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A novel TBP-interacting zinc finger protein represses transcription by inhibiting the recruitment of TFIIA and TFIIB☆

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Abstract

We isolated a novel gene encoding a zinc finger protein from *Xenopus laevis*, designated NZFP that interacts with the TATA-binding protein (TBP). NZFP contains a highly conserved sequence designated finger associated box (FAX) and SUMO-1 consensus-binding motifs at the N-terminal half and 10 C2H2 type zinc finger motifs at the C-terminal half, respectively. Deletion mutants of NZFP fused with the Gal4 DNA binding domain were used to determine the function of NZFP during gene transcription by transfecting them into a *Xenopus* kidney cell line. Both full-length NZFP and the FAX domain repressed transcription activity by 3–5-fold. Moreover, an in vitro pull-down assay showed that the C-terminal core domain of TBP makes direct contact with the N-terminal portion of NZFP. We also found through chromatin immunoprecipitation experiments that the interaction between NZFP and TBP inhibits binding of TFIIA and TFIIB. These data strongly suggest that the repression by NZFP occurs through its binding to both DNA and TBP and the resulting NZFP–TBP–promoter complex inhibits preinitiation complex assembly by preventing binding of TFIIA and TFIIB.

Keywords: Chromatin immunoprecipitation; FAX; TBP; Transcription; Transcriptional repressor; *Xenopus laevis*; Zinc finger protein

Initiation of gene transcription by RNA polymerase II requires the assembly of the preinitiation complex (PIC) at the core promoter region through its interaction with the general transcription factors (GTFs) [1]. The TATA-binding protein (TBP) was identified as a major GTF that binds initially to the basal promoter (reviewed in [2–4]). Besides TBP, other GTFs, including TFIIA, TFIIB, TFIIF, and the TBP-associated factors (TAFs), are also required for basal transcription by RNA polymerase II. The rate of transcription of most polymerase II transcribed genes is regulated by transcription activator or repressor proteins [5,6].

Repression of transcription occurs by similar mechanisms as those involved in activation. The mechanisms of repression may be classified into at least two types. One is the mechanism of histone remodeling [7]. For example, the Sin3–Rpd3 complex and NuRD complex contain members of the histone deacetylase complex (HDAC) family. These complexes downregulate expression of their target gene by causing local histone deacetylation and inhibition of PIC assembly [8,9].

In the second type of transcription repression, repressors such as NC2 downregulate transcription through protein–protein interactions with the components of GTFs, primarily TBP or TAFs [10,11]. The repressor–TBP–promoter ternary complex prevents the binding of both TFIIA and TFIIB to TBP [12,13]. In

☆ *Abbreviations:* ChIP, chromatin immunoprecipitation; FAX, finger associated boxes; GTFs, general transcription factors; HDAC, histone deacetylase complex; KLF, Krüppel-like factors; NZFP, negatively regulating zinc finger protein; PCR, polymerase chain reaction; PIC, preinitiation complex; SUMO-1, small ubiquitin modifier-1; TBP, TATA-binding protein; ZFP, zinc finger protein.

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addition, repressors like *Drosophila* Eve directly interact with the TATA box binding surface of TBP, and thus the repressor–TFIID complex is not capable of binding to the promoter [14,15].

Zinc finger proteins (ZFPs) can bind to DNA so that they regulate the expression of their target gene and apparently can serve as either positive or negative regulators (reviewed in [16]). The entire family of ZFPs commonly found in many eukaryotic transcriptional factors can be visualized to contain two main domains. One is the putative DNA-binding domain (zinc finger) and the other is the transcriptional regulatory region. Some zinc finger proteins such as Krüppel and Krüppel-related proteins regulate gene expression during development or differentiation [17]. The proteins contain zinc finger motifs at their C-terminus and a repression domain at their N-terminus to negatively regulate their target genes.

We have been investigating the mechanism of selenocysteine tRNA gene transcription in *Xenopus laevis* [18]. Our previous studies have suggested the existence of novel transcription factor(s) that interact with TBP [19]. To further investigate such factors, we employed a yeast two-hybrid system by using the core domain of TBP as bait and detected a gene encoding a ZFP. More recently, this gene was found to be expressed primarily in *Xenopus* head tissues during early development (manuscript submitted). In this study, we provide insights into the molecular function of NZFP. The N-terminal domain of this protein contains a negatively regulating domain that binds to TBP. To our knowledge, this is the first report to show that the zinc finger transcription factor represses transcription by interacting with TBP.

