Human TFDP3, a novel DP protein, inhibits DNA binding and transactivation by E2F

Huan Qiao1,4*, Luisa Di Stefano3,5*, Chan Tian1, Yun-Yan Li1, Yan-Hui Yin1, Xiao-Ping Qian1, Xue-Wen Pang1, Yan Li1, Michael Allen McNutt2, Kristian Helin3,6, Yu Zhang3, Wei-Feng Chen3

1Department of Immunology, and 2Department of Pathology, Peking University Health Science Center, Beijing 100083, China
3Department of Experimental Oncology, European Institute of Oncology, 20141, Milan, Italy
Current address: 4Department of Medicine, Vanderbilt University School of Medicine, 2215 Garland Avenue, 539 Light Hall, Nashville, TN 37232-0275, USA, 5Massachusetts General Hospital Cancer Center, Bldg 149, 13th Street, Charlestown, MA 02129, USA, and 6BRIC, University of Copenhagen, 2100 Copenhagen, Denmark

Running title: Functional analysis of human TFDP3 protein

*These two authors contributed equally to this paper.

Address correspondence to: Yu Zhang or Wei-Feng Chen, Department of Immunology, Peking University Health Science Center, 38 Xue Yuan Road, Beijing, China, 100083. Tel. 86-10-82802593; Fax. 86-10-82801436; E-mail: zhangyu007@hsc.pku.edu.cn or wfchen@public.bta.net.cn

The two known DP proteins, TFDP1 and 2, bind E2Fs to form heterodimers essential for high-affinity DNA binding and efficient transcriptional activation/repression. Here we report the identification of a new member of the DP family, human TFDP3. In spite of the high degree of sequence similarity, TFDP3 is apparently distinct from TFDP1 in function. While TFDP3 retained the capacity to bind to E2F proteins, the resulting heterodimers, however, failed to interact with the E2F consensus sequence. In contrast to the stimulatory effect of TFDP1, TFDP3 inhibited E2F-mediated transcriptional activation. Consistent with this observation, we found that ectopic expression of TFDP3 impaired cell cycle progression from G1 to S phase instead of facilitating such a transition as TFDP1 does. Sequence substitution analysis indicated that the DNA binding domain of TFDP3 was primarily responsible for the lack of DNA binding ability of E2F/TFDP3 heterodimers and the inhibition of E2F-mediated transcriptional activation. Fine mapping further revealed four amino acids in this region, which were critical for the functional conversion from activation by TFDP1 to suppression by TFDP3. In conclusion, these studies identify a new DP protein and a novel mechanism whereby E2F function is regulated.

The E2F transcription factors are involved in the regulation of a wide variety of fundamental life processes ranging from cell cycling and growth, to apoptosis, and cell differentiation and development. Typically, E2F activity is mediated by a group of heterodimers composed...
of an E2F protein and a DP protein. The E2F family includes eight known members, which can be divided into four distinct subfamilies based upon their structural features, their transcriptional properties, and the molecules with which they interact. The first subfamily consists of E2F1–3. They are periodically expressed during the cell cycle, interact exclusively with the retinoblastoma (Rb) tumor suppressor protein, and are required for S-phase entry in the cell cycle (1–3). An additional role specific for E2F1 is the induction of apoptosis (4–6). The second subfamily is composed of E2F4 and 5, whose function is mainly regulated by p130 and p107 (7–11). These two E2F proteins are expressed at nearly constant levels through the cell cycle and are generally considered to be critical for cell cycle exit and differentiation (12, 13). The third subfamily contains a single member, E2F6. It lacks the typical transactivation/pocket protein (Rb, p130 and p107) binding domain, but retains the dimerization domain for DP proteins (14–17). E2F7 and E2F8 belong to the fourth subfamily. They possess two distinct DNA binding domains only, which are organized to mimic an E2F/DP heterodimer (18–22). E2F6–8 primarily act as transcription repressors, and are capable of blocking E2F-mediated transcripational activation of a subset of E2F targets. Thus, the multiple E2F proteins constitute a complicated regulatory network with diversified functions.

The DP family contains two well characterized members, TFDP1 and 2. These two proteins share high homology in the DNA binding/heterodimerization domain, but diverge from each other in the C-terminus (23–26). Due to the lack of a transactivation domain, DP proteins themselves have no transcriptional activity. Instead, they exert a regulatory function by dimerizing with E2F proteins. In fact, the heterodimerization of E2F/DP is essential for both high affinity DNA binding and efficient transcriptional regulation by E2Fs (24, 27–29). As heterodimers, the E2F/DP complexes bind to the consensus E2F DNA recognition site TTTc/gGCGCc/g identified in a large number of cellular promoters. This could lead to either activation or repression of the target genes depending on the specific E2F members involved. E2F1–3, for example, usually lead to the activation of genes critical for DNA synthesis and cell cycle progression. E2F4 and 5, on the other hand, recruit Rb and related proteins to E2F-regulated promoters and actively repress gene expression (30, 31).

In consideration of the pivotal role of E2F in cell cycle control, it has been speculated that deregulated E2F activity contributes to tumor development. As cofactors for E2Fs, DP proteins have been reported to cause transformation of cells in conjunction with activated ras, indicating a proto-oncogenic potential (32). Studies by Bargou et al, however, suggest that DPs may also act as a tumor suppressor. In their hands, cell transformation was achieved using a dominant negative TFDP1 mutant (33). The underlying mechanism for these apparently opposing effects is not well understood.

In the search for tumor associated antigens expressed in hepatocellular carcinoma, we identified a protein sharing high homology with human TFDP1 (34). In the present study, functional characterization of this novel member of the DP family (TFDP3) was pursued. First, its interaction with E2F proteins was analyzed using GST pull-down and co-immunoprecipitation assays. The putative E2F/TFDP3 complex was then examined for the capacity to bind to the E2F consensus sequence and to regulate E2F-dependent transcription.
Subsequently, a series of substitution constructs were created to reveal the structural basis for the functional differences between TFDP3 and TFDP1. Finally, the influence of TFDP3 on E2F-driven cell cycle progression and cell growth was explored by cotransfection of E2F3 with TFDP3. As a result, our studies have identified a new negative regulator of the E2F transcription factors.

