorylation, ubiquitination and degradation of IκB proteins [1]. This unmasks a nuclear localisation signal in the NF-κB proteins, enabling them to translocate to the nucleus where they subsequently bind specific κB sites in the promoters of target genes [4]. The transcriptional activity of NF-κB is also regulated by phosphorylation of the p65 subunit [5]. NF-κB transcription factors regulate a large number of genes in response to inflammation, infection and stressful conditions [5].

The NF-κB proteins differ in their ability to activate transcription [1]. The p65 subunit has a strong transcriptional activation potential and has two independent transcription activation domains at its C-terminal end [6]. One way in which transcription activation domains are thought to mediate the activation of transcription is by interacting with one or more components of the basal transcription machinery [7]. Transcription initiation requires the formation of a preinitiation complex (PIC) that consists of RNA polymerase II and the basal transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH [8]. TFIID is a complex of the TATA box binding protein (TBP) and many TBP associated factors (TAFs). Binding of TBP to the TATA box initiates the formation of the PIC and this is followed either by the stepwise assembly of the other basal factors [8] or by the recruitment of a holoenzyme [9]. The basal factors assemble into the PIC in the following order: TFIIA, TFIIB, TFIIF along with RNA polymerase II, TFIIE and TFIIH [8].

The basal factor TFIIB is a common target of proteins that activate transcription. Herpes simplex virus type 1 protein VP16 [10], vitamin D receptor [11], retinoid X receptor [12], papillomavirus E2 protein [13] and Tat [14] are some of the activators that functionally interact with TFIIB. TFIIB plays a critical role in PIC formation by providing a physical link between TBP and RNA polymerase II [8]. TFIIB also binds to a sequence element located immediately upstream of the TATA box called the IIB recognition element [15] and is thought to contribute to the orientation of PIC assembly [16].

In this study we investigate whether NF-κB p65 regulates the transcription of target genes via an interaction with TFIIB. We describe the interaction of p65 with TFIIB and map the region of p65 that is involved. In addition, we show specific residues of p65 which facilitate a functional interaction with TFIIB.

2. Materials and methods

2.1. Plasmid construction

The expression vectors for full length NF-κB p65 and its deletion

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Abbreviations: NF-κB, nuclear factor κB; TBP, TATA binding protein; TFIIB, transcription factor IIB

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mutants were constructed by cloning the appropriate regions of p65 cDNA into pBluescript II KS as described previously [17]. The glutathione *S*-transferase (GST)-TFIIB plasmid was a kind gift from Dr T. Kouzarides, Wellcome Trust/Cancer Research UK Institute, Cambridge, UK. NF-κB p65(1–309) and its point mutants were generated using the QuikChange site directed mutagenesis kit from Stratagene. The single point mutants were created by converting 41 R to 41 A (p65(1–309)-41A), 42 S to 42 A (p65(1–309)-42A) and 53 D to 53 A (p65(1–309)-53A). The triple mutant was generated by converting 41 R, 42 S and 53 D to 41 A, 42 A and 53 A (p65(1–309)-AAA). The expression vectors for HA-p65-wt, HA-p65-41A, HA-p65-42A, HA-p65-53A and HA-p65-AAA were made in three steps. Firstly, the point mutations were introduced into full length p65 cloned in pBluescript II KS. Secondly, a HA epitope tag was generated at the amino-terminal end of these constructs using the QuikChange site directed mutagenesis kit from Stratagene. The primers were 5′-ATAAGCTTCACCATGTACCCATACGATGTTCCAGATTACGCTGGGGACGAACTGTTCC-3′ and 5′-GGAACAGTTCGTCCCCAGCGTAATCTGGAACATCGTATGGGTACATGGTGAAGCTTAT-3′. Thirdly, the HA epitope tagged p65 cDNAs were sub-cloned into the pBK-RSV expression vector (Stratagene). The constructs were verified by sequencing and restriction enzyme analysis.