Materials and methods

Plasmids. The effector plasmid pHKG-NZFP and its derivatives were constructed by fusing NZFP cDNA to the Gal4 DNA-binding domain. The control plasmid, pHKG, contains only the Gal4 DNA-binding domain driven with SV40 promoter. The reporter plasmid (pGALTKCAT) was provided by Dr. H. Ro, which contains five Gal4-binding sites upstream of the TATA box of the thymidine kinase (TK) promoter and carries the chloramphenicol acetyl transferase (CAT) gene. His-tagged protein of full-length TBP (residues 1–297), a partial deletion of the N-terminal domain (TBPΔ1/2N, residues 70–297), a complete deletion of the N-terminal domain (TBPΔN, residues 117–297), and a complete deletion of the C-terminal core domain (TBPΔC, residues 1–116) were provided by Dr. P. Labhart. For construction of the green fluorescent protein (GFP)-NZFP expression vector, NZFP (amino acids 1–613) was subcloned into the pEGFP-C1 expression vector (Clontech).

Isolation of the full-length NZFP cDNA. Yeast two-hybrid screen was carried out in strain Y190 as described by the manufacturer's protocol (Clontech). As bait, the C-terminal core domain of the *Xenopus* TBP gene was subcloned into pAS2 that contains the DNA-binding domain of GAL4 (residues 1–147). Approximately 10^6 transformants from a *Xenopus* oocyte cDNA library (Clontech) were screened. The isolated clone was subcloned and sequenced. The NZFP nucleotide sequence has been submitted to the GenBank database under Accession No. AY211520.

Coimmunoprecipitation. Coimmunoprecipitation was carried out as described by Seong et al. [20] with the expectation that 293T cells were transfected with the expression vector for GFP-NZFP or GFP, lysed, and immunoprecipitated by using anti-GFP antibody (Santa Cruz Biotechnology).

In vitro pull-down assay. TBP (full-length 297 residues), TBPΔ1/2N (residues 70–297), TBPΔN (residues 117–297), and TBPΔC (residues 1–116) were expressed in *Escherichia coli*, respectively. Each *E. coli* extract was mixed with Ni–NTA agarose and then with ^{35}S-labeled NZFP synthesized in vitro [21] for detailed procedure. The bound proteins were separated on a 10% SDS–polyacrylamide gel.

Filter overlay assay. Filter overlay assays were performed as described by Niethammer et al. [22] with the expectation that the truncated mutants were fused to glutathione-*S*-transferase, transferred onto nitrocellulose, and incubated with his-TBPΔN (see *Plasmids* described within Materials and methods). After incubation, bound his-TBPΔN was detected by Western blotting.

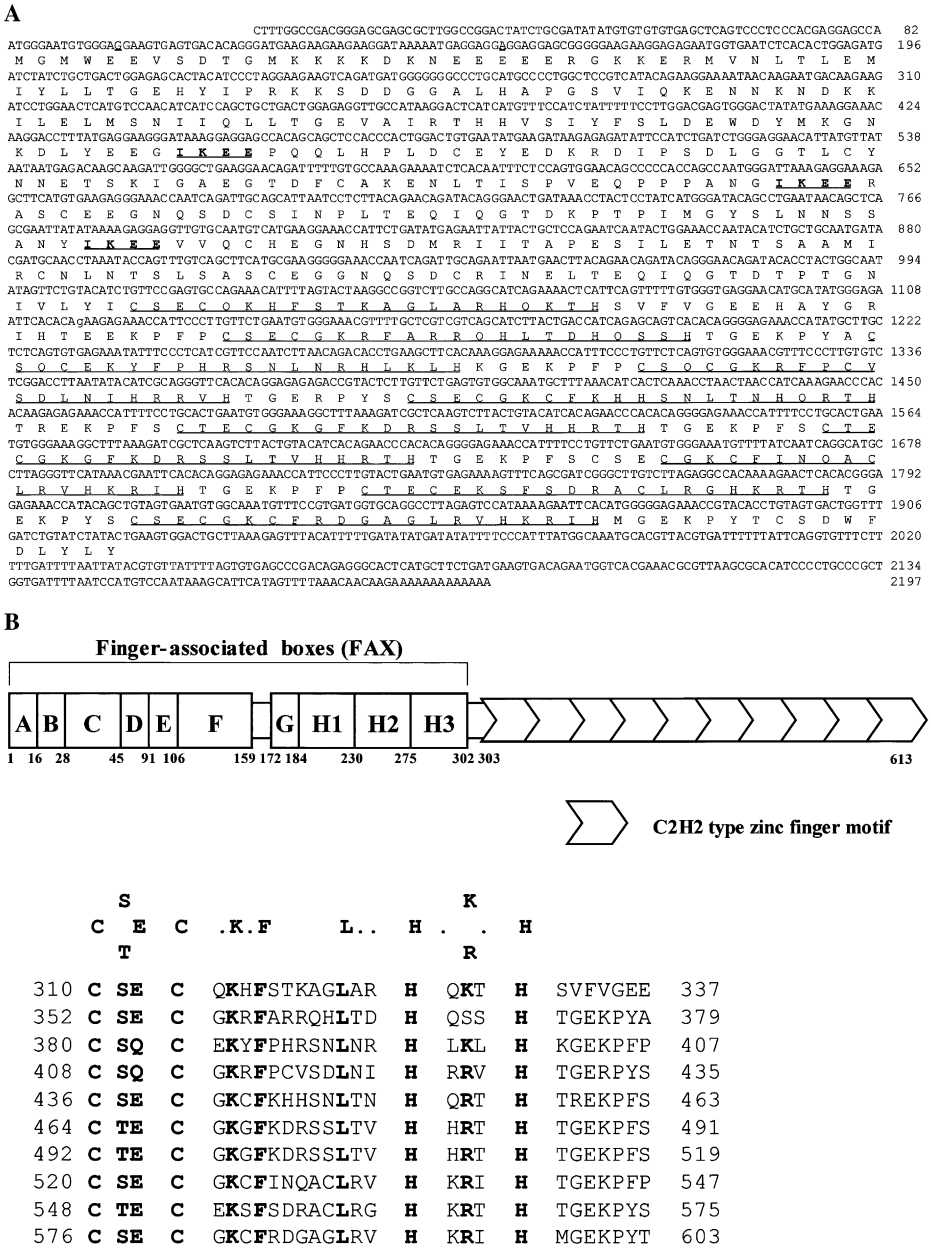
Transcription activity assay. *Xenopus* kidney cells (A6) were transfected with effector plasmid (see the *Plasmids* in the Materials and methods) and pGALTKCAT (reporter plasmid) along with pLacZ (a CMV promoter-driven β-galactosidase expression plasmid). CAT assays were performed to measure the transcription activity of the reporter gene by the effector. To normalize transfection efficiency, β-galactosidase activity was measured [23].