Experimental Procedures

Cell culture and transfection
COS-7, HeLa, HEK-293 and the human liver cell line L02 were maintained in Dulbecco’s modified Eagle’s Medium (DMEM) with 10% (v/v) newborn calf serum (NCS). LipofectAMINE 2000 reagent (Invitrogen) was used for the delivery of plasmids into cells.

Plasmid constructs
To generate the expression constructs for TFDP3, TFDP1 and the various TFDP3/TFDP1 substitution mutants as indicated in Figure 7, full-length coding sequences were amplified by polymerase chain reaction (PCR). Upon sequence verification, the PCR products were inserted into the Hind III-Bam HI sites of pCDNA3-FLAG. pCMV-HA-E2F1, pCMV-HA-E2F2, pCMV-HA-E2F3, and pGL3 TATA basic 6xE2F luciferase constructs have been described previously (18, 35). pCDNA3-HA-E2F4 and pCDNA3-HA-E2F5 were provided by Dr. H. B. Shu (Peking University, China). pGEX-4T2 was used to express Glutathione S-transferase (GST) fusion proteins of E2F1–6 for the in vitro binding assay. pEGFP-N1 (Clontech) was used to express E2F proteins fused to the enhanced green fluorescent protein (EGFP) for subcellular localization of E2F.

In vitro binding assay
GST and GST-E2F fusion proteins were prepared by following standard procedures. 35S-labeled TFDP3 protein was produced by in vitro transcription (T7 polymerase) and translation in the presence of radioactive 35S-methionine using TNT® Quick Coupled Transcription/Translation system (Promega) according to the manufacturer’s protocol in a final volume of 50 µl per assay. For the in vitro binding reaction, appropriately purified GST or GST-E2F fusion proteins bound to glutathione-agarose beads were added to in vitro translated TFDP3 in lysis buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 10 mg/ml lysozyme, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 50 mg/ml leupeptin, 50 mg/ml protease inhibitor, 50 mg/ml aprotinin, and 50 mM dithiothreitol. After incubation for 2.5 h at 4°, the beads were collected and washed four times in lysis buffer to remove unbound protein. The protein bound to the beads was then released and fractionated on a 12.5% polyacrylamide gel. TFDP3 was detected by exposing the dried gel to an X-ray film.

Co-immunoprecipitation and Western blot analysis
To analyze the interaction of TFDP3/TFDP1 with E2F in vivo, HeLa cells were co-transfected with E2F and TFDP3/TFDP1. The transfected cells were washed twice in phosphate-buffered saline (PBS) and resuspended in lysis buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 5 µg/ml aprotinin, 5 µg/ml leupeptin, and 2 mM PMSF. The expression levels were monitored by Western blot of the cell lysate with antibodies specific for the hemagglutinin (HA) tag (for E2Fs), or the FLAG tag (for DPs). For immunoprecipitation, the lysate was incubated with anti-FLAG or anti-HA antibodies at a final concentration of 2 µg/ml with 25 µl of protein.
A-agarose (Roche) for at least 2 h at 4°C. The precipitates were separated on polyacrylamide gels, and blotted onto nylon membranes. These blots were then probed anti-HA (for samples immunoprecipitated with anti-FLAG) or anti-FLAG antibodies (for samples immunoprecipitated with anti-HA).

Immunofluorescence staining
COS-7 cells in 24-well plates were transfected with E2F and TFDP1/TFDP3. After 24 h, cells were fixed for 20 min at -20°C with methanol (precooled at -70°C), and permeabilized with 0.2% Triton X-100 in PBS. After blocking, the fixed cells were incubated with 1 µg/ml anti-FLAG antibody for 1 h at room temperature, thoroughly washed, and stained with tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG antibody (diluted to 1:100 with 1% BSA in PBS) for 1 h at room temperature. E2Fs were traced by EGFP, and the nuclei were counterstained with 10 µg/ml Hoechst 33342 (Sigma).

Electrophoretic mobility shift assay (EMSA)
EMSA was performed as previously described (36).

6xE2F-luciferase reporter assay
L02 cells were transfected with E2F and TFDP3/TFDP1 as indicated in the figure legends, together with a 6xE2F luciferase reporter gene. To normalize the transfection efficiency, 140 ng of pRL-SV40 Renilla luciferase reporter plasmid was added to each transfection as an internal control. The luciferase activities were determined using a dual specific luciferase assay kit (Promega).

Fluorescence-activated cell sorter (FACS) analysis
An asynchronous population of HEK-293 cells in log phase was transfected with the indicated plasmids together with Us9-GFP (37). After 48 h, cells were harvested and stained with propidium iodide (PI). Green fluorescent protein (GFP)-positive cells were gated for analysis of DNA content according to standard protocols.

Colony formation assay
HEK-293 cells were transfected with the indicated plasmids, along with pEGFP as a marker. 24 h after transfection, GFP-positive cells were isolated by FACS, and added into culture medium containing 0.4% (w/v) agar and 10% (v/v) NCS. Cells were then poured onto a 6-well plate with a bottom layer of 0.8% agar at 200 cells/well. These plates were maintained at 37°C under 5% CO2 for two weeks. Colonies containing more than 50 cells were counted.

Statistical analysis
Statistical evaluation for Colony formation was performed by the Student's t-test. P<0.05 was accepted as statistically significant.