2.2. Purification of GST-TFIIB

GST and GST-TFIIB were expressed in *Escherichia coli* BL21 (DE3) (Invitrogen). Overnight bacterial cultures, grown in 2×YTG medium, were diluted 1:10 in fresh medium and grown at 37°C until the OD_{600} reached 1. Protein expression was induced by the addition of 0.1 mM isopropyl β-D-thiogalactoside and the cultures were grown for a further 2.5 h at 30°C. The bacteria were harvested and resuspended in phosphate buffered saline (PBS) containing 1 mM EDTA and Complete protease inhibitors (Roche). The cells were sonicated for 4×30 s bursts and 1% Triton X-100 was added to solubilise the GST proteins. The lysates were cleared by centrifugation and the supernatants applied directly to columns of glutathione Sepharose 4B (Amersham Pharmacia). After extensive washing, the beads were stored at 4°C in PBS containing 1 mM EDTA and Complete protease inhibitors (Roche). The purity of the GST proteins was checked by eluting a small fraction in sodium dodecyl sulfate (SDS) sample buffer and analysing the proteins by SDS–polyacrylamide gel electrophoresis (PAGE) and Coomassie staining.

2.3. In vitro translation

The in vitro translations were performed using the TNT coupled reticulocyte lysate system from Promega. pBluescript vectors containing cDNAs for wild-type and mutant NF-κB p65 were used as the templates for the in vitro translation. The 50 μl reaction mixture included 1 μg DNA, 25 μl rabbit reticulocyte lysate, 40 U RNasin ribonuclease inhibitor, 1 μl T3 RNA polymerase, 2 μl TNT buffer, 1 μl amino acid mix minus methionine and 20 μCi [^{35}S]methionine (Amersham). The reaction was carried out at 30°C for 90 min. The ^{35}S labelled in vitro translated proteins were separated by SDS–PAGE and visualised by autoradiography.

2.4. GST pull down assays

Glutathione Sepharose 4B beads with or without bound GST proteins were equilibrated in binding buffer (20 mM HEPES pH 7.8, 30 mM NaCl, 2.5 mM $MgCl_2$, 1 mM EDTA, 0.05% NP-40, 1% Triton X-100, 1 mM dithiothreitol and Complete protease inhibitors (Roche)). 10 μl ^{35}S labelled in vitro translated protein and 150 μl binding buffer were incubated with 40 μl glutathione Sepharose 4B beads on a rotary wheel for 2 h at 4°C. After this clearing step, the supernatant was incubated with 40 μl GST or GST-TFIIB bound to glutathione Sepharose 4B beads on a rotary wheel for 12 h at 4°C. The beads were washed five times with binding buffer and twice with PBS containing 1% Triton X-100. The bound proteins were eluted in 20 μl glutathione elution buffer (Amersham Pharmacia), separated by SDS–PAGE and the ^{35}S labelled proteins visualised by autoradiography.

2.5. Cell transfection and luciferase assays

CV-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated foetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-glutamine (Gibco). The cells were seeded at 1.25×10^5 cells/well in 6 well plates 24 h before transfection. The cells were transfected overnight using a standard calcium phosphate method. 0.5 μg pBK-RSV vector or HA-p65 (wild-type or mutant) was co-transfected with 0.8 μg NF-κB luciferase reporter plasmid (gift from Dr Iain Uings, GSK, Stevenage, UK) and 0.1 μg *Renilla* luciferase control reporter plasmid (Promega). The cell growth medium was replaced with DMEM containing 0.5% serum and the cells were harvested 24 h later. The cells were washed once with PBS and incubated at room temperature in 200 μl 1×Passive Lysis Buffer (Promega) for 20 min. The cell lysates were subjected to one freeze/thaw cycle and centrifuged at 14 000 rpm for 2 min to pellet the cell debris. 20 μl of cell lysate was combined with 50 μl Luciferase Assay Reagent II (Promega) and the firefly luciferase activity monitored using a luminometer (TD-20e model, Turner). 50 μl Stop and Glo Reagent (Promega) was then added and the *Renilla* luciferase activity monitored using a luminometer. The ratio of firefly to *Renilla* luciferase activity was calculated for each sample. Each assay was performed in triplicate.