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assays were performed as described [24]. After A6 cells were transfected with pHKG-NZFP or pHKG and pGALTKCAT, they were cross-linked with formaldehyde, washed, and resuspended in ChIP lysis buffer. The lysates were sonicated and immunoprecipitated with appropriate antibodies. DNA was recovered from the immune complex and used as a template for PCR (24 cycles) using pGALTKCAT promoter-specific oligonucleotides (5′-GCGGAGA CTCTAGAGGA-3′ and 5′-GGCCACACGCGTCACCT-3′). pHKG promoter (SV40)-specific oligonucleotides were used as an internal control. PCR products were separated by agarose gel electrophoresis and quantified using a gel image analyzer.

Results

Isolation and characterization of the NZFP cDNA

We used the yeast two-hybrid system to isolate a protein that interacts with TBP. A TBP mutant with partial deletion of the N-terminal domain (residues 117–297, TBPΔN) was used as bait, because it eliminated false positives observed frequently with full-length TBP. Seven clones were isolated from approximately one million transformants obtained from the *Xenopus* oocyte cDNA library. One of them containing 613 amino acids (Fig. 1A) revealed that the sequence is highly homologous with members belonging to the FAX-ZFP subfamily, especially with XLcGF53.1 [25]. We observed that this clone is expressed mostly in *Xenopus* head tissues such as brains, eyes, otic vesicles, and branchial arches during early development (manuscript submitted). Like other FAX-ZFPs, NZFP is composed of a highly conserved FAX domain at the N-terminus and numerous heterogeneous zinc finger motifs at its C-terminus. NZFP contains 10 conserved regions in the FAX domain from the A box to the H3 box and 10 C2H2 type zinc fingers (see Fig. 1B). In the N-terminal



	C	S/T E	C	.K.F	L..	H	. . R	H	
310	C	SE	C	QKHFSTKAGLAR		H	QKT	H	SVFVGEE 337
352	C	SE	C	GKRFARRQHLTD		H	QSS	H	TGEKPYA 379
380	C	SQ	C	EKYFPHRSNLNR		H	LKL	H	KGEKPFP 407
408	C	SQ	C	GKRFPCVSDLNI		H	RRV	H	TGERPYS 435
436	C	SE	C	GKCFKHHSNLTN		H	QRT	H	TREKPFS 463
464	C	TE	C	GKGFKDRSSLTV		H	HRT	H	TGEKPFS 491
492	C	TE	C	GKGFKDRSSLTV		H	HRT	H	TGEKPFS 519
520	C	SE	C	GKCFINQACLRV		H	KRI	H	TGEKPFP 547
548	C	TE	C	EKSFSDRACLRG		H	KRT	H	TGEKPYS 575
576	C	SE	C	GKCFRDGAGLRV		H	KRI	H	MGEKPYT 603