Results
Characteristics of TFDP-3
Human TFDP3 (also called HCA 661 with NCBI nucleotide accession number CAI42694) was initially isolated as a novel cancer-testis antigen in our screening for tumor associated antigens (34, 38). Located on chromosome X, TFDP3 contains a single exon encoding a 405-amino-acid protein. As shown in Figure 1, TFDP3 shares a high degree of sequence homology with TFDP1 (75.2% amino acid identity). In addition, TFDP3 resembles TFDP1 in overall structure, which includes a heterodimerization domain, a DNA binding domain, and a typical RRXYD E2F DNA recognition motif in the DNA binding domain. Moreover, similar to TFDP1 and 2, TFDP3 possesses a C-terminus composed of multiple acidic amino acids (15 out of the last 20 amino acids are acidic). These features strongly suggest that TFDP3 is a third member of the DP family.
We subsequently performed a thorough search of NCBI genomic database for human TFDP3 homolog in other species. In rhesus, three TFDP-like genes, represented by XR013178, XM001112439 and XP001097146, respectively, were identified. While XM001112439 encodes TFDP2, XR013178 and XP001097146 had been previously named TFDP1 despite the apparent sequence variations. More careful analysis revealed that XR013178 encodes a protein almost identical to human TFDP1 whereas the protein dictated by XP001097146 is more closely related to human TFDP3 than TFDP1 (86% vs. 76% identity). Therefore, we believe that XP001097146 is actually the rhesus homolog of human TFDP3. Another potential TFDP3-coding sequence, although incomplete, was found in chimpanzee. Mouse and rat, on the other hand, seem to have no TFDP3-like gene, suggesting that TFDP3 might arise later in evolution. Phylogenetic analysis indicated that TFDP3 segregates from TFDP1 and TFDP2, forming a distinct subfamily (Figure 1C). Alignment of the DNA binding domain of various DP proteins revealed that some of the well conserved amino acids in TFDP1 and 2 are no longer retained in TFDP3 (Figure 1D). The significance of such substitutions will be further discussed in later sections.

Interaction of TFDP3 with E2F1~6
As a member of the DP family, TFDP3 would be expected to directly interact with the E2F proteins. To test for such a potential, *in vitro* translated, $^{35}$S-labeled TFDP3 was incubated with GST-E2F fusion proteins, and the complexes were then isolated using glutathione beads. As shown in Figure 2A, TFDP3 was pulled down with GST proteins fused with each member of E2F family (E2F1~6) (lane 3 to 8) but not with GST protein itself (lane 2), suggesting that TFDP3 was able to heterodimerize with individual E2Fs.

We next analyzed TFDP3 association with E2F1~6 *in vivo*. HeLa cells were co-transfected with expression plasmids of TFDP3 and E2Fs in pairs, and the expression levels of these proteins were monitored by immunoblotting the lysates with antibodies against the tags (HA for E2Fs and FLAG for TFDP3) (Figure 2B, middle and bottom panels). TFDP3 was immunoprecipitated from the cell lysate with anti-FLAG antibodies, and the precipitates were then probed with anti-HA antibody. E2Fs were observed to co-precipitate with TFDP3 (Figure 2B, top panel), indicating that TFDP3 and E2Fs can interact *in vivo*.

To further determine the relative affinity of TFDP3 versus TFDP1 for E2Fs, cells were co-transfected with HA-tagged E2F3, and Flag-tagged TFDP1 and 3. Upon immunoprecipitation of E2F3 with anti-HA antibodies, the precipitates were examined for the presence of TFDP1 and 3, which were distinguished by size. We found that TFDP3 effectively competed with TFDP1 for E2F in a dose-dependent manner (Figure 2C, top panel). Taken together, these data show that TFDP3 can bind with a high affinity to each member of the E2F family, most likely through the heterodimerization domain to which TFDP1 has been shown to bind.

Subcellular localization of TFDP3 and its translocation induced by E2F
To reveal the subcellular distribution of TFDP3, COS-7 cells were transfected with FLAG-tagged DP and/or EGFP-tagged E2F expression constructs. Localization of E2F and DP proteins was followed by autofluorescence and immunofluorescence, respectively. When applied alone, both TFDP3 and 1 were localized in the cytoplasm (Figure 3A; similar results...
were obtained in HeLa cells, data not shown). Also found in the cytoplasm are E2F4 (Figure 3A) and 5 (data not shown), whereas E2F3 (Figure 3A), E2F1 and 2 (data not shown) were restricted to the nucleus.

We subsequently investigated how the localization of TFDP3 was affected by interaction with E2F proteins. Similar to TFDP1, TFDP3 translocated to the nucleus of transfected cells in the presence of E2F3 (Figure 3B), and E2F1 or 2 (data not shown). In contrast, both TFDP3 and 1 remained in the cytoplasm after co-transfection with E2F4 (Figure 3B) or 5 (data not shown). TFDP3 therefore appears to be directed to the same cellular compartments as TFDP1 whether expressed alone or in combination with E2Fs.

**DNA binding defect of the E2F/TFDP3 complex**

Having confirmed the direct interaction between TFDP3 and E2F, we next examined the lysate of TFDP3-tranfected cells for the presence of binding activity to the consensus E2F DNA recognition sequence by EMSA. A basal level of binding activity was detected in HeLa cells, which was not significantly altered following transfection with TFDP3 or any of the individual E2F members tested (Figure 4, compare lanes 2, 3, 6 and 9 with lane 1). However, consistent with previous studies (23, 26-28), co-transfection of E2F with TFDP1 resulted in a marked increase in DNA binding activity (Figure 4, compare lane 5 with lane 3, lane 8 with lane 6 and lane 11 with lane 9). On the other hand, co-transfection with TFDP3 caused no change (Figure 4, compare lane 4 with lane 3, lane 7 with lane 6 and lane 10 with lane 9) in spite of similar expression levels of exogenous proteins in the cell lysate (data not shown). Therefore, even though TFDP3 interacted with E2Fs as efficiently as TFDP1, the resulting E2F/TFDP3 complexes were defective in DNA binding.

**Inhibition of transcriptional activity of E2F by TFDP3**

The DNA binding defect of the E2F/TFDP3 complex prompted us to explore the impact of TFDP3 on E2F-mediated transcriptional activation. Different combinations of the E2F (E2F1–5) and DP (TFDP1 and 3) expression vectors were introduced into L02 cells together with a reporter plasmid containing 6 E2F DNA binding sites. As shown in Figure 5A, E2F alone generated a remarkable increase in luciferase activity over the empty-vector control (10- to 30-fold, depending on the specific E2F members tested), and the enzymatic activity was further increased by co-transfection with TFDP1. In sharp contrast to the synergistic effect of TFDP1, TFDP3 demonstrated an inhibitory effect on E2F-mediated transcriptional activation of the reporter gene as suggested by the much reduced luciferase activity in cells co-transfected with E2F and TFDP3.