The level of protein expression of wild-type and mutant HA-p65 in the CV-1 cell lysates was assessed by immunoprecipitation followed by Western blotting. 5 μg HA antibody 12CA5 was combined with the remainder of the CV-1 cell lysates and tumbled at 4°C for 2 h and then for a further 1 h with protein G Sepharose beads (Amersham Pharmacia). The beads were washed three times with lysis buffer and the proteins eluted by boiling in SDS sample buffer. The eluted proteins were separated by SDS–PAGE and transferred onto nitrocellulose by Western blotting. The HA tagged proteins were detected using a HA antibody (Roche). The antigen–antibody complex was visualised using the ECL detection system (Amersham Pharmacia).

2.6. Statistical analysis

The *t*-test was used to compare levels of luciferase between constructs. Statistical significance was taken to be at the 0.05 level.

2.7. Protein docking and molecular surface modelling

The protein docking study of NF-κB p65 and TFIIB was performed with the FTDOCK program using shape complementarity and electrostatics information [18]. The protein structures used were 1vkx.pdb (chain a) for NF-κB p65 [19] and 1vol.pdb (chain a) for the core domain of TFIIB [20].

The molecular surface modelling of NF-κB p65 was performed using a minimum perturbation procedure using 1vkx as a template. The graphics representations were generated in WebLab Viewer.

3. Results and discussion

Previous reports show that the basal transcription factor TFIIB functionally interacts with proteins that activate transcription such as herpes simplex virus type 1 protein VP16 [10], vitamin D receptor [11], retinoid X receptor [12], papillomavirus E2 protein [13] and Tat [14]. We decided to examine whether NF-κB also regulates the transcription of target genes via an interaction with TFIIB. The p65 subunit of NF-κB was used in this study because it has a strong transactivation potential [6].

GST pull down assays were used to show that the p65 subunit of NF-κB interacts with TFIIB. In addition, deletion mutants were constructed to determine which region of p65 binds to TFIIB. A schematic diagram of the deletion mutants is shown in Fig. 1A. Wild-type and mutant p65 cDNAs, cloned in pBluescript, were used as templates to generate ^{35}S labelled p65 proteins in vitro. SDS–PAGE analysis and autoradiography showed that the in vitro translated p65 proteins have the correct predicted molecular weights and were produced at similar levels (Fig. 1B). The ^{35}S labelled p65 proteins were incubated with equal quantities of purified GST or GST-TFIIB bound to glutathione Sepharose beads. After extensive washing, the bound proteins were eluted and separated by SDS–PAGE. The ^{35}S labelled p65 proteins were visualised by autoradiography (Fig. 1C). The GST protein

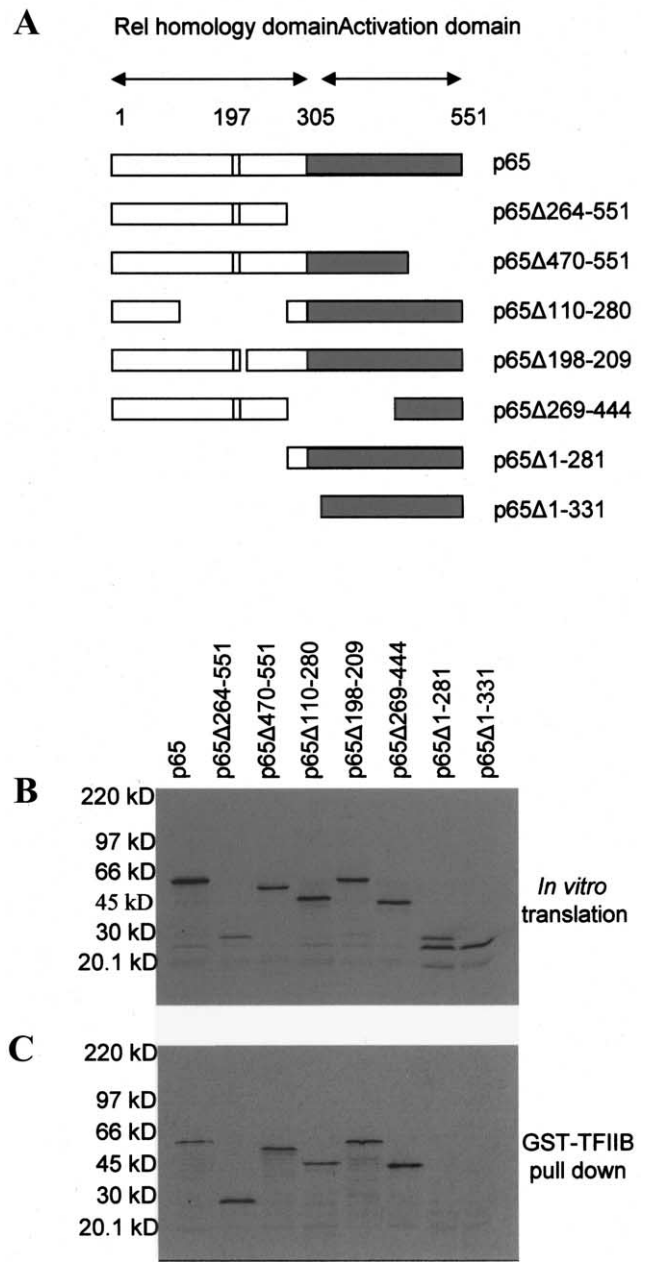

Fig. 1. Identification of the regions of NF-κB p65 that interact with TFIIB. A: Schematic diagram of wild-type p65 and its deletion mutants. The various cDNAs were cloned into the pBluescript II KS vector as described previously [17]. B: In vitro translated and ^{35}S labelled p65 proteins. C: GST-TFIIB pull down assays. The labelled p65 proteins were incubated with purified GST-TFIIB bound to glutathione Sepharose beads. After extensive washing, the bound proteins were eluted and separated by SDS–PAGE. The ^{35}S labelled p65 proteins were visualised by autoradiography.

was used as a negative control for the GST pull down assays and no p65 proteins were observed to bind to GST (data not shown).

The GST pull down assays suggest that the first half of the Rel homology domain of p65, from amino acids 1 to 110, is involved in mediating the interaction with TFIIB. As shown in Fig. 1C, p65 proteins that contain this region (p65, p65Δ264–551, p65Δ470–551, p65Δ110–280, p65Δ198–209 and p65Δ269–444) are able to bind to GST-TFIIB. In contrast, p65 proteins that lack amino acids 1–110 (p65Δ1–281 and p65Δ1–331) do not interact with GST-TFIIB. Some of these findings differ from those reported previously by another group [21]. They showed that a fusion protein of Gal4 and amino acids 471–551 of p65 can interact with ^{35}S labelled in vitro translated TFIIB, whereas here we report that a p65 deletion mutant that lacks amino acids 470–551 can bind GST-TFIIB. Moreover, we were unable to pull down in vitro translated TFIIB with GST-p65(470–551) (data not shown). The reason for this discrepancy is not clear but may be due to variations in the experimental procedures.

To gain further insight into how NF-κB p65 binds to TFIIB, protein docking by Fourier correlation was employed

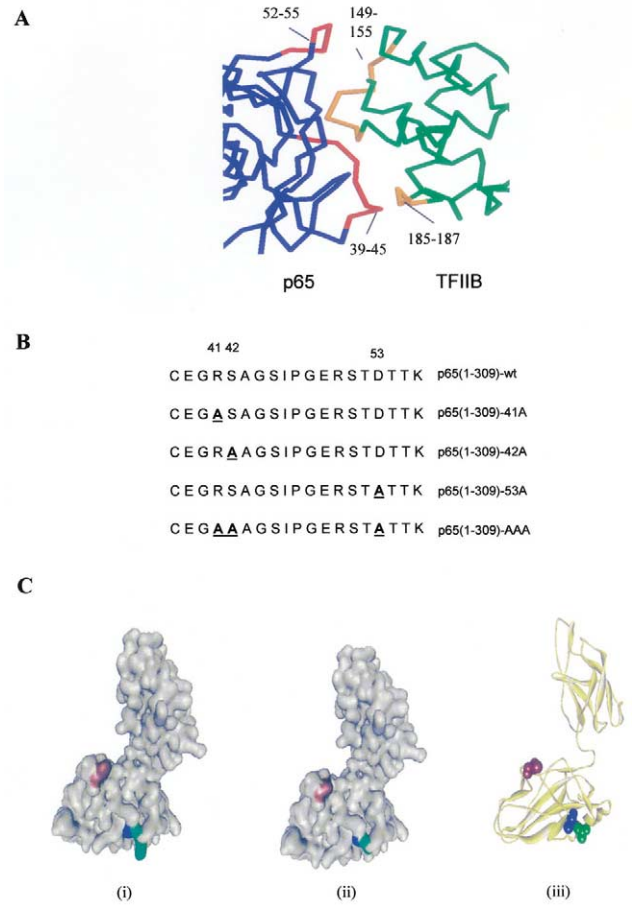

Fig. 2. Predicted sites of interaction between NF-κB p65 and TFIIB. A: Backbone trace of the predicted interaction loops of p65 (blue) and TFIIB (green). The interaction loops are shown in red and orange respectively. The protein docking study was performed with the FTDOCK program using the protein structure 1vkx.pdb (chain a) for p65 and 1vol.pdb (chain a) for the core domain of TFIIB. B: Mutants used to examine the predicted sites of contact between p65 and TFIIB. The single point mutants were created by converting 41 R to 41 A (p65(1–309)-41A), 42 S to 42 A (p65(1–309)-42A) and 53 D to 53 A (p65(1–309)-53A). The triple mutant was generated by converting 41 R, 42 S and 53 D to 41 A, 42 A and 53 A (p65(1–309)-AAA). C: (i) Molecular surface model of wild-type p65. 41 R (green), 42 S (blue) and 53 D (red). (ii) Molecular surface model of the triple alanine mutant. Colouring of the alanine replacements at positions 41, 42 and 53 corresponds to the equivalent wild-type residues in (i). (iii) Ribbon diagram of the triple alanine mutant, colour scheme as in (i) and (ii). The mutations were modelled by a minimum perturbation procedure using 1vkx as a template.

to model the interactions [22]. The three-dimensional structure of the NF-κB p50/p65 heterodimer bound to the NF-κB site of the intronic enhancer of the immunoglobulin light chain gene has been resolved at 2.9 Å [19]. The p50/p65 heterodimer makes specific contacts along the NF-κB site mainly through the loops that connect the secondary structural elements in both subunits. The C-terminal region of the Rel homology domain forms the dimerisation interface, using residues that are strongly conserved in the Rel family. Both p50 and p65 adopt variable conformations in a DNA sequence dependent manner. The three-dimensional structure of the TFIIB–TBP–TATA element ternary complex has been resolved at 2.7 Å [20]. TBP displays a two domain DNA binding fold and its approximate two-fold intramolecular symmetry forms a saddle shaped structure with a curved antiparallel β-sheet that provides a concave DNA binding surface. TATA element recognition occurs through an induced fit mechanism involving a dramatic distortion of the DNA. The seat of the saddle presents a large convex surface to which other transcription factors bind during transcriptional initiation. TFIIB is a two domain α-helical protein with a cleft made up of a short random coil peptide separating the domains. TFIIB acts as a clamp binding the C-terminal 'stirrup' of TBP in its cleft and interacting with the phosphoribose backbone of the promoter.

Protein docking was performed with the FTDOCK program using shape complementarity and electrostatics information and refined by MULTIDOCK [18]. NF-κB p65 and TFIIB were predicted to interact through flexible loop regions, most likely through an induced fit mechanism. The most favourable mode of interaction predicted for p65 and TFIIB, consistent with steric constraints in the complex, involved amino acids 39–45 (S1-H1 loop, in particular 41 R and 42 S) and 52–55 (H1-S2 loop, in particular 53 D) of p65 and residues 149–155 (H2-H3 loop) and 185–187 (H4-H5 loop) of TFIIB (Fig. 2A). The cleft region in TFIIB can be expected to provide additional flexibility for the positioning of the interface in the N-terminal sub-domain of p65.

Sequence comparison of the region in p65 predicted to interact with TFIIB with those of the other NF-κB members indicates that residues 39–45 are conserved within the transcriptionally active members of the Rel family, such as p65, c-Rel and RelB, but not in the transcriptionally inactive p50 or p52 (Fig. 3). The conservation of residues 39–45 in transcriptionally active members of the NF-κB family may indicate that these are functionally important.

The model of interaction was tested by generating specific point mutants of the Rel homology domain of p65 and assessing their ability to bind to TFIIB. Single point mutants were created by converting 41 R to 41 A (p65(1–309)-41A), 42 S to 42 A (p65(1–309)-42A) and 53 D to 53 A (p65(1–309)-53A. A triple mutant was also generated by converting 41 R, 42 S and 53 D to 41 A, 42 A and 53 A (p65(1–309)-AAA). Fig. 2B shows an overview of wild-type p65(1–309) and its point mutants.

Fig. 3. Sequence comparison of the NF-κB p65 with other members of the Rel proteins. Residues in green are involved in DNA binding. Residues underlined are predicted to interact with TFIIB by protein docking study.

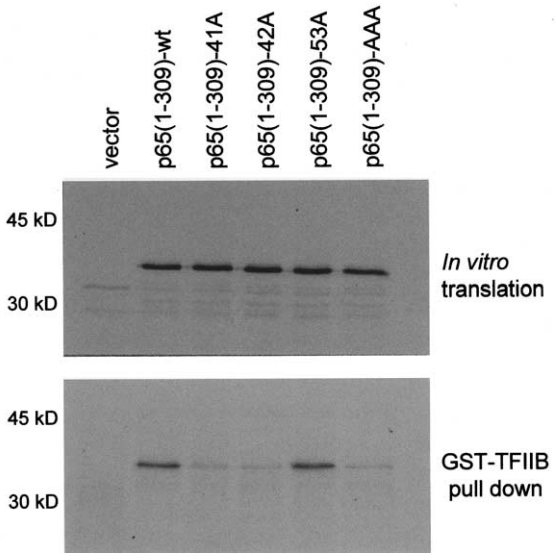

Fig. 4. Identification of the residues in NF-κB p65(1–309) that interact with TFIIB. A: In vitro translated and ^{35}S labelled wild-type and mutant p65(1–309) proteins. B: GST-TFIIB pull down assays. The labelled p65 proteins were incubated with purified GST-TFIIB bound to glutathione Sepharose beads. After extensive washing, the bound proteins were eluted and separated by SDS–PAGE. The ^{35}S labelled p65(1–309) proteins were visualised by autoradiography.

The effect of mutating amino acids 41, 42 and 53 to alanine residues was examined at a structural level. Molecular surface models of wild-type p65 and its triple alanine mutant were generated using a minimum perturbation procedure using 1vkx as a template. The models were represented as space-fill and ribbon diagrams using WebLab Viewer (Fig. 2C). As expected, the models show that mutating amino acids 41, 42 and 53 to alanine residues alters the surface of NF-κB p65. The alanine substitutions both affect surface shape and remove two charged residues (41 R and 53 D) that may play important roles in intramolecular interaction. To assess whether these residues facilitate the interaction between NF-κB p65 and TFIIB, a GST-TFIIB pull down assay was performed with the various alanine mutants of p65. ^{35}S labelled p65(1–309) proteins were translated in vitro. SDS–PAGE analysis and autoradiography showed that the in vitro translated p65(1–309) proteins have the correct predicted molecular weights and were produced at similar levels (Fig. 4A). The ^{35}S labelled p65 proteins were incubated with equal quantities of purified GST or GST-TFIIB bound to glutathione Sepharose beads. After extensive washing, the bound proteins were eluted and separated by SDS–PAGE. The ^{35}S labelled p65 proteins were visualised by autoradiography (Fig. 4B). The GST protein was used as a negative control for the GST pull down assay and no p65(1–309) proteins were observed to bind to GST (data not shown).

The GST pull down assay shows that mutating either residue 41 R to 41 A or 42 S to 42 A prevents the binding of

p65(1–309) to GST-TFIIB. This suggests that both these residues are important in mediating the interaction between p65 and TFIIB. This agrees well with the predicted model. The triple alanine mutant, p65(1–309)-AAA, also did not interact with GST-TFIIB. Presumably, this is because it contains mutations of residues 41 and 42. In contrast, mutating residue 53 D to 53 A does not affect the binding of p65(1–309) to GST-TFIIB. Although amino acid 53 D was predicted to be involved in the interaction between p65 and TFIIB, the GST pull down data suggest that this is unlikely.

Immunoprecipitation experiments were also performed to study the interaction between p65 and TFIIB in HeLa and HepG2 cells stimulated with the proinflammatory cytokine interleukin-1, known to activate NF-κB. These immunoprecipitation experiments provided no conclusive results, which we ascribed to the probable breakdown of the complexes caused by the high salt conditions required to extract nuclear TFIIB.

The functional effects of mutating amino acids 41, 42 and 53 to alanine residues on the transcriptional activity of NF-κB were investigated. Full length NF-κB p65 and its alanine mutants were cloned into the pBK-RSV vector and epitope tagged with HA at the N-terminal end. The p65 proteins were tested for their transcriptional activity by transiently expressing them in CV-1 cells along with a NF-κB luciferase reporter and a *Renilla* luciferase control. CV-1 cells were used because it was previously reported that these cells lack endogenous NF-κB activity, which would facilitate the characterisation of the p65 mutants [23]. The NF-κB reporter construct was composed of the firefly luciferase gene driven by a promoter containing five copies of the NF-κB consensus sequence. The *Renilla* luciferase activity was used to control for transfection efficiency. The cells were harvested 36 h after transfection and the luciferase activities measured using the dual luciferase reporter assay system and a standard luminometer. The firefly luciferase activities were normalised to the *Renilla* luciferase activities and the data are presented in Fig. 5A. The luciferase assays show that the CV-1 cells do have some endogenous NF-κB activity and that expression of wild-type NF-κB p65 induces a 4.3 fold increase in luciferase activity. Interestingly, expression of the 41 A mutant or the 42 A mutant of p65 caused a statistically significant 1.3 and 1.5 fold reduction of luciferase activity, respectively. The 53 A mutant of p65 produced a similar, if not slightly greater, luciferase activity as wild-type p65. The triple alanine mutant of p65 caused a 1.2 fold reduction of luciferase activity compared to wild-type p65. The triple alanine mutant of p65 had a comparable effect in 293T cells of a 1.3 fold reduction of luciferase activity (data not shown). The p65 proteins were also examined to determine if they were expressed at similar levels in CV-1 cells. The proteins were immunoprecipitated with a HA antibody and analysed by SDS–PAGE and Western blotting. Fig. 5B shows that the p65 proteins were expressed at similar levels in CV-1 cells.

The fold differences in luciferase activity suggest that amino acids 41 R and in particular 42 S play a role in NF-κB p65 driven transcription. In contrast, amino acid 53 D does not seem to be involved. These results correlate well with the GST-TFIIB pull down data and suggest that amino acids 41 R and 42 S facilitate a functional interaction with the basal transcription factor TFIIB. One possible explanation for the observed subtle differences in transcription activity between

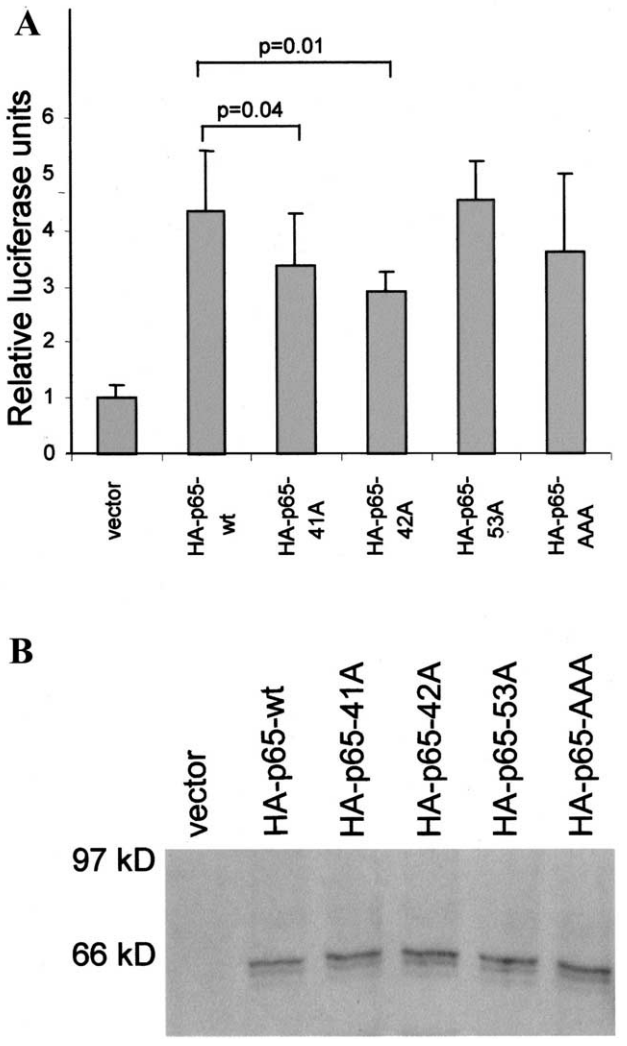

Fig. 5. Effect of the NF-κB mutants on transcription. A: CV-1 cells were co-transfected with a NF-κB reporter plasmid, a *Renilla* luciferase control vector and various p65 proteins. The cells were harvested 36 h later and the luciferase activities measured using the dual luciferase reporter assay system (Promega). The firefly luciferase activities were normalised to the *Renilla* luciferase activities. Each data point represents the mean of six separate experiments with three replicates in each experiment. The values are expressed as mean ± S.D. fold increase relative to the vector alone. B: The level of protein expression of wild-type and mutant HA-p65 in the CV-1 cell lysates. The HA-p65 proteins were immunoprecipitated with a HA antibody, separated by SDS–PAGE and analysed by Western blotting.

wild-type p65 and mutant p65 which does not interact with TFIIB is that the proteins used in these assays contained functional activation domains. We (unpublished data) and others [21] have found that the C-terminal region of the activation domain of p65 binds to another basal transcription factor TBP. This interaction may mask the effects of the p65 mutants on transcription. Thus p65 interacts with more than one component of the transcription machinery. We postulate that the binding of p65 to TFIIB stabilises its interaction with TBP, thereby enhancing the transcriptional activity of p65.

In conclusion, we show a novel function of the Rel homology domain of NF-κB p65 in facilitating transcription by interacting with the basal transcription factor TFIIB. Amino acids 41 R and in particular 42 S that are located in the Rel

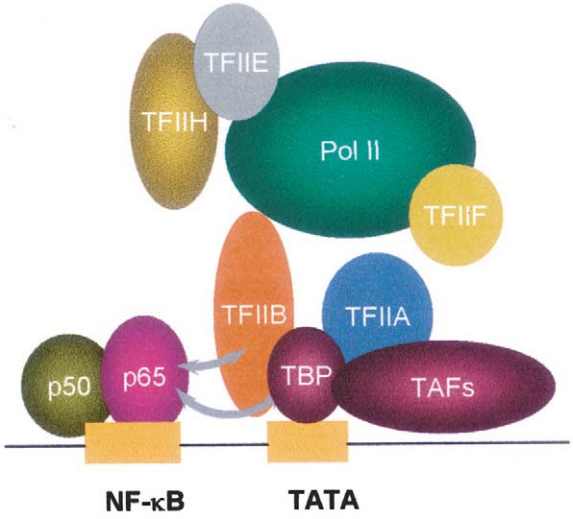

Fig. 6. Model showing the protein–protein interactions between NF-κB and the RNA polymerase II transcription machinery. Genes transcribed by RNA polymerase II typically contain gene specific DNA elements that are recognised by regulatory transcription factors and common core promoter elements that are recognised by basal transcription factors. The regulatory transcription factors are thought to modulate the function of the basal transcription factors. NF-κB is a regulatory transcription factor and is represented as a heterodimer of p50 and p65 subunits. The RNA polymerase II transcription machinery comprises polymerase II and a set of basal transcription factors including TFIID (TBP and TAFs), TFIIB, TFIIA, TFIIH, TFIIF and TFIIE. The grey arrows show the protein–protein interactions between NF-κB p65 and the RNA polymerase II transcription machinery.

homology domain of p65 are important mediators of this interaction. A model showing the protein–protein interactions between NF-κB p65 and the RNA polymerase II transcription machinery is shown in Fig. 6. Thus, both the Rel homology domain and the activation domain of NF-κB p65 are required for its interaction with the RNA polymerase II transcription machinery for full activation.

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