Fig. 1. Sequence of the *Xenopus* NZFP gene. (A) NZFP cDNA sequence and deduced amino acid sequence. The zinc finger domains are underlined. The SUMO-1 binding sequences are underlined in bold letters. (B) Schematic representation of NZFP and sequences of the C2H2 zinc finger motifs. The A–H boxes in the upper diagram designate the conserved FAX domain proposed by Knöchel et al. [25]. Numbers under the boxes represent the amino acid residues within each FAX domain. Chevrons on the right portion of the upper diagram (from amino acid residues 303 to 613) designate C2H2 type finger motifs. Consensus sequences of C2H2 zinc finger motifs are aligned in the lower portion of the figure with inclusive amino acid residue numbers. The conserved residues are in bold. The upper portion of the lower figure shows the conserved amino acid residues in bold.

region, there are three SUMO-1 consensus sequences (ΨKXE; Ψ stands for a large hydrophobic amino acid and X for any amino acid) (see Fig. 1A and its legend, [26]). The first one is within the F box (residues 122–125), the second within the H1 box (residues 186–189), and the third within the H2 box (residues 232–235), respectively. It should be noted that the negatively regulating region of NZFP and the TBP interacting domain are located within the H box (see the following sections).

Interaction between TBP and NZFP in vivo and in vitro

Physical interactions between TBP and NZFP were analyzed by coimmunoprecipitation and in vitro pull-down experiments with his-tagged proteins or GST fusion proteins as shown in Fig. 2. To determine if NZFP interacts with TBP in vivo, we performed coimmunoprecipitation experiments using 293T cells transfected with GFP-NZFP or GFP expression vector (Fig. 2A).