As E2F4 and 5 were found to be predominantly restricted to the cytoplasm and TFDP1 and 3 had no significant impact on their subcellular localization (Figure 3), the transactivation of the reporter by E2F4 and 5 and the modifying effect of TFDP1 and 3 on their activities is somehow surprising. Nevertheless, similar effects have been reported in previous studies with E2F4 and TFDP1 (10). One may assume that non-detectable amounts of E2F4 and TFDP1 are in the nucleus, which are sufficient to activate transcription.

The mechanism by which TFDP3 repressed E2F-dependent transcription remains to be determined. As one possibility, TFDP3 may compete with endogenous TFDP1 to form inactive complexes with E2F proteins. To test this hypothesis, we compared the luciferase
activities in L02 cells transfected with E2F and TFDP1, together with increasing amounts of TFDP3. The transcriptional activity of all combinations of E2F/TFDP1 was significantly inhibited in the presence of TFDP3. Moreover, there was clearly a dose-dependent response. At a 4-fold excess, TFDP3 completely abolished the enhancing effect of TFDP1 on E2F activity (Figure 5B). These results support the notion that TFDP3 functions as a competitive inhibitor.

Up to this point, we had shown that TFDP3 could counteract the transcriptional activation induced by E2F transfection. Is endogenous E2F activity also affected by TFDP3? Indeed, we found that the baseline level of E2F activity in L02 cells was inhibited by TFDP3 in a dose-dependent manner, which is the reverse of the dose-dependent stimulatory effect of TFDP1 (Figure 5C). Similar results were obtained using a luciferase reporter containing the E2F responsive element from the Cyclin A2 promoter, a natural target of E2F (data not shown), ruling out the possibility of an artifact somehow associated with the synthetic E2F promoter construct.

**TFDP3 induces G1 arrest and cell growth inhibition**

The biochemical studies described above established TFDP3 as a negative regulator of the E2F activity. Given the complexity of the E2F family of transcription factors, it was next of interest to determine how TFDP3-mediated inhibition affected the behavior of intact cells. To this end, we first evaluated the effect of TFDP3 on cell cycle progression. Cells were transfected with E2F3, along with TFDP3 or TFDP1. The transfected population was then analyzed to determine the percentage of cells in G1, S and G2/M phases. As shown in Figure 6A, transfection with E2F3 resulted in a significant decrease in the percentage of cells in G1 and a concomitant increase in the percentage of S phase cells. Moreover, such changes were further enhanced by co-transfection with TFDP1. In contrast, the E2F3-mediated effect was largely abolished by co-transfection with TFDP3. Next, we sought to determine the influence of TFDP3 on colony formation of transfected cells. As demonstrated in Figure 6B, TFDP3 significantly reduced the colony-forming capacity of HEK-293 cells. More intriguing, similar inhibition was also observed even in the presence of exogenous TFDP1, suggesting that TFDP3 plays a dominant role. Taken together, these data are consistent with a model in which TFDP3 can act as a negative regulator of E2F function.

**The molecular basis of TFDP3-mediated inhibition of E2F activity**

To dissect the molecular basis of the functional difference between TFDP3 and TFDP1, we created a panel of constructs with sequence substitutions between TFDP3 and TFDP1 (Figure 7). Following introduction of these constructs into L02 cells, the expression of mutant proteins was confirmed by Western blotting with anti-FLAG antibody (Figure 8A). Thereafter, we estimated the effect of the mutants on the transcriptional activities of a representative E2F member, E2F4.

First, we tested a series of constructs with the substitution of individual functional domains to grossly map the region that dictates the stimulatory or inhibitory function. Activities similar to those of the parent proteins were maintained in the constructs generated from replacement of the heterodimerization domain (TFDP3SH and TFDP1SH) or the C terminal half (1D3H/3D1H). On the other hand, each of the constructs with the DNA binding domain substituted, including TFDP3SD/TFDP1SD, and TFDP3SDH/TFDP1SDH, demonstrated a
function opposite to that of the wild-type molecule. TFDP3 harboring a DNA binding domain derived from TFDP1 acquired the capacity to stimulate E2F activity, whereas TFDP1 with a TFDP3 DNA binding domain showed inhibitive activity (Figure 8B). Therefore, it is the DNA binding domain that distinguishes TFDP3 from TFDP1 in function.

Next, we sought to determine the critical amino acids, which confer the inhibitory effect of TFDP3 within the DNA binding domain. Based on the analysis of sequence differences between TFDP3 and TFDP1, we performed a single-amino-acid substitution (TFDP3<sup>164</sup> and TFDP1<sup>169</sup>) in the RRXYD DNA recognition motif and the replacement of a 13-amino acid region (TFDP3<sup>109-121</sup> and TFDP1<sup>114-126</sup>) proposed to be involved in the heterodimerization contacts and DNA backbone contacts (39, 40). Surprisingly, these substitutions had no effect on the function of either TFDP3 or TFDP1 (Figure 8B). More substitution mutants were then made, covering virtually all the remaining regions showing amino acid differences within the DNA binding domain, including TFDP3<sup>130-145</sup>, TFDP3<sup>148-161</sup>, and TFDP3<sup>179-190</sup>. While the last two substitutions failed to affect the inhibitory function of TFDP3, TFDP3<sup>130-145</sup> substitution led to the loss of inhibition (Figure 8C). Among the 16 amino acid residues in this region, TFDP3 and TFDP1 show differences in 7. To evaluate the contribution of each of these residues, we created 7 single-amino-acid substitution mutants (TFDP3<sup>130</sup>, TFDP3<sup>131</sup>, TFDP3<sup>134</sup>, TFDP3<sup>135</sup>, TFDP3<sup>140</sup>, TFDP3<sup>142</sup>, and TFDP3<sup>145</sup>). It was of interest that none of these were significantly different from wide-type TFDP3 in function (Figure 8C). Therefore, it is likely that the abolition of TFDP3-mediated inhibition will require simultaneous substitutions of multiple amino acids in this region.

Although the TFDP3<sup>130-145</sup> mutant no longer inhibited E2F activity, this substitution was not sufficient to convert TFDP3 into a stimulatory molecule like TFDP1, suggesting that other residues outside this region may be required for the optimal function of TFDP3 or TFDP1. We therefore carried out a series of combination substitutions involving aa130-145 in the α<sub>2</sub> helix and the appropriate regions in α<sub>1</sub> (aa109-121) or α<sub>3</sub> (aa148-161) helix (Figure 7B and 9A). TFDP3<sup>130-145,148-161</sup> was similar to TFDP3<sup>130-145</sup> in activity, showing that the α<sub>3</sub> helix is not directly involved in the functional divergence of TFDP3 and TFDP1. On the other hand, TFDP3<sup>109-121,130-145</sup> not only lost the inhibitory function of the parent molecule, but also acquired a new stimulatory property similar to that of TFDP1 (Figure 8D). Further studies showed that a single key amino acid residue at position 121 in the α<sub>1</sub> helix, and three essential residues at position 130, 131 and 140 in the α<sub>2</sub> helix were responsible for these effects. TFDP3<sup>121,130,131,140</sup> is therefore functionally comparable to TFDP3<sup>109-121,130-145</sup> as well as wild-type TFDP-1 (Figure 8D).

To summarize these findings, we showed that the distinctive functions of TFDP3 and TFDP1 are largely determined by their DNA binding domains. More specifically, this function involves 4 key amino acid residues, including one at position 121 in the α<sub>1</sub> helix, and three others at positions 130, 131 and 140 in the α<sub>2</sub> helix. Substitution of these residues in TFDP3 with those at the corresponding positions of TFDP1, Thr→Lys at 121, Cys→Tyr at 130, Gln→Asn at 131, and Lys→Glu at 140, were sufficient to convert TFDP3 into a stimulatory molecule like TFDP1.
Discussion

In this study, we have characterized a novel human protein, TFDP3. This new member of the DP family shares a high degree of sequence homology with TFDP1 and 2. Moreover, it has demonstrated certain functional properties common to DP proteins. Specifically, TFDP3 interacts with E2F1–6 in vitro and in vivo, and co-localizes with E2F1–3 in the nucleus, and with E2F4 and 5 in the cytoplasm in transfected cells. Nevertheless, TFDP3 appears to exert an influence different from that of TFDP1 on E2F activities. While dimerization with TFDP1 dramatically increases the DNA binding capacity of E2F, the E2F/TFDP3 complex fails to bind to the consensus E2F DNA recognition sequence. More intriguingly, the transcriptional activation driven by either endogenous or exogenous E2F is substantially suppressed by overexpression of TFDP3, which is opposite to the enhancing effect of TFDP1. Hence our studies have identified a new and functionally distinctive member of the DP family.

The original member of the DP family, TFDP1, was first isolated in 1993 (23). Two years later, the second member of this family, TFDP2 (also called DP3 in mouse), was identified (25, 26). TFDP1 and TFDP2 both function to enhance the DNA binding and the transcriptional activities of E2F. By targeting the DNA binding domain, Wu et al. generated a series of TFDP1 mutants which were dominant-negative in function (39). The new DP family member identified in this study, TFDP3, resembles these mutants in many ways. In particular, these molecules all retained the capacity to interact with E2F, but the resulting complexes failed to bind to DNA, and their ectopic expression caused the suppression of E2F activities and a G1 arrest in cell cycle progression. With a view to these latter features, we propose a model in which TFDP3 acts as an endogenous negative regulator for E2F, where TFDP3 competes with the activating DP family members for E2F binding, leading to the formation of transcriptionally inactive heterodimers and hence the inhibition of cellular E2F activities. In support of this model, we found that TFDP3 directly competed with TFDP1 in E2F binding and in transcriptional activation by E2F in a dose-dependent manner. Moreover, co-transfection of TFDP1 was able to partially overcome the inhibitory effect of TFDP3 on colony formation. In this context, it is worth mentioning a recent report on the identification of a novel and inhibitory isoform of TFDP1, DP1α (41). Inhibition by DP1α is obviously via a different mechanism as it fails to bind to E2F due to the lack of the C-terminal heterodimerization domain. It therefore seems clear that DP activities are fine-tuned by multiple mechanisms.

In the analysis for the molecular basis of the functional divergence between TFDP3 and TFDP1, we found that it is the DNA binding domain that confers the capacity for inhibition by TFDP3 or stimulation by TFDP1 since an interchange of the DNA binding domains in these two molecules led to complete reversal in function. Fine mapping revealed that the inhibitory effect of TFDP3 involves four key amino acid residues, including Thr121, Cys130, Gln131 and Lys140. Substitution of these residues with those at the corresponding positions of TFDP1 (Lys126, Tyr135, Asn136 and Glu145) was sufficient to render TFDP3 stimulatory to E2F activity. Notably, these residues are well conserved in all known DP proteins in a whole variety of species except for a substitution of Glu145 by a similar residue Asp in DPL-1 of *C. elegans* and AtDPα of *A. thaliana*. On the other
hand, three out of the four substations in human TFDP3 (Thr121, Cys130 and Lys140) are also identified in the putative TFDP3 protein of rhesus (Figure 1D). The phylogenetic conservation of these residues further highlights their importance in specification of stimulatory versus inhibitory function of DP proteins.

To understand how these four residues may contribute to the unique function of TFDP3, we performed computer-aided modeling of the tertiary structures of the DNA binding domains of DP proteins making use of Swiss-Pdbviewer and SWISS-MODEL (42) with TFDP2 (PDB entry 1cf7B) (40) as a template. The DNA binding domain of TFDP3 was predicted to assume a winged helix structure composed of three α helices and three β sheets, which is typical of DP proteins (Figure 9).

In TFDP1 (Lys126 and Glu145) or TFDP-2 (Lys81 and Glu100), residues Lys and Glu, which correspond in position to Thr121 and Lys140 in TFDP3, are spatially close to each other with a distance of 2.81 Å or 2.82 Å, allowing the formation of a salt bridge that links the α1 and α2 helices. This type of interaction, however, is not favored in TFDP3 because of the relatively long distance between Thr121 and Lys140 (6.23 Å). Single amino acid substitution of Thr121 with Lys (as in the TFDP3121 mutant) or Lys140 with Glu (as in TFDP3140) reduces the distance to 4.07 and 5.41 Å, respectively. Distance in this range is still non-permissive for the formation of a salt bridge. On the other hand, simultaneous substitution of both residues, Thr121 with Lys and Lys140 with Glu, reduces the distance to 2.81 Å, and an effective interaction is thus restored (Figure 9B).