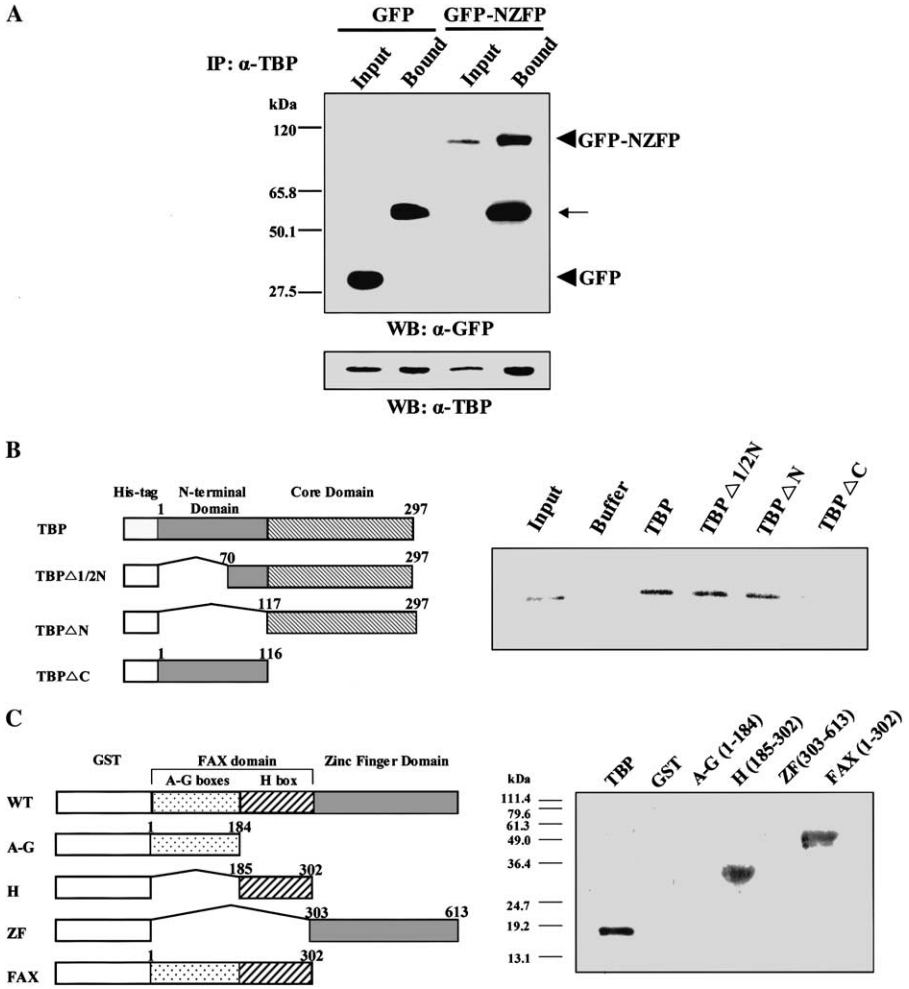


Fig. 2. Interaction of NZFP with TBP. (A) Interaction of NZFP with TBP in vivo. The 293T cells transfected with GFP or GFP–NZFP expression vector were harvested and lysed. Total cell lysates were immunoprecipitated by anti-TBP antibody and Western blot analysis was carried out against anti-GFP antibody (upper panel) and the same blot was analyzed with anti-TBP antibody as control (lower panel). Arrow designates immunoglobulin heavy chain. (B) Determination of the NZFP interaction domain in TBP. Structures of wild type and mutant his-TBP constructs are depicted in the left portion of the figure. Open boxes represent the His-tag region, filled boxes the N-terminal domain, and hatched boxes the core domain. The numbers of the first and last amino acid residues of undeleted portions of the constructs are given. An autoradiogram of [^{35}S]methionine-labeled NZFP bound to TBP constructs is shown in the right portion of the figure. NZFP was synthesized in vitro and mixed with Ni–NTA agarose saturated with each TBP construct, the resulting complexes were washed extensively, the retained proteins were collected and analyzed by SDS–PAGE, and the developed gel was subjected to autoradiography as described in Materials and methods. Input: the in vitro translated NZFP used for the assay. (C) Mapping of the TBP interaction domain in NZFP by filter binding assay. Structures of the GST–NZFP fusion proteins are depicted in the left portion of the figure. Open boxes indicate GST, dotted and hatched boxes, the A–G and H boxes, respectively, of the FAX domain, and filled boxes, the zinc finger domain of NZFP. The numbers of the first and last amino acid residues of undeleted portions of the constructs are given. GST-fusion proteins of various NZFP deletion mutants were prepared, separated by SDS–PAGE, and transferred to nitrocellulose filters, and the filters were incubated with his-TBPΔN, analyzed by Western blotting as described in Materials and methods, and shown in the right portion of the figure. Numbers on the left side of the panel showing the gel of the Western blot data indicate positions of molecular weight markers in kDa.

Endogenous TBP was immunoprecipitated from cell lysates and Western blot analysis showed that TBP was precipitated (Fig. 2A, lower panel). The binding of NZFP was subsequently analyzed by Western blotting against anti-GFP antibody. As shown in Fig. 2A (upper right panel), NZFP was detected in the immunoprecipitants, but not in the control GFP immunoprecipitants, demonstrating that NZFP interacts with TBP in vivo.

To verify further the NZFP interaction domain in TBP, his-tagged TBP derivatives were used in the in vitro pull-down assay. His-tagged proteins with full-length TBP (residues 1–297), a partial deletion of the N-terminal domain of TBP (TBPΔ1/2N, residues 70–297), a complete deletion of the N-terminal domain of TBP (TBPΔN, residues 117–297), and a complete deletion of the C-terminal core domain of TBP (TBPΔC,

residues 1–116) were used as affinity matrices for binding with radiolabeled full-length NZFP that was produced with the rabbit reticulocyte lysate system (Fig. 2B). This in vitro pull-down assay with various deletion mutants of TBP showed that NZFP is capable of interacting with the C-terminal domain of TBP (Fig. 2B).

To determine the region of NZFP that interacts with TBP, the filter overlay assay was performed with GST fusion proteins harboring various regions of NZFP (Fig. 2C). TBPΔN was used as the TBP fragment that consisted of residues 117–297. Only the GST fusions with the FAX domain containing the H boxes (H1–H3, residues 185–302) were able to bind the TBP fragment (Fig. 2C). No interactions were detectable with both the upstream (residues 1–184) and downstream (residues 303–613) of the H box.

NZFP is a transcriptional repressor

Since NZFP interacts with TBP, it is most likely that NZFP regulates the transcription efficiency of the target gene. To determine how NZFP affects the efficiency of transcription, we constructed fusion proteins of the FAX domain (residues 1–302) or zinc finger domain (residues 303–613) of NZFP fused to the GAL4 DNA-binding domain present in the eukaryotic expression vector pHKG that was modified from pSG424. pHKG, which only expresses the GAL4 DNA-binding domain, was used as a negative control. In cotransfection