Tyr of TFDP1 (Tyr135) and TFDP2 (Tyr90) is positioned to interact with another Tyr of TFDP1 (Tyr170) and TFDP2 (Tyr125) which is in direct contact with DNA. The π stacking created by these two aromatic residues allows an optional occupancy of space, and creates a strong hydrophobic core in the structure (Figure 9C). In TFDP3, the first Tyr is replaced by Cys130, leading to the disruption of the favored configuration.

Still another residue critical for the distinct functions of DP proteins is Gln131 in TFDP3, or Asn in TFDP1 (Asn136) and TFDP2 (Asn91). Although these two residues are structurally conserved, substitution of Gln131 by Asn was absolutely required for the full functional conversion of TFDP3. At this point, we have no notion as to the specific contribution of Asn136/Asn91 to the maintenance of the appropriate structures of TFDP1 and TFDP2. Neither do we know how the introduction of Gln131 leads to a disruption. Answers to these questions may rely on the resolution of the crystal structures of TFDP1 and TFDP3.

Among the diverse activities attributed to E2Fs, the best studied is their role in regulation of the G1/S transition and S-phase entry during the cell cycle (13, 30, 31). Many genes encoding various DNA replication proteins and cell cycle regulators are known to be under direct control by E2Fs. In G0 and early G1 phase, repressor E2Fs (E2F-4 and 5) are prevalent, forming complexes with pocket proteins p107 and p130 at most E2F-regulated promoters. The pocket proteins recruit histone deacetylases, and the chromatin-remodeling induced by these enzymes results in a compact structure which is refractory to transcription initiation. Meanwhile, the activator E2Fs (E2F1–3), which are expressed at low levels, are sequestered by Rb. In late G1 and S phase, pocket proteins are phosphorylated by cyclin D/cdk4, 6 and cyclin E/cdk2 and dissociate from E2Fs. The repressor E2Fs are relocated to the cytoplasm, whereas the activator E2Fs, which are induced to high levels,
bind the vacated promoters and restore histone acetylation by recruiting histone acetyltransferases. As an essential component of E2F activity, DP proteins would be expected to play an active role in cell cycle regulation. Studies by Wu et al showed that overexpression of a dominant TFDP1 mutant blocks G1 progression in human osteosarcoma cells (39). Similar G1 arrest is also observed following TFDP1 knock-down by siRNA in human fibroblasts (43). In contradiction to these in vitro studies, loss of Tfdp1 in mouse does not compromise the expression of cell cycle-related genes, and Tfdp1−/− ES cells can give rise to most tissues other than the extra-embryonic tissues (44, 45). Our finding of a new DP family member demonstrates a further level of complexity in the E2F transcriptional network. Consistent with its role as a negative regulator, overexpression of TFDP3 inhibits the enhanced cell cycle progression and growth of HEK293 cells induced by E2F3. But as in all studies in which proteins are overexpressed, one should keep in mind that the results obtained may not accurately reflect the physiological functions of endogenous products. Thus, for better understanding of the biological role of TFDP3, it would be important to determine whether and how the expression and localization of endogenous TFDP3 is regulated during the cell cycle.

Defects in the Rb/E2F pathway are believed to be present in most if not all human cancers. Mutations frequently involve components upstream of E2Fs, such as inactivation of the Rb gene and altered Cdk activity. These mutations lead to deregulated E2F activity, suggesting the importance of E2Fs in tumor development (46). In order to define the exact roles of E2Fs in tumor development, a number of mouse models have been developed by gene manipulation. However, results obtained so far defy any easy classification of E2Fs, particularly the activating members, into the conventional “oncogenes” or “tumor suppressors”. For example, on the one hand, forced expression of E2f1 in epidermal or hepatocellular cells induces spontaneous skin or liver tumors (47, 48), implying an oncogenic potential. On the other hand, mice deficient in E2f1 also develop a broad spectrum of tumors (49), supporting a tumor-suppressive function. These bimodal activities may stem from the fact that E2Fs can affect both cell proliferation and cell death (50). Under normal circumstances, the coupling of these two events ensures a fail-safe mechanism for apoptosis to occur in the event of any irreversible damage in the cell cycle. In case of E2F deregulation, such a mechanism could result in either uncontrolled cell proliferation or accumulation of cell mutants, either of which may lead to the development of tumors.

TFDP3 was initially identified as a gene highly expressed in hepatocellular carcinomas but not in normal liver tissues (34). This restricted pattern suggests a potential role in tumorigenesis. Surprisingly, the present study showed that overexpression of TFDP3 in HEK293 cells counteracted the enhancing effect of E2F3 on G1-S transition and colony formation in soft agar. A putative explanation for this paradox is that the induction of TFDP3 in tumor tissues is a response to hostile microenvironments such as hypoxia and nutrition-deprivation, and the consequent slow-down in growth actually favors tumor development in the long-run. In a preliminary experiment, we observed the induction of TFDP3 transcription in several non-expressing cell lines following serum starvation (data not shown). Efforts are being undertaken to identify the functional relevance of this induction. Alternatively, the “forced” expression of TFDP3
does not necessarily fully recapitulate the function of endogenous proteins within a specific cellular context. Previous studies have demonstrated that the known DP proteins can display both tumor promoting and suppressing activities, and the specific action is highly context dependent (32, 33). In line with this thinking, one may speculate that TFDP3 preferentially interacts with the repressor E2Fs in tumor cells. By displacing/preventing them from binding to target promoters, TFDP3 could block E2F-mediated repression, and thereby promoting tumorigenesis. Therefore, it would be interesting to determine if TFDP3 binds to specific E2F members in normal and tumor cells.

In conclusion, we have identified a new and functionally distinct member of the DP family. In contrast to the enhancing effect of known DP proteins, TFDP3 suppresses E2F-mediated transcriptional activation and cell cycle progression. This functional conversion seems to result from amino acid substitutions at a few critical positions. Further clarification of its biological functions may influence our current perspective of how E2F activities are orchestrated in normal cells and how they are disturbed in such pathological conditions as cancer.

Acknowledgements

We thank Dr. N. Zheng (Washington University, USA) for his helpful comments about protein structure in the mutational analysis, and Dr. L. Enquist (Princeton University) for providing Us9-GFP plasmid. This work was supported by grants from National Natural Science Foundation of China (No.30531160045), National 863 Program in China (No.2003AA215110) National Basic Research Program of China (No. 2006CB504300) and Beijing Natural Science Foundation (No.7061003).

Footnote

While this manuscript was in preparation, A. Milton et al. published their studies on TFDP3, which they called DP4. Results from both their study and ours demonstrated a distinct inhibitory function of TFDP3 on the E2F activities. However, our study defined in detail the structural basis for the functional divergence between TFDP3 and TFDP1. Moreover, we tested this new protein against almost the entire family of E2F proteins. As to the nomenclature, we adopted the name TFDP3 recommended by the HUGO Gene Nomenclature Committee as it maintains the consecutive order of this family of proteins.

The abbreviations used are: Rb: retinoblastoma; DMEM: Dulbecco’s modified Eagle’s Medium; NCS: newborn calf serum; PCR: polymerase chain reaction; GST: glutathione S-transferase; EGFP: enhanced green fluorescent protein; PMSF: phenylmethylsulfonyl fluoride; PBS: phosphate-buffered saline; HA: hemagglutinin; TRITC: tetramethylrhodamine isothiocyanate; EMSA: electrophoretic mobility shift assay; FACS: fluorescence-activated cell sorter; PI: propidium iodide; GFP: green fluorescent protein.
References


Figure legends

Figure 1. Sequence features of TFDP3. (A) Amino acid sequence alignment of TFDP3 and TFDP1. Identical residues are marked with an asterisk; the conserved substitutions with a colon; the semi-conserved substitutions with a dot, and the missing residues with a dash. The spanning of the DNA binding domain and the heterodimerization domain is labeled, and the RRXYD DNA recognition motif is shaded. (B) Schematic representation of structural domains of TFDP3 and TFDP1. Percentage of amino acid identity within specified regions is indicated. (C) Phylogenetic relationship of DP proteins. Multiple alignment was performed using ClustalW program and the graphical output was prepared using DRAWTREE and DRAWGRAM programs. Scale bar at the bottom of the tree represents 10% difference in amino acid sequences. (D) Amino acid sequence alignment of the DNA binding domain of DP proteins. Shaded uppercase characters indicate fully conserved residues.

Figure 2. Interaction of TFDP3 with E2F1–6 in vitro and in vivo. (A) GST pull-down assay for interaction in vitro. GST-E2F fusion proteins (lanes 3 to 8) immobilized on glutathione-agarose beads were incubated with in vitro-translated, 35S-labeled TFDP3. The bound TFDP3 proteins were visualized by exposure to X-ray film following SDS-gel electrophoresis. TFDP3 (lane 1) and GST protein (lane 2) served as positive and negative controls, respectively. (B) Co-immunoprecipitation for interaction in vivo. HeLa cells were co-transfected with TFDP3-FLAG and E2F-HA constructs, and the cell lysate was analyzed. Top panel, cell lysate was immunoprecipitated with anti-FLAG, and the blot was probed with anti-HA; Middle and bottom panel, cell lysate was analyzed for E2F and TFDP3 expression using anti-HA and anti-FLAG, respectively. Arrowheads indicate the mobility of the specific E2F species and the TFDP3 protein. (C) Competitive E2F binding by TFDP3 and TFDP1. Cells were transfected with 3 µg of E2F3, 3 µg of TFDP1, and an increasing amount of TFDP3 at 3, 6 or 12 µg. Empty vectors were used to compensate for a total of 18 µg plasmid DNA per transfection. Top panel, cell lysate was immunoprecipitated with anti-HA, and the blot was probed with anti-HA; Middle and bottom panel, cell lysate was analyzed for TFDP3/TFDP1 and E2F3 expression using anti-FLAG and anti-HA, respectively. Arrowheads indicate the mobility of the TFDP3, TFDP1 and E2F3 proteins.

Figure 3. Subcellular localization of DP proteins in conjunction with E2Fs. Following transient transfection of COS-7 cells with various constructs, TFDP3/TFDP1 was detected in fixed cells by indirect immunofluorescence using anti-FLAG antibody and TRITC-conjugated secondary antibody (red), whereas E2F proteins were located by autofluorescence with the EGFP tag (green). Hoechst 33342 (blue) was used to stain the nuclei. Images were taken with a 20X objective len. (A) Localization of E2F or DP proteins when expressed alone. pEGFP-N1 vector served as a control. (B) Co-localization of E2F and DP. From top to bottom, cells were co-transfected with TFDP3+E2F3, TFDP1+E2F3, TFDP3+E2F4, and TFDP1+E2F4.

Figure 4. DNA binding activity in transfected cells. HeLa cells were transfected with the indicated
expression plasmids. Cell lysates were examined for binding activity with a $^{32}$P-labeled oligonucleotide probe containing an E2F DNA recognition site in EMSA.

**Figure 5.** Inhibition of E2F transcriptional activity by TFDP3. L02 cells were transiently transfected with 140 ng of 6xE2F-luciferase reporter construct and 140 ng of pRL-SV40 Renilla luciferase reporter construct, with or without E2F and/or DP constructs as indicated. The firefly luciferase activity in each transfectant was normalized to the corresponding Renilla luciferase activity, and presented as a multiple of that in cells transfected with the reporter construct alone (mock). Data shown are averages of at least three independent experiments. (A) E2F activity in the presence of TFDP3 or TFDP1. A total of 560 ng of plasmid DNA was used for each transfection. 20 ng of E2F1 or E2F3; 70 ng of E2F4; 140 ng of E2F5, and 140 ng of TFDP3 or TFDP1 was used, and when necessary, empty vectors were used for compensation. (B) Dose-dependent inhibitory effect of TFDP3. E2Fs and TFDP1 were used at the doses specified above. TFDP3 was added at 0, 140, 280 or 560 ng to give a total of 1,120 ng per transfection. (C) Inhibition of endogenous E2F activity by TFDP3. 0, 140, 280, or 560 ng of TFDP3 or TFDP1 were used per transfection.