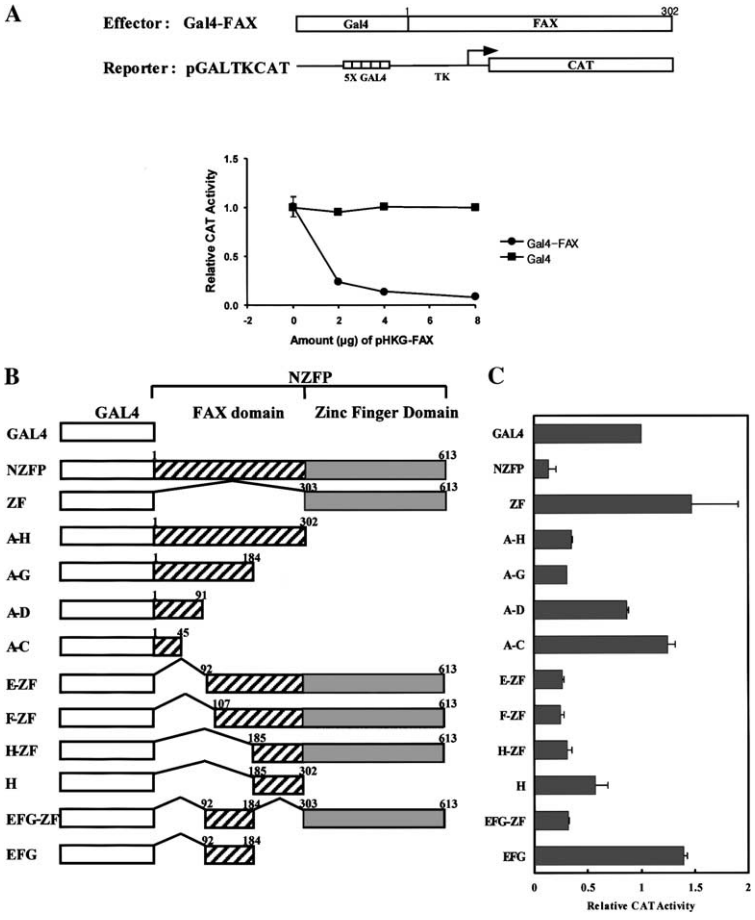


Fig. 3. NZFP is a transcriptional repressor. (A) Dosage-dependent transcriptional repression by NZFP. The Gal4-FAX (effector) and the pGALTKCAT (reporter) constructs are depicted in the upper portion of the figure. pGALTKCAT (2 μg) was cotransfected in the presence of increasing amounts of the Gal4-FAX plasmid with equal concentration adjustment of total DNA made by the plasmid pBlueScript (a total of 8 μg). The ordinate in the graph of the lower portion of the figure is the percentage change in CAT activity due to cotransfection with the Gal4-FAX plasmid by the amount indicated on the abscissa. The value represents the average of two independent transfections after normalization for the internal control of β-galactosidase activity. (B) GAL4–NZFP wild type and deletion constructs. Wild-type and deletion constructs of the GAL4–NZFP fusion protein are depicted. The numbers of the first and last amino acid residues of undeleted portions of the constructs are given. Open boxes indicate GAL4 and hatched and filled boxes, the FAX and zinc finger domains, respectively, of NZFP. (C) Effect of NZFP deletion mutants on transcription efficiency. Effector polypeptides fused to the Gal4 DNA-binding domain are designated on the ordinate and correspond to the deletion mutant constructs shown in (B). The relative amounts of CAT activity are shown by the filled boxes within the graph. Results were obtained from three different transfection experiments and experimental variations are indicated as shown.

experiments, pGALTKCAT was used as a reporter. We investigated the regulatory activity of the FAX domain in A6 cells by increasing the amount of the effector plasmid (pHKG-FAX) containing the FAX domain of NZFP. As shown in Fig. 3A, the relative amount of reporter gene (CAT activity) following transfection with the effector plasmid (pHKG-FAX) was decreased in a dose-dependent manner compared to that obtained by GAL4 alone. This result indicates that the FAX domain plays a role as a repressor during transcription.

To identify the repression domain, a series of truncated mutants were constructed and their repression activity was measured. As shown in Figs. 3B and C, the N-terminal FAX domain was sufficient to repress transcription of the target gene, while the C-terminal zinc finger domain did not show any repression activity (see GAL4 and ZF constructs and resulting transfection activities). We, therefore, examined the repression activity of mutants deleted from the C-terminus of NZFP (Δ185–613, Δ92–613, and Δ46–613, which correspond to A–G, A–D, and A–C boxes, respectively). Although Δ185–613 retained repression activity, further deletion of upstream regions (Δ92–613 and Δ46–613) abolished repression activity. These results indicate that the repression domain is located between residues 92 and 184 which corresponds to the E–G boxes of FAX protein family.

Deletions from the N-terminus, however, manifested a different result than that obtained with the C-terminus deletion mutants (see E-ZF through EFG constructs and the resulting transcription activities in Figs. 3B and C). Transcription was repressed in the mutants deleted up to residue 184 from the N-terminus (E-ZF, F-ZF, and H-ZF, respectively). The mutant containing only the residues between 185 and 302 also showed repression activity. These results suggest that there is another repression domain in this region, which corresponds to the H box of the FAX protein family. It should be noted that this region interacts with TBP (see Fig. 2).

The mutant containing residues 92–184 (E–G boxes) did not show any repression activity, but when the zinc finger domain was fused to this mutant at its C-terminus, repression activity was restored (Fig. 3C). These results suggest that there are two distinct repression domains in NZFP, one is located at residues 185–302 (H box) and interacts with TBP. The other is located at residues 92–184 (E–G boxes) and is size dependent for repression. The molecular mechanism of the latter one seems to be more complicated, since an extra protein sequence at either its C- or N-terminus is required to obtain repression activity (see Discussion).

Molecular mechanism of NZFP repression: inhibition of TFIIA and TFIIB for binding to TBP

The results presented in the above section suggest that the binding of NZFP to TBP may sterically inhibit the recruitment of GTFs such as TFIIA and/or TFIIB.

ChIP assay was employed to further examine whether the interaction between NZFP and TBP inhibits the binding of TFIIA and TFIIB. Chromatin fragments from lysates of A6 cells transfected with pGALTKCAT and pHKG or pHKG-NZFP were precipitated with anti-TFIIA antibody and anti-TFIIB antibody, respectively (Fig. 4). DNA from the immunoprecipitant was subjected to PCR analysis using primers specific to the TATA box-containing TK promoter of pGALTKCAT. As shown in Fig. 4A, the chromatin levels were dramatically reduced when the cells were transfected by GAL4–NZFP and the chromatins immunoprecipitated with anti-TFIIA or anti-TFIIB antibodies, suggesting that TFIIA and TFIIB recruitment to the promoter was inhibited by NZFP. On the other hand, when the chromatin was immunoprecipitated by anti-TBP anti-

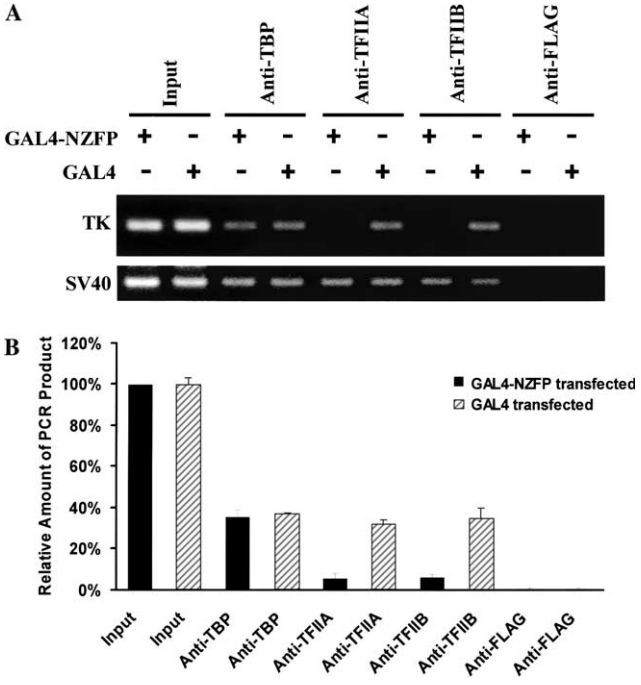


Fig. 4. TFIIA and TFIIB binding to TBP in the presence and absence of NZFP. A6 cells were transfected with pHKG or pHKG-NZFP and chromatin immunoprecipitation assays were performed on the resulting transiently transfected cells using antibodies to TBP, TFIIA, TFIIB, and FLAG as described in Materials and methods and shown in the figure. (A) Representative ChIP assays are shown; input indicates the chromatin solution used for the ChIP assay in which anti-TBP antibody and anti-FLAG antibody were used as positive and negative controls, respectively. PCR amplifications of the SV40 promoter region of pHKG or pHKG-NZFP were performed as an internal control. (B) Quantification of PCR products by gel image analysis. After PCR amplification, the intensity of each band was measured by using Gel-Pro Analyzer (Media Cybernetics, USA). The data are expressed as percentages of the input samples with the intensity of the input samples from each transfected cell line as 100% and the values shown represent means ± SE of three independent experiments.

body, the levels of precipitants from both GAL4 and GAL4–NZFP expressed cells were not significantly different. The expression of neither GAL4 nor GAL4–NZFP showed any appreciable effect on the binding of transcription factors to SV40 promoter region which was used as internal control (Fig. 4A). The intensity of the amplified DNA fragment was measured by gel image analysis. The mean value of the relative band intensity was at least fivefold higher in pHKG than that in pHKG-NZFP transfected cells, when precipitated by anti-TFIIA and anti-TFIIB antibodies (Fig. 4B). This observation provides strong evidence that the binding of NZFP with TBP inhibits the recruitment of GTFs such as TFIIA and TFIIB to further generate the PIC on the target gene promoter.

Discussion

In this study, we have examined the molecular mechanism of NZFP. Our study provides evidence that NZFP functions as a transcriptional repressor by inhibiting the recruitment of TFIIA and TFIIB through interaction with TBP.

NZFP has a high degree of sequence homology with XLCGF53.1 that also contains a FAX domain at its N-terminus [25]. NZFP is likely the full-length protein of XLcGF53.1, since the sequence of NZFP shows 96% amino acid sequence homology with XLcGF53.1 from its N-terminus FAX domain. However, the C-terminal 97 amino acid sequence of NZFP was deleted in XLcGF53.1. FAX-ZFPs are composed of a highly conserved FAX domain at the N-terminus and a zinc finger domain at the C-terminus [25]. Zinc finger domains are heterogeneous among FAX-ZFPs [25]. Molecular functions of FAX-ZFPs have not been defined so far. The zinc finger domain of FAX-ZFPs including NZFP can be assumed to be a DNA-binding domain, because most zinc finger transcription factors use this domain for DNA binding [16]. Therefore, the FAX domain would be the functional domain. In the present study, we show that the FAX domain functions as a transcriptional repressor.

To identify the repression domain of NZFP, we tested its transcription activity using a series of truncated mutants of this protein. The NZFP deletion that retained its H box (residues 185–302) showed significant repression activity. Interestingly, the H box also interacts with TBP, suggesting that NZFP transcriptional repression occurs by interaction with TBP. On the other hand, the E–G boxes of NZFP (residues 92–184) could not repress transcription by themselves, but were able to repress transcription when fused to the zinc finger domain or to the A–D boxes. The E–G boxes therefore require additional sequences to effectively cause repression. These results suggest that the H box of NZFP serves as a core repression domain within NZFP. Repression may occur by inhibiting PIC formation through interaction with TBP. The E–G boxes may function as an auxiliary domain which assists in repression by causing steric hindrance that disrupts the interaction between TBP and GTFs.

A certain group of repressors have been known to inhibit transcription by a quenching mechanism through interaction with certain specific activators or GTFs [11,14,27]. For example, NC2 and Eve interact with TBP subsequent to DNA binding and then interfere with binding of other GTFs such as TFIIA and TFIIB [13,15]. Our results suggest that NZFP repression involves a similar mechanism as that observed with Eve and NC2 [13,14], whereby NZFP perturbs the interaction between GTFs and TBP. That is, NZFP may compete with TFIIA for binding to TBP and sterically inhibit the recruitment of TFIIB. This hypothesis is supported by our observation that NZFP inhibits the binding of TFIIA and TFIIB with TBP in the ChIP assay.

On the basis of these findings, we propose the following model to account for the mechanism of transcriptional repression mediated by NZFP (see Fig. 5). The zinc finger domain of NZFP binds to the promoter sequence that is just upstream of the TATA promoter box of the target gene. The binding of NZFP through its zinc finger domain would bring this protein at a juxtaposition to interact with TBP preventing the binding of TFIIA and TFIIB (Fig. 5A). We have also shown that the FAX domain of NZFP, in addition to its ability to

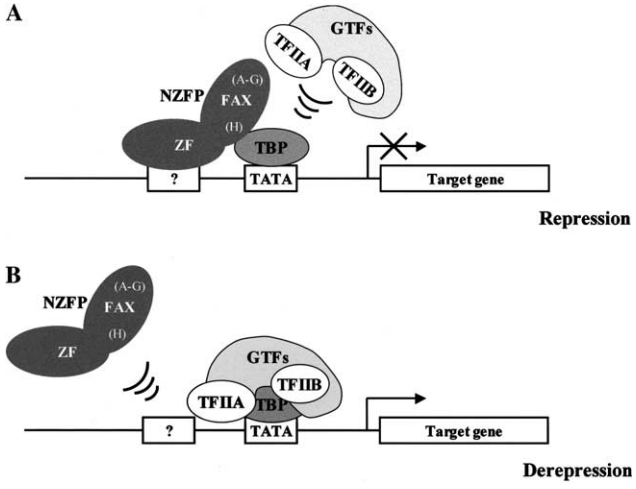


Fig. 5. Transcriptional repression–derepression model involving NZFP. (A) Repression model. NZFP is bound to the promoter of the target gene through its zinc finger motifs, and the H box of the FAX domain interacts with TBP presumably at the site where TFIIA binds. Therefore, this NZFP–TBP–promoter complex inhibits the recruitment of TFIIA and TFIIB to make the preinitiation complex (PIC) with general transcription factors (GTFs). (B) Derepression model. NZFP does not form a complex with the TBP–promoter and GTFs are recruited to make the PIC.

bind to the core domain of TBP, has as a major role in transcriptional repression. As far as we know, this is the first example to show that the zinc finger transcription factor may have a role in repressing transcription by interacting with TBP. We further propose that NZFP is removed from the promoter during derepression so that the GTFs can form a complex with TBP to initiate transcription (Fig. 5B). It remains to be elucidated whether NZFP requires other corepressors for efficient repression and what is (are) the target gene(s).

Acknowledgments

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