**Figure 6.** Inhibition of cell cycle progression and cell proliferation by TFDP3. (A) Effect of TFDP3 on cell cycle progression. HEK-293 cells were transfected with 1 µg of E2F3 and 2 µg of DP expression plasmid together with 1 µg of Us9-GFP, and the GFP-positive cells were analysed by PI staining to determine the cell cycle profiles. A representative result from one of three independent experiments is shown. The numbers indicate the proportion of cells at different phases of the cell cycle. (B) Effect of TFDP3 on colony formation in soft agar. HEK-293 cells were transfected with constructs as indicated. The GFP-positive cells were sorted and plated in soft agar at 100 cells/well. Colonies were counted at day 14. The y axis shows the average number of colonies/well from three independent experiments with three wells for each trial (n=9). *P = 0.0085 (E2F-3 vs. Mock); **P = 1.5 x10^{-7} (E2F3+TFDP1+TFDP3 vs. E2F3) or P = 1.2x10^{-9} (E2F3+TFDP1+TFDP3 vs. E2F3+TFDP1); ***P = 5x10^{-10} (E2F3+TFDP3 vs. E2F3).

**Figure 7.** Schematic illustration of TFDP3 and TFDP1 mutants. The transcriptional property of the mutants is indicated to the right: “+” indicates transcriptional activation at the level of wild-type TFDP1; “-” indicates transcriptional inhibition similar to wild-type TFDP3; “+/-” indicates a transcriptional activity close to E2F4 alone. (A) Mutants derived from gross substitution of individual domains. UNT: unknown functional N-terminus; DBD: DNA binding domain; HD: heterodimerization domain; UCT: unknown functional C-terminus. (B) Mutants derived from substitution of specific regions or residues in the DNA binding domains. The regions or residues which originated from TFDP1 were shaded in grey.

**Figure 8.** Effect of TFDP3 and TFDP1 mutants on E2F-mediated transcriptional activation. (A) Expression of TFDP3 and TFDP1 mutants. The protein expression was confirmed by Western blot using antibodies against the tag. (B), (C) and (D) L02 cells were transfected with 70 ng of E2F4, together with 210 ng of either TFDP3, TFDP1, one of the substitution mutants, or the empty vector to give a total of 560 ng of DNA. The experimental procedures and data processing are as specified in Figure 5A.

**Figure 9.** Computer modeling of the structure of the DNA binding domains in TFDP2, TFDP1, TFDP3 and TFDP3 mutants. (A) Sequence alignment of the DNA binding domains of DP proteins. Amino acid residues involved in the formation of the $\alpha$ helix and $\beta$ sheet are marked as “h” and “s”, respectively. Also labeled are those residues in heterodimerization contact, DNA backbone contact and DNA base contact. Other labels have been described in Fig. 1A. (B) The positioning of residues T121 and K140 in TFDP3,
and that of equivalent pairs in other DP proteins and mutants. The distance between the two residues is shown on the top. (C) The positioning of residues C130 and Y165 in TFDP3, and that of equivalent pairs in other DP proteins and mutants. (D) Crystal structure of the E2F4-TFDP2 heterodimer DNA complex (PDB entry 1cf7B) showing the relative positions of the key amino acids in the complex. The complex is viewed with the DNA axis either in horizontal position (left) or in vertical position (right).
Figure 1

A

B

DNA-binding domain

C

Heterodimerization domain

D

TFDP1 Mouse (123-196)

TFDP3 Human (123-196)

TFDP2 Mouse (68-150)

XIDP-2 X.laevis (69-153)

TFDP1 Human (68-151)

TFDP2 Mouse (129-211)

TFDP_D.melanogaster (163-242)

TFDP1 Human (108-191)

TFDP3 Human (108-191)

TFDP2 Rhesus (68-150)

XIDP-2 X.laevis (69-153)

ThDPb A.thaliana (101-185)

DPL-1 C.elegans (71-155)

TFDP3 Human (108-191)

TFDP3 Rhesus (108-191)

TFDP2 Rhesus (113-196)

TFDP1_Rhesus (113-196)

TFDP1 Mouse (113-196)

XIDP-2 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)

XIDP-1 X.laevis (132-197)
Figure 2

A

B

C

TFDP1  62KD
TFDP3  47.5KD

TFDP1  62KD
TFDP3  47.5KD

E2F3  62KD

E2F3  - + + + +
TFDP3  - - + + +

IPα-FLAG
Blot α-HA

Lysate
Blot α-FLAG

Lysate
Blot α-HA

83KD  62KD  47.5KD
83KD  62KD  47.5KD

Downloaded from http://www.jbc.org/ on November 15, 2017 by guest
Figure 3
Figure 5

A

Fold activation

B

Fold activation

C

Fold activation

TFDP3

TFDP1
Figure 6

A

DNA content
mock

E2F3

E2F3+TFDP1

E2F3+TFDP3

G1: 63.43%
S: 22.64%
G2/M: 13.92%

G1: 50.16%
S: 33.76%
G2/M: 16.08%

G1: 44.59%
S: 30.02%
G2/M: 25.39%

G1: 56.78%
S: 27.77%
G2/M: 15.45%

B

mock
E2F3
E2F3+H4D3
E2F3+TFDP3
E2F3+TFDP1+TFDP3

0
10
20
30

* * * **
Figure 8
Figure 9

A

<table>
<thead>
<tr>
<th>TFDP1</th>
<th>TFDP2</th>
<th>TFDP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>115...196</td>
<td>68...181</td>
<td>180...191</td>
</tr>
</tbody>
</table>

Secondary structure: **H** indicates helix, **E** indicates extended conformation.

- Heterodimerization contacts
- DNA backbone contacts
- DNA base contacts

B

![Graphs](image)

C

![Graphs](image)

D

![Graphs](image)
Human TFDP3, a novel DP protein, inhibits DNA binding and transactivation by E2F
Huan Qiao, Luisa Di Stefano, Chan Tian, Yun-Yan Li, Yan-Hui Yin, Xiao-Ping Qian, Xue-Wen Pang, Yan Li, Michael Allen McNutt, Kristian Helin, Yu Zhang and Wei-Feng Chen

J. Biol. Chem. published online October 24, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M606169200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts