Two Domains of the Human bZIP Transcription Factor TCF11 Are Necessary for Transactivation*

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TCF11 is a bZIP transcription factor of the CNC subfamily. It has been implicated in the regulation of the antioxidant response and is vital during embryonic development, but its precise biological functions have not yet been fully worked out. Structural characterization of the gene and several of its products indicates that complex regulatory mechanisms are employed. To investigate how altering the structure of the gene products might influence their activity we have mapped functional domains within the protein. We show that two separate domains are required for transactivation by full-length TCF11: an N-terminal acidic domain and a serine-rich stretch adjacent to the CNC-bZIP domains. A naturally occurring shorter isoform (identical to LCR-F1) produced by internal initiation of translation is unable to transactivate in our assay. However, the shorter form could interfere with the transactivating ability of the longer form, which indicates a control mechanism for keeping the activity of TCF11 at a desired level. We show that TCF11 and the closely related CNC-bZIP factor p45 NF-E2 show different cell type-specific activation patterns with full-length TCF11 being active in COS-1 cells but silent in erythroid cells (K562), whereas p45 NF-E2 is active in K562 cells and silent in COS-1 cells. Domain swapping experiments show that cell type-specific activity is not fully determined by dimerization/DNA binding domains or transactivation domains alone. The resulting profile of activity is most likely achieved by interaction of the domains and their cell-specific environment.

TCF11 (and the isoform Nrf1) is a widely expressed human transcription factor of the CNC-bZIP family (1, 2), most closely related to the family members p45 NF-E2, Nrf2, and Nrf3 (3–5). The importance of TCF11 was demonstrated by two independent inactivations of the gene in mouse, showing that TCF11 is vital during development (6, 7), but the precise biological role of TCF11 has not yet been defined. It has been shown that fibroblasts derived from TCF11/Nrf1-null embryos have enhanced sensitivity to the toxic effects of oxidant compounds (8). This is likely to be because of reduced glutathione level in these cells. TCF11 binding and transactivation of the promoter of the catalytic subunit of γ-glutamylcysteine synthetase have recently been observed.[1] In addition, TCF11 has been shown to transactivate through the hARE response element in the NAD(P)H:quinone oxidoreductase-1 promoter (9), a site that is important for the induction of NAD(P)H:quinone oxidoreductase-1 in response to xenobiotics and antioxidants. These observations indicate a role for TCF11 in the regulation of the antioxidant response. In addition, TCF11 has also been suggested to play a role in tumor necrosis factor-α regulation, because specific interaction with the tumor necrosis factor-α promoter has been demonstrated in the cell line DC18 (10).

TCF11 is widely expressed (1) and has the potential of complex regulation. Among the different cDNA clones that were originally isolated, both alternative transcription start sites and alternative poly(A) sites were observed. Several different isoforms caused by alternative splicing were isolated (1), one of them identical to the cDNA sequence of Nrf1 (2). In a later study another isoform was identified without the serine-rich domain, which specifically interacts with the tumor necrosis factor-α promoter to stimulate transcription (10). These observations indicate that differential processing of TCF11 transcripts is a prominent feature and may be important in regulating TCF11 activity.

The activity of TCF11 may also be regulated at a number of additional levels. First, *tcf11* transcripts are translated as two major isoforms with the possibility for translational initiation from an internal methionine cluster to yield a short isoform of 447 amino acids (originally described as LCR-F1 (11)). Full-length TCF11 ranges from 728 to 772 amino acids depending on alternative splicing within an acidic N-terminal domain (1). Second, dimerization with different partners may alter the transactivating activity of TCF11. The small Maf proteins MafF, MafG, and MafK have been identified as putative partners of TCF11 (12, 13). More recent results show that these factors, originally thought to be widely expressed, are differentially expressed during development and might therefore regulate TCF11 in a tissue-specific manner (14, 15). Both MafG and MafK repress transactivation when coexpressed with TCF11 compared with the level of reporter gene transcription induced by TCF11 alone in transient transfections (13, 16). However, these factors are also partners of several other bZIP proteins (for an overview, see Ref. 17), such as the erythroid-specific transcription factor p45 NF-E2 (13, 18) and more widely expressed family members. Competition between partners might thus regulate TCF11 activity. Third, post-translational modifications such as phosphorylation may play a role in regulating of TCF11 activity. TCF11 has several potential phosphorylation sites, and casein kinase II has been shown to phosphorylate an isoform of TCF11 specifically and thereby render it active in binding to the tumor necrosis factor-α promoter (10). Fourth, TCF11 might interact with other tissue-specific, yet unidentified cofactors.

Given the potential for complex regulation and interaction, we wished to map functional domains within TCF11, using a transactivation assay in cultured cells. The activities of three

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naturally occurring differential translation products have been compared, and we used deletion and fusion constructs to examine subdomains more extensively. Two different regions in the TCF11 protein are required to gain full transcription factor activity in transient transfections in COS-1 and HeLa cells. However, even full-length TCF11 is inactive in K562 cells, whereas the closely related protein p45 NF-E2 is active under the same conditions. We demonstrate that even though TCF11 and p45 NF-E2 have a high degree of similarity in their DNA binding domains, one does not interfere with the transactivation abilities of the other through the NF-E2 site when coexpressed in transient transfections. Chimeric fusions between TCF11 and p45 NF-E2 show that the DNA binding and dimerization domains from p45 NF-E2 can replace the corresponding domains from TCF11 and give a protein with the functional characteristics of TCF11. However, this occurs only when the fusion includes the serine-rich stretch, a domain that is not present in p45 NF-E2.

EXPERIMENTAL PROCEDURES

Expression Constructs

The expression construct pcA20 producing TCF11 longer form was made by cloning the full-length sequence of *tcf11*, clone pZcEA20 (5′ to the *Eco*RV site at bp 3550, accession no. X77366) (1) into the expression vector pcDNA3 (Invitrogen). In the same way, clone pZcEA56 was used to make an expression construct pcA56 producing a variant of the TCF11 longer form, and pZcEA52 was used for the construct pcA52 producing only the shorter form. An expression construct pcA20IntMut producing only the full-length TCF11 was made by mutating the four internal methionine residues (Met-318, Met-321, Met-323, and Met-326) to leucine residues (QuickChange site-directed mutagenesis, Stratagene). All deletion constructs were made from the full-length sequence of *tcf11* (pcA20). In the TCF11Δ173–315 construct (numbers referring to the amino acid residues deleted) the sequence between the two *Bgl*II sites was deleted (restriction sites at bp 1108 and 1537 in the *tcf11* sequence). In the TCF11Δ12–125 construct the sequence between the *Xmn*I site (bp 624) and the first *Xho*I site (bp 962) was deleted. In the TCF11Δ12–315 construct the sequence between the *Xmn*I (bp 624) site and the second *Bgl*II site (bp 1537) was deleted, and in the TCF11Δ469–558 construct the sequence between a *Pvu*II site (bp 1996) and the *Nar*I (bp 2569) site was deleted. The p45 NF-E2 expression construct was kindly provided by Dr. Paul Ney. In this construct the murine *p45* NF-E2 is under the control of the polypeptide chain elongation factor 1α (19, 20). For *in vitro* translation, *p45* NF-E2 was subcloned into the pcDNA3 vector using the *Eco*RI and the *Not*I sites.

Fusion Constructs

TCF11 and p45 NF-E2 fusion constructs were made by cloning PCR-generated *p45* NF-E2 fragments (verified by sequencing) into the appropriate sites in the TCF11 expression construct pcA20 as follows.

The Transactivating Domain (TAD) from p45 NF-E2 Fused to the DNA Binding Domain (DBD) of TCF11 without the Serine-rich Domain—The sequence coding for the TAD fragment was PCR amplified with primers 1 and 2 and ligated to the *Hin*dIII (polylinker of pcDNA3)/*Bam*HI (bp 2263) fragment of pcA20. The construct was named NATDB (p45 *N*F-E2 *a*ctivating domain fused to *T*CF11 *D*NA *b*inding domain).

The TAD from p45 NF-E2 Fused to the DBD of TCF11 Including the Serine-rich Domain—The sequence coding for the TAD fragment was PCR amplified with primers 1 and 3 and ligated to the *Hin*dIII/*Bsg*I (bp 2003) fragment of pcA20 (named NASerTDB).

TAD from TCF11 without the Serine-rich Domain Fused to DBD from p45 NF-E2—The sequence coding for the DBD fragment was PCR amplified with primers 4 and 5 and ligated to the *Bsr*GI (1599)/*Xba*I (polylinker of pcDNA3) fragment of pcA20 (named TANDB).

TAD from TCF11 Including the Serine-rich Domain Fused to DBD from p45 NF-E2—The sequence coding for the DBD fragment was PCR amplified with primers 6 and 5 and ligated to the *Pml*I (bp 2302)/*Xba*I fragment of pcA20 (named TASerNDB).

Primer 1: 5′-GCG C<u>AA GCT T</u>GG ACA CTA CCC GCA GCC TCA TCT C-3′, covering bp 215–238 in the p45 NF-E2 sequence (AC L09600 (3)) and containing an additional *Hin*dIII site.

Primer 2: 5′-CGC AGC T<u>GG ATC C</u>CC CTG ATG CAG G**TC ATA AGA TGG TGG GGG AAG GGA GAA GC**-3′, covering bp 586–559 and containing an additional *Bam*HI site.

Primer 3: 5′-AAA TTC CTC C<u>TC CAC CTG GGA GGC CTG CAC</u> **CTC ATA AGA TGG TGG GGG AAG GGA GAA GCC TGC**-3′, covering bp 586–555 and containing an additional *Bsg*I site.

Primer 4: 5′-GGA ACC T<u>TG TAC A</u>CA CCC **TTG GCC TTA GAG TCA TCC TCC GGT CC**-3′, covering bp 942–967 and containing an additional *Bsr*GI site.

Primer 5: 5′-**GCA CCA GTG TCC ACT C**<u>TC TAG A</u>CC-3′, covering bp 1484–1461 with the *Xba*I site.

Primer 6: 5′-TTA ATT <u>CAC GTG</u> **GCC TTA GAG TCA TCC TCC**-3′, covering bp 943–962 and containing an additional *Pml*I site.

The part of the *p45* NF-E2 sequence in the different primers is shown in bold, and the enzyme sites are underlined.

Cell Culture

COS-1, HeLa, and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (1 g/liter glucose, Life Technologies, Inc.) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 50 units/ml penicillin-streptomycin at 37 °C in 5% CO_2. K562 cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 units/ml penicillin-streptomycin, sodium pyruvate, and 0.25% glucose at 37 °C in 5% CO_2.

Transient Transfections

COS-1, HeLa, or HepG2 cells were transfected at 50–80% confluence either using a standard calcium phosphate procedure (16) or FuGENE 6, following the supplier's instructions (Roche Molecular Biochemicals). For calcium phosphate transfection, 5 μg of TCF11 expression construct, 2 μg of PBGD3.2Luc reporter (16), and 1 μg of internal control (pRSVCAT)/10-cm dish were used. The luciferase gene in the PBGD3.2Luc reporter is under the control of a part of the porphobilinogen deaminase (PBGD) gene promoter. It has been shown previously that TCF11 activates the reporter through the single NF-E2 site in the promoter region (16). A total of 1.0–2.2 μg of DNA/3.5-cm dish was used during FuGENE 6 transfections together with 2–6 μl of transfection reagent. The DNA mixture consisted typically of 0.2 μg of PBGD3.2Luc reporter construct, various amounts of different TCF11 and/or p45 NF-E2 expression constructs, and empty pcDNA3 vector to the required total amount of DNA.

K562 cells were transfected with DEMRIE-C transfection reagent (Life Technologies, Inc.) following the supplier's instructions. A mixture of 4 μg of DNA together with 4 μl of DEMRIE-C transfection reagent was used to transfect 2.0×10^6 cells. The DNA mixture consisted typically of 0.8 μg of PBGD3.2Luc reporter construct, 0.4 μg of internal control (pRSVCAT), various amounts of TCF11 and/or p45 NF-E2 expression constructs, and empty vector to achieve the required total amount of DNA.

Luciferase, CAT, and Protein Assay

The cells were harvested at two days post-transfection in a lysis buffer (50 mM Tris-MES, pH 7.8, 1 mM dithiothreitol, 0.1% Triton X-100). The luciferase activity was measured on a MicroLumat Plus luminometer (EG&G Berthold). 5–50 μl of cell extract was diluted in luciferase mixture buffer (10 mM Mg(OAc)$_2$, 50 mM Tris-MES, pH 7.8, 2 mM ATP) to a total volume of 200 μl. 100 μl of 1 mM luciferin (Roche Molecular Biochemicals 1626353) was added to each sample. CAT activity was measured using a standard protocol (16). Luciferase activity was normalized either by comparison with CAT activity or with the total protein content in the sample, determined by the Bio-Rad protein assay with 3–5 μl of the cell extract.

The levels of luciferase induction after transfection with TCF11 or other expressing constructs are given as relative activity compared with the activity obtained by the empty vector alone.

Western Blot Analysis

COS-1, HeLa, or K562 cells were transfected as described previously. After 2 days of incubation the cells were harvested using standard methods for cells growing either in monolayer or in suspension (21). Protein extracts were separated using 10% SDS-polyacrylamide gel electrophoresis (running buffer: 25 mM Tris, 200 mM glycine, 0.1% SDS) then electroblotted onto nitrocellulose membranes (blotting buffer: 20% (v/v) methanol, 200 mM glycine, and 25 mM Tris). After transfer, the membranes were blocked with 5% bovine serum albumin in TBST (165 mM NaCl, 10 mM Tris, pH 8, 0.1% Tween 20) for 1 h at room temperature. Blots were incubated with primary antibody (anti-TCF11 diluted 1:16,000 in TBST containing 0.5% bovine serum albumin (12)) at 4 °C overnight, followed by incubation with secondary antibody (anti-rabbit horseradish peroxidase diluted 1:5,000 in TBST containing 0.5% bovine
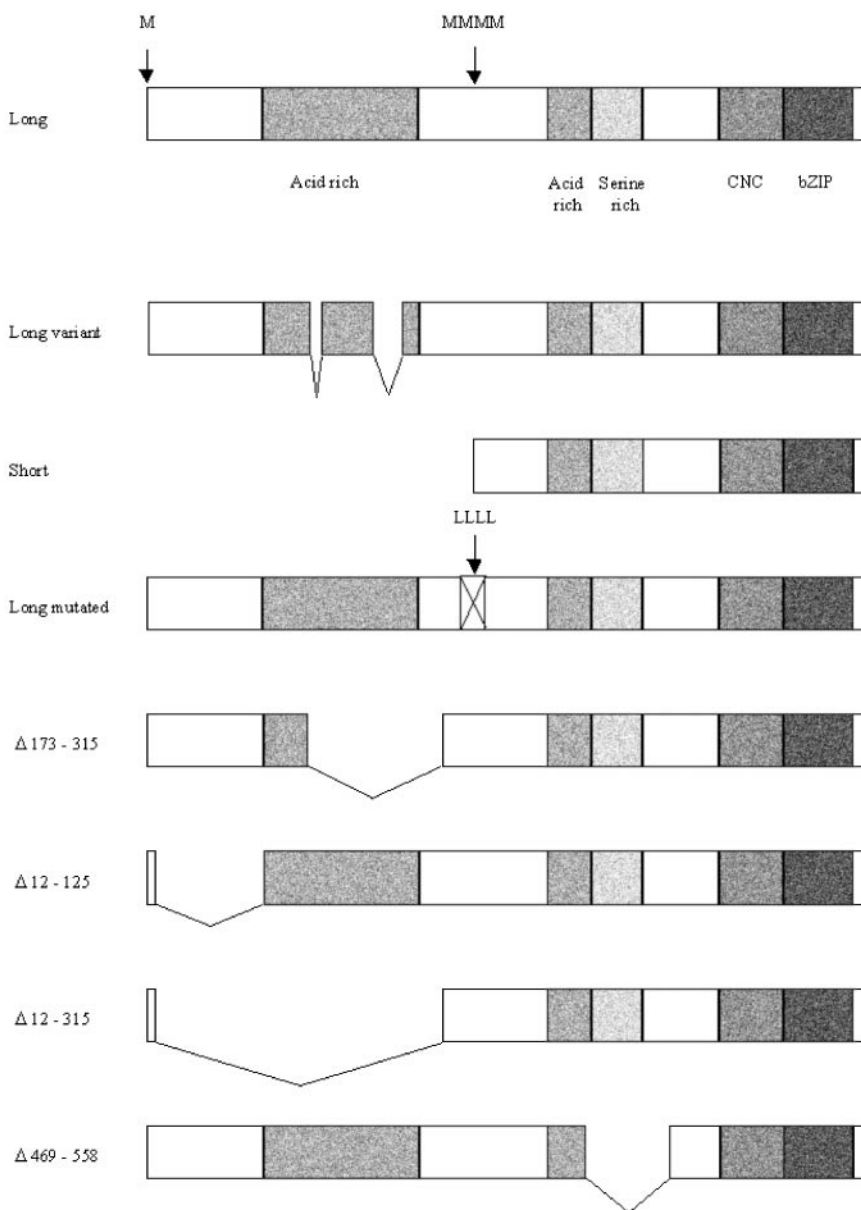
FIG. 1. **Schematic representation of the different isoforms and mutated or deleted versions of TCF11 indicating structural motifs within the protein.** The putative N-terminal transactivating domain (acid-rich), an internal acid-rich domain (acid-rich), the serine-rich domain, the CNC domain (CNC, highly conserved among the family of the CNC-bZIP factors), and the basic (DNA binding domain) leucine zipper (dimerization domain) (bZIP) are shown. The two different translation initiation start sites, which are the initial methionine (M) and the internal methionine cluster (MM-MM), are also marked. Full-length TCF11 (long), a natural variant of the long form (long variant), the internal initiated shorter protein form (short), a mutated version of the long form where the four internal methionine residues are changed to leucine residues (LLLL, long mutated), and four deletion constructs are represented. The long variant lacks a total of 41 amino acids in the N-terminal acid-rich domain because of alternative splicing. In Δ173–315, amino acid residues 173–315 have been removed in the N-terminal acid-rich domain. In Δ12–125 most of the protein N-terminal to the acid-rich domain is deleted, and Δ12–315 has a deletion covering the whole acid-rich domain in addition to most of the N terminus. The serine-rich domain has been deleted in Δ469–558.

serum albumin (Amersham Pharmacia Biotech)) for 1 h at room temperature. The immune complexes were visualized using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) and detected with x-ray film.

MBP Pulldown Assay

In vitro dimerization between TCF11 or MafG and different TCF11 variants, p45 NF-E2, or chimeric proteins was performed as described previously (12). Briefly, MBP, MBP-TCF11-A, and MBP-MafG were bacterially expressed and purified on amylose resin (New England Biolabs). The different variants of TCF11, p45 NF-E2, and the chimeric proteins were translated *in vitro* using the TNT-coupled *in vitro* transcription/translation system (Promega) in the presence of [^{35}S]methionine and incubated with the desired MBP fusion by gently mixing overnight at 4 °C in dimerization buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40) containing protease inhibitors. Unbound proteins were removed by washing three times with the dimerization buffer. Bound proteins were eluted by denaturation in SDS-PAGE sample buffer (2% SDS, 0.1 M dithiothreitol, 60 mM Tris-HCl, pH 6.8, 10% glycerol, and 0.001% bromphenol blue), separated by SDS-PAGE, and analyzed by autoradiography.

RESULTS

Two Alternative Translation Products of TCF11 Show Different Transactivating Ability—In vitro assays have previously revealed two major translation isoforms of TCF11 (2), the second, smaller isoform presumably being produced by translation initiation from an internal cluster of ATGs (Fig. 1). We confirm this observation both in cell culture and with *in vitro* transcription/translation assays. This is not unexpected because there is a non-optimal Kozak sequence at the initial methionine (22). TCF11 produced from a full-length cDNA, where both isoforms can potentially be produced, can specifically bind to and transactivate through the NF-E2 site in the PBGD promoter (16). We wished to investigate if either or both of the major translation products were involved and if they showed any differences in their ability to induce expression of a reporter. To test this, we have used a transient transfection assay expressing two differentially spliced cDNAs that can produce both full-length and internally initiated protein and a truncated cDNA that can only produce the short isoform. The reporter induction occurs through a single NF-E2 site in the assay.

Fig. 1 shows a schematic representation of the full-length TCF11 protein with functional domains indicated, a variant of the protein caused by alternative splicing (long variant), and the shorter protein resulting from internal translation initiation. Initiation from the internal methionine cluster yields a

FIG. 2. *Panel a*, luciferase activity induced in transient transfected COS-1 cells expressing TCF11 long form, the TCF11 long variant, and the TCF11 short form. The PBGD3.2Luc reporter and internal control (pRSVCAT) were cotransfected with either empty pcDNA3 vector (*first bar* from *left*) or one of the TCF11 expression constructs (*second* through *fourth bars*). The cells were transfected by a standard calcium phosphate method using a total of 10 μg of DNA/10-cm dish. Luciferase activity has been normalized to CAT activity and is shown relative to the activity given with cotransfection of the empty vector. The induction shown is the average of eight experiments, and the *error bars* reflect the standard deviation of each mean value. *Panel b*, detection of the different translation products using Western blot analysis. Whole cell extracts were prepared from COS-1 cells transiently transfected with the different TCF11 constructs expressing wild type isoforms as shown in Fig. 1. After SDS-PAGE and transfer to a nitrocellulose membrane, immunodetection with a polyclonal antibody against the C terminus of TCF11 (12) revealed the different translation products as indicated (●). The *arrowheads* on the *left* (▶) indicate the position of size standards with the size of each shown in kDa. Extracts of cells transfected with pcDNA3 show background staining with this antibody. *Panel c*, *in vitro* dimerization between MBP-TCF11-A (12) and different *in vitro* translated variants of TCF11 was tested in an MBP pulldown assay (*sixth* through *ninth lanes*). TCF11 long form incubated with MBP alone is used as a control for binding specificity (*fifth lane*). 10% of the input of ^{35}S-labeled proteins is shown in the *first* through *fourth lanes*.
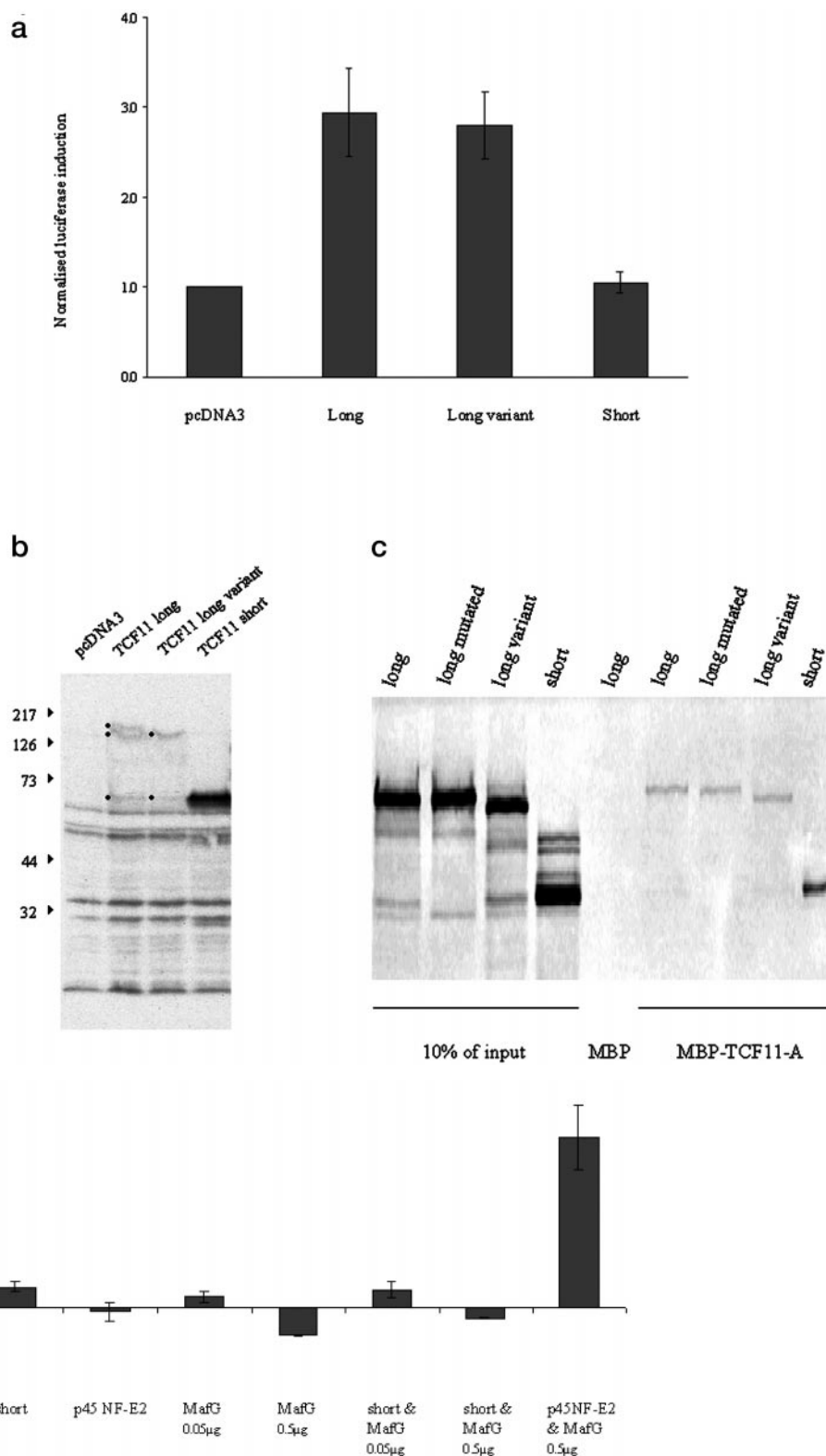

FIG. 3. **Luciferase activity induced in transient transfected COS-1 cells expressing TCF11 short form, p45 NF-E2, and MafG either alone or in combinations.** The PBGD3.2Luc was cotransfected with either empty pcDNA3 vector (*first bar*), the incomplete TCF11 expression construct (*second bar*), the p45 NF-E2 expression construct (*third bar*), the MafG expression construct (*fourth* and *fifth bars*), a combination of TCF11 with different amounts of MafG (*sixth* and *seventh bars*), or a combination of MafG and p45 NF-E2 (*eighth bar*). Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The induction shown is the average of three experiments, and the *error bars* reflect the standard deviation of each mean value. The cells were transfected by using FuGENE 6 transfection reagent and a total of 1.3 μg of DNA/3.5-cm dish.

short isoform of 447 amino acids, whereas full-length TCF11 is 772 amino acids (1). The long variant transcript yields a product of 731 amino acids (Fig. 1) because of the omission of exons 3a and 4 in the N-terminal acid-rich domain (23).

In a transient transfection assay the different protein products show variation in their ability to induce luciferase expression. COS-1 cells transiently transfected with clones that produced either of the longer translation products (long and long

a

b

c

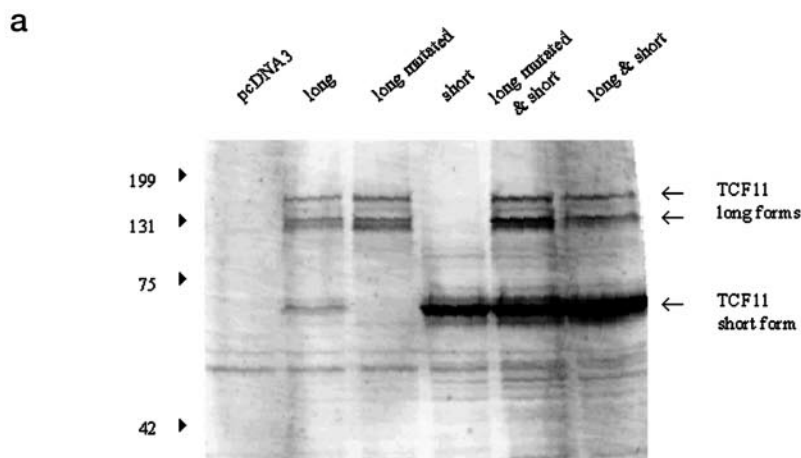| pcDNA3 | 2.0 | 1.5 | 1.5 | 1.0 | 0.5 | 1.5 | 1.0 | 0.5 | 1.0 | 0.5 | - | 0.5 | 1.0 | 0.5 | - | 0.5 |
Short	-	0.5	-	-	-	-	-	-	0.5	0.5	0.5	1.0	0.5	0.5	0.5	1.0
Long	-	-	0.5	1.0	1.5	-	-	-	0.5	1.0	1.5	0.5	-	-	-	-
Long mutated	-	-	-	-	-	0.5	1.0	1.5	-	-	-	-	0.5	1.0	1.5	0.5

FIG. 4. *Panel a*, detection of the different translation products using Western blot analysis. Whole cell extracts were prepared from COS-1 cells transiently transfected with different TCF11 constructs expressing either TCF11 long form, long mutated, or short form as shown in Fig. 1. After SDS-PAGE and transfer to a nitrocellulose membrane, immunodetection with a polyclonal antibody against the C terminus of TCF11 (12) revealed the different translation products as indicated (←). The *arrowheads* on the *left* (▶) indicate the positions of size standards with the size of each shown in kDa. Extracts of cells transfected with pcDNA3 show background staining with this antibody. *Panel b*, luciferase activity induced in transient transfected COS-1 cells expressing TCF11 long form, long mutated, and short form. The PBGD3.2Luc was cotransfected with either empty pcDNA3 vector (*first bar*) or one of the TCF11 expression constructs (*second* through *fourth bars*). Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The induction shown is the average of three experiments, and the *error bars* reflect the standard deviation of each mean value. The cells were transfected by using FuGENE 6 transfection reagent and a total of 2.2 μg of DNA/3.5-cm dish. *Panel c*, luciferase activity induced in transient transfected COS-1 cells expressing TCF11 long form, long mutated, and short form either alone or in combinations. The PBGD3.2Luc was cotransfected with either empty pcDNA3 vector (*first bar*), one of the TCF11 expression constructs (*second* through *eighth bars*), or a combination of the incomplete clone together with either the full-length (*ninth* through *twelfth bars*) or the mutated construct (*thirteenth* through *sixteenth bars*). Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The induction shown is the average of three experiments, and the *error bars* reflect the standard deviation of each mean value. The cells were transfected by using FuGENE 6 transfection reagent and a total of 2.2 μg of DNA/3.5-cm dish. The different amounts of DNA used in each transfection are shown.
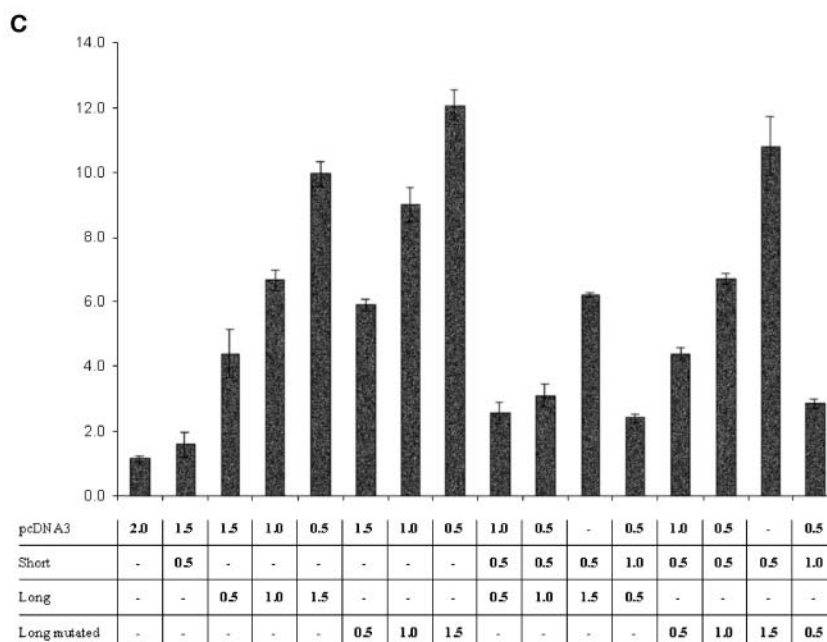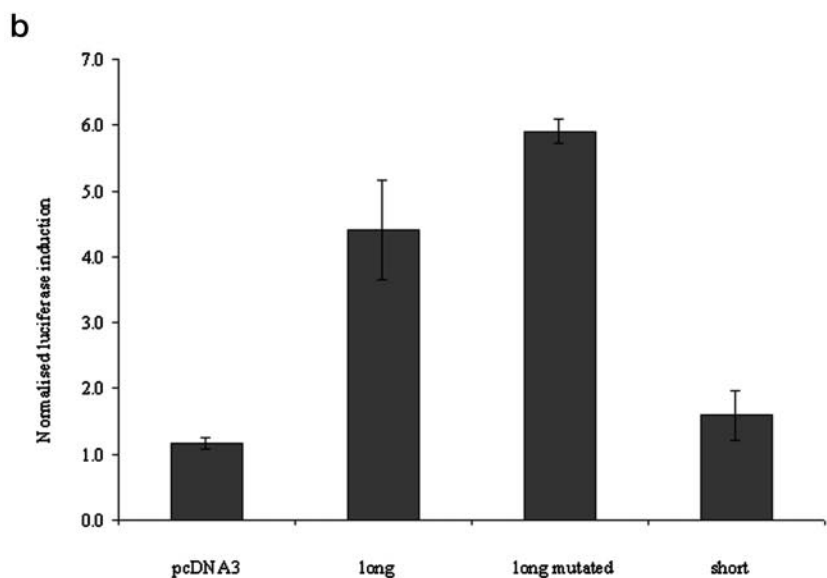
variant) showed a significant luciferase induction (Fig. 2*a*, *second* and *third bars*). In contrast, in cells where only the shorter translation product can be produced, no luciferase in-

duction above background level was observed (Fig. 2*a*, *fourth bar*). The same results were obtained with transiently transfected HeLa or HepG2 cells (data not shown). The omission of

FIG. 5. *Panel a*, luciferase induction by full-length TCF11 compared with the four deletion constructs (Δ173–315, Δ12–125, Δ12–315, and Δ469–558) represented in Fig. 1. The 3.2PBGDLuc reporter was co-transfected with either empty pcDNA3 vector (*first bar*) or one of the TCF11 constructs (*second* through *sixth bars*) in COS-1 cells. Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The induction shown is the average over a number of experiments (*n*), and the *error bars* reflect the standard deviation of each mean value. The cells were transfected by using FuGENE 6 transfection reagent and a total of 1 μg of DNA/3.5-cm dish. *Panel b*, detection of the different translation products using Western blot analysis. Whole cell extracts were prepared from COS-1 cells transiently transfected with TCF11 long form and the different TCF11 deletion constructs shown in Fig. 1. After SDS-PAGE and transfer to a nitrocellulose membrane, immunodetection with a polyclonal antibody against the C terminus of TCF11 (12) revealed the different translation products as indicated (●). The *arrowheads* on the *left* (▶) indicate the position of size standards with the size of each shown in kDa. Extracts of cells transfected with pcDNA3 show background staining with this antibody. Expression from the transfected constructs is seen in addition to this background pattern. *Panel c*, *in vitro* dimerization between MBP-TCF11-A (*upper figure*) or MBP-MafG (*lower figure*) (12) and different *in vitro* translated mutant proteins of TCF11 were tested in an MBP pulldown assay (*seventh* through *tenth lanes*). TCF11 long form incubated with MBP alone is used as a control for binding specificity (*upper figure*, *fifth lane*). 10% of the input of ^{35}S-labeled proteins is shown in the *first* through the *fourth lanes* in the *upper figure* and the *first* through the *fifth lanes* in the *lower figure*.
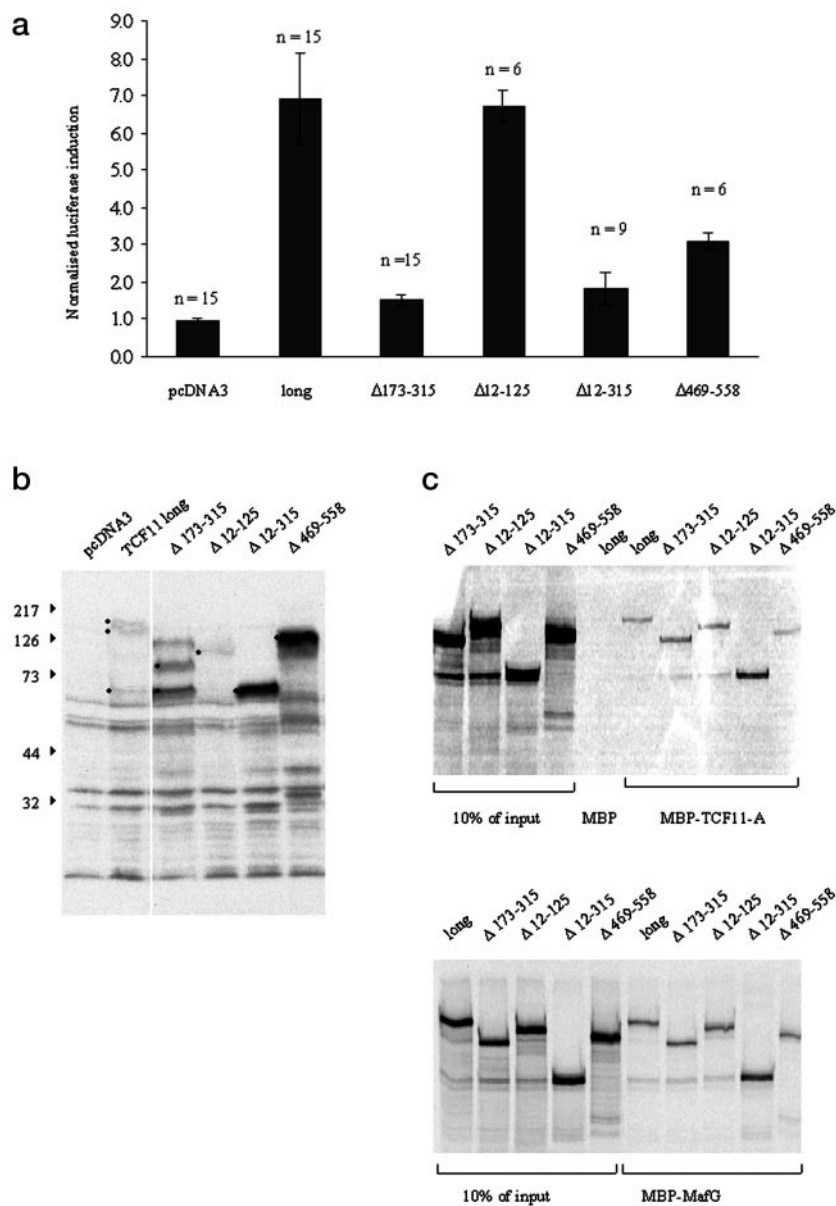
the two small exonic sequences (exons 3a and 4; 41 amino acids) in the acid-rich domain of the long variant of TCF11 did not show a detectable difference in the ability to activate the reporter under the conditions used (Fig. 2*a*, compare *second* and *third bars*).

To examine the expression of the different translation products the proteins were analyzed by Western blot (Fig. 2*b*). The full-length clone gave rise to three different equally expressed protein forms: two large proteins of ~160 and 140 kDa, and a shorter protein of 65 kDa, which is the expected size for the internally initiated translation product (Fig. 2*b*, *second lane*) (2). The alternatively spliced clone gave rise to two different translation products: a longer form of 140 kDa and the internally initiated product (Fig. 2*b*, *third lane*). The incomplete clone gave only the shorter protein form (Fig. 2*b*, *fourth lane*). The total amount of TCF11 protein detected was much higher when only the shorter protein form was produced.

To examine if the observed inability of TCF11 short form to activate the reporter in the transient transfection assay was caused by a difference in dimerization ability, an MBP pulldown assay was performed. The dimerization ability of different *in vitro* translated variants of TCF11 was tested (Fig. 2*c*). The long, long variant, and the short form all showed specific

dimerization with both the MBP-TCF11-A fusion protein (Fig. 2*c*, *sixth*, *eighth*, and *ninth lanes*) and MBP-MafG (data not shown) (12). No dimerization was observed in the control reaction between long TCF11 and MBP (Fig. 2*c*, *fifth lane*). This implies that dimerization ability is not altered in the various forms.

To characterize further the activity of the short form, a transient transfection assay with coexpression of TCF11 short form and MafG was performed (Fig. 3). This showed that TCF11 short form in the presence of MafG was still not able to transactivate expression under these conditions (Fig. 3, *sixth* and *seventh bars*), although an MBP pulldown assay showed that the two proteins could dimerize (data not shown). On the other hand MafG expressed alone repressed reporter expression (Fig. 3, *fifth bar*) whereas coexpression with TCF11 short form reduced the repression caused by MafG (Fig. 3, *seventh bar*). However, MafG coexpressed with p45 NF-E2 activated reporter expression (Fig. 3. *eighth bar*).

Because transactivation in our transient transfection assay in COS-1, HeLa, and HepG2 cells was achieved only under conditions where either of the longer proteins was produced, we wished to examine if the observed activation was dependent on the expression of a longer form alone or a combination of a
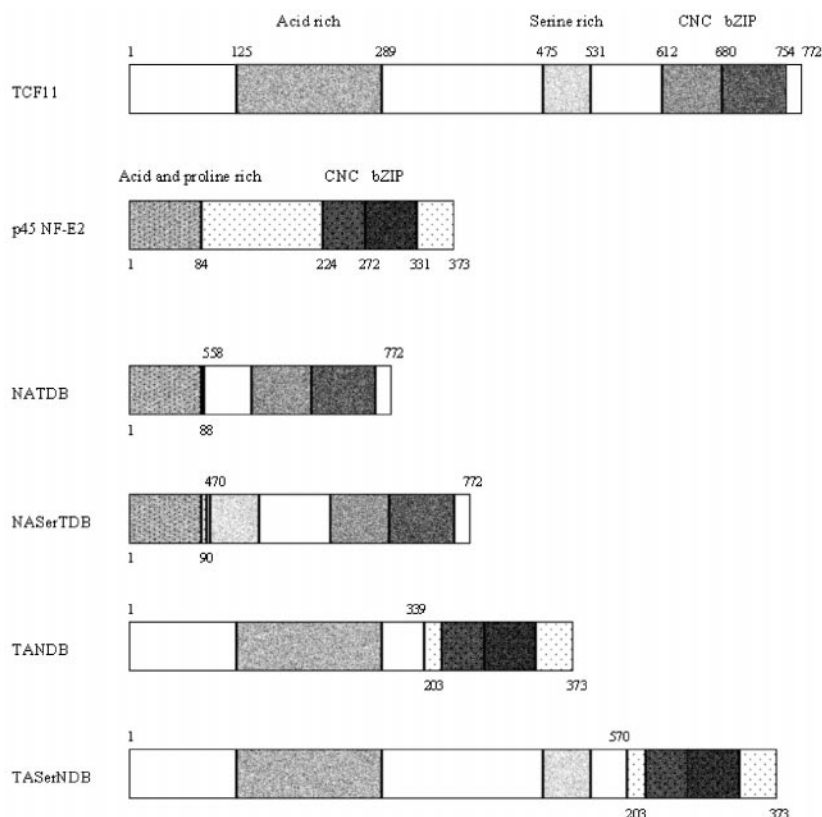
Fig. 6. **Schematic representation of the complete TCF11 long form, p45 NF-E2, and the different chimeric TCF11/NF-E2 proteins.** The different *shaded boxes* indicate the same domains as given in Fig. 1. In the chimeric constructs the *numbers* indicate the amino acid residues in the protein of origin. *Numbers* above the drawing indicate domains from TCF11, whereas *numbers* below the drawing indicate a domain from p45 NF-E2 (also *dot-filled*). The names of the different constructs are given as p45 NF-E2 or TCF11 (*N* or *T*) *A*ctivating domain fused with p45 NF-E2 or TCF11 (*N* or *T*) *D*NA *B*inding domain. When the serine-rich region is incorporated, Ser is added to the name of the chimeric protein.

longer and the shorter form. To address this we produced a mutated construct that produces only the longer protein form (Fig. 1, long mutated). In the mutated form four methionine residues (Met-318, Met-321, Met-323, and Met-326), which are possible internal translation initiation start sites, were changed into leucine residues, and thereby internal initiation was prevented. This was confirmed by *in vitro* expression (Fig. 2*c*, *second lane*) and Western blotting (Fig. 4*a*, *third lane*), and the protein product was shown to dimerize with both MBP-TCF11-A (Fig. 2*c*, *seventh lane*) and MBP-MafG (data not shown). Expression of the mutated form in the transient transfection assay showed an enhanced transactivation ability compared with TCF11 long form (Fig. 4*b*, compare *second* and *third bars*). This enhanced activity was observed when several different amounts of DNA were used in the transfections (Fig. 4*c*, compare *sixth*, *seventh*, and *eighth bars* with *third*, *fourth*, and *fifth bars*). When either the longer form or the longer mutated form was coexpressed with TCF11 short form the transactivation abilities were reduced (Fig. 4*c*, compare *ninth* through *twelfth bars* with *third* through *fifth bars*, and *thirteenth* through *sixteenth bars* with *sixth* through *eighth*). This implies that the presence of short form TCF11 can actually interfere with the activity of TCF11 long form.

To examine if the introduced leucine residues had any effect on the protein expression, extracts from transiently transfected cells were analyzed by Western blot. The mutated long form was expressed at the same level as TCF11 long form (Fig. 4*a*, compare *third* with *second lane*). Coexpression of the short form with either long or long mutated form had no effect on the level of protein expression (Fig. 4*a*, *fifth* and *sixth lanes*).

From these results we conclude that the region N-terminal to the internal methionine cluster is necessary for transactivation under these conditions. Within this stretch there is an acid-rich region that may function as a transactivation domain. However, omission of two small stretches within this region did not influence the transactivation. Interestingly, there is an addi-

tional acidic domain just N-terminal of the serine stretch (amino acids 428–474, Fig. 1) which is also present in the shorter protein produced by internal initiation. This is clearly not sufficient to induce reporter expression in our assay in COS-1, HeLa, or HepG2 cells although it may have an additional effect.

Domains of TCF11 Important for Transactivating Potential—Because the long variant of TCF11, lacking 41 amino acids in the N-terminal acidic domain, showed the same activity as the full-length protein in transient transfections, we constructed plasmids that express mutant proteins to determine more closely which regions of TCF11 are important for the transactivating ability. In three of these constructs, areas around the N-terminal acidic region were deleted (Fig. 1: Δ173–315, Δ12–125, Δ12–315), and one construct lacks a region spanning the serine-rich domain (Fig. 1: Δ469–558). When these constructs were used in transient transfections in COS-1 cells, significant differences in luciferase induction were observed. Although no reduction in the reporter activity was seen when amino acids 12–125 were deleted (Fig. 5*a*, *fourth bar*), the activity was abolished when part of (amino acids 173–315) or the complete (amino acids 12–315) N-terminal acidic domain was deleted (Fig. 5*a*, *third* and *fifth bars*). These results indicate that the acid-rich domain is necessary for the transactivation ability of TCF11 and that deletion of the last 143 amino acids in the region (amino acids 173–315) was enough to abolish most of the transactivating ability. On the other hand omission of exons 3a and 4 at the N terminus had no effect. In addition, the serine-rich domain, a stretch of 56 amino acids with more than 60% serine residues (35 serine residues out of 56 amino acids), was also shown to be essential for the full transactivating capacity of TCF11. Construct Δ469–558 showed a significant reduction in the induced luciferase level compared with wild type (Fig. 5*a*, *sixth bar*).

The same results were obtained when these four deletions were used in transient transfections of HeLa cells (data not shown).
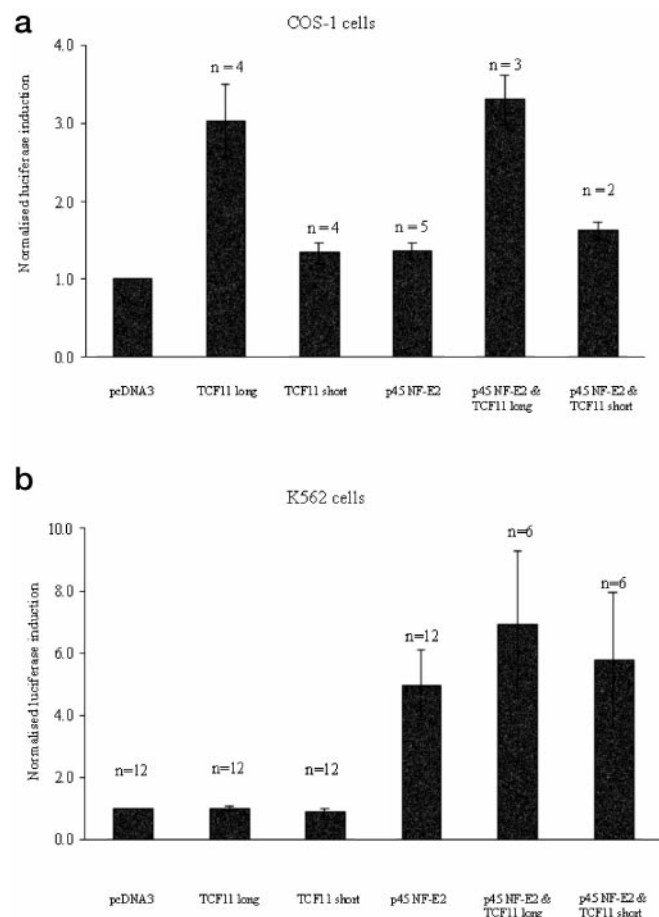
FIG. 7. *Panel a*, transient transfection of COS-1 cells showing the effect on luciferase induction when TCF11 is coexpressed with p45 NF-E2. The PBGD3.2Luc reporter was cotransfected with either empty pcDNA3 vector (*first bar*), TCF11 longer or shorter form alone (*second* and *third bars*, respectively), p45 NF-E2 alone (*fourth bar*), or TCF11 together with p45 NF-E2 (*fifth* and *sixth bars*). Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The induction shown is the average over a number of experiments (n), and the *error bars* reflect the standard deviation of each mean value. The cells were transfected by using FuGENE 6 transfection reagent and a total of 1.3 μg of DNA/3.5-cm dish. *Panel b*, transient transfection of K562 cells measuring the effect on luciferase induction when TCF11 is coexpressed with p45 NF-E2. The PBGD3.2Luc reporter and internal control (pRSVCAT) were co-transfected with either empty pcDNA3 vector (*first bar*), TCF11 long or short form alone (*second* and *third bars*, respectively), p45 NF-E2 alone (*fourth bar*), or TCF11 together with p45 NF-E2 (*fifth* and *sixth bars*). Luciferase activity was normalized to the CAT activity and is relative to the activity obtained with cotransfection of the empty vector. The induction shown is the average over a number of experiments (n), and the *error bars* reflect standard deviation of each mean value. The cells were transfected by using DEMRIE-C (Life Technologies, Inc.) transfection reagent and a total of 1.3 μg of DNA/2.0×10^6 cells.

To confirm that the reduced or absent activity was not caused by a lower level of TCF11 protein, extracts of cells transfected with the wild type or mutant constructs were analyzed by Western blot. All of the mutant proteins that were less active than the wild type in the transfection assay were present in approximately equal (Δ173–315) or higher amounts (Δ12–315 and Δ469–558) compared with the wild type (Fig. 5*b*, compare *third*, *fifth*, and *sixth lanes* with second lane). Interestingly, the only deletion construct that showed a reduced protein level was the N-terminal deletion Δ12–125 (Fig. 5*b*, compare *fourth lane* with the *second lane*) where the transactivating ability was the same as for the wild type (Fig. 4*a*, compare *fourth bar* with *second bar*).

In addition, an MBP pulldown assay was used to analyze the ability of the mutant proteins to dimerize with MBP-TCF11-A or MBP-MafG (Fig. 5*c*). All four mutant proteins showed the same dimerization abilities as TCF11 long form (Fig. 5*c*, compare *seventh* through *tenth lanes* with *sixth lane* in both the upper and the lower figure).

Taking these results together we conclude that the presence of both the serine-rich domain and the N-terminal acid-rich domain is necessary for TCF11 to maintain full transactivating capacity in COS-1, HeLa, and HepG2 cells.

Opposite Transactivation Ability of TCF11 and p45 NF-E2 in COS-1 and K562 Cells—Structural comparisons between TCF11 and p45 NF-E2 (Fig. 6) show a high degree of similarity in the C-terminal parts of the proteins. The DNA binding domain shows 85% (22 amino acids identical in a stretch of 26 amino acids (2)) identity in amino acid residues. Immediately N-terminal to this region, the CNC domain shows 67% (29 amino acids of 43 amino acids (11)) identity. The dimerization domain is less conserved: 39% (14 amino acids out of 36 amino acids (2)). It is therefore not surprising that both factors can bind to and transactivate through the same DNA sequence if they bind as homodimers or heterodimerize with the same partners (16, 18). In contrast to the widely expressed TCF11 (1, 2), p45 NF-E2 is specifically expressed in hematopoietic cells (3, 24). It was postulated that p45 NF-E2 could regulate the expression of globin genes during development together with MafK (25). However, more recently it was shown that p45 NF-E2-deficient mice only show a mild erythroid defect (26, 27), and it has therefore been speculated that other members of the CNC-bZIP family might compensate for the p45 NF-E2 deficiency (6). Two different reports show that a compound deficiency of Nrf2 and p45 NF-E2 in mice did not introduce any additional defects in globin gene expression compared with p45 NF-E2 deficiency alone (28, 29). We therefore wanted to compare directly the activity of TCF11 and p45 NF-E2 proteins through the NF-E2 site in COS-1 cells and in an erythroid cell line (K562).

TCF11 longer form induced luciferase activity in COS-1 cells (Fig. 2*a*, *second bar*, and Fig. 7*a*, *second bar*) as well as in several other cell lines (16). In contrast, p45 NF-E2 did not induce the reporter through the NF-E2 site when transiently transfected into COS-1 cells (Fig. 7*a*, *fourth bar*), although analyses of cell extracts by Western blot showed the presence of p45 NF-E2 protein (see Fig. 9*a*, *fourth lane*). The lack of reporter induction in COS-1 cells was consistent with earlier reports that in non-hematopoietic cells, p45 NF-E2 has to be coexpressed with one of the small Maf proteins to achieve detectable transactivation in a transient transfection assay (Fig. 3) (13, 18), indicating that p45 NF-E2 lacks an activation partner in COS-1 cells. When both TCF11 and p45 NF-E2 were coexpressed in COS-1 cells, the level of luciferase induction did not change compared with that of TCF11 alone (Fig. 7*a*, compare *fifth* and *sixth bars* with *second* and *third bars*, respectively). Analysis of cell extracts showed that both TCF11 and p45 NF-E2 were expressed (see Fig. 9*a*, *fifth* and *sixth lanes*). This implies that p45 NF-E2 in this context does not compete directly with TCF11 for binding to the NF-E2 site in the reporter construct in COS-1 cells, and p45 NF-E2 does not appear to interfere with the transactivation activity of TCF11.

In the erythroid K562 cell line the ability of TCF11 and p45 NF-E2 to induce a reporter through a single NF-E2 site was reversed compared with the results from COS-1 cells. K562 cells transiently transfected with p45 NF-E2 expression vector showed significant luciferase induction above background level (Fig. 7*b*, *fourth bar*). In these cells the dimerization partner MafK is known to be present (13). In contrast, neither TCF11 long nor short form showed any ability to influence the produc-

FIG. 8. *Panel a*, transient transfection of COS-1 cells measuring the effect on luciferase induction of the different TCF11/p45 NF-E2 chimeric proteins (Fig. 6). The PBGD3.2Luc reporter was co-transfected with either empty pcDNA3 vector (*first bar*), TCF11 long form (*second bar*), or one of the chimeric constructs (*third* through *sixth bars*). Luciferase activity was normalized to the total protein content and is relative to the activity given with the empty vector. The cells were transfected by using FuGENE 6 transfection reagent and a total of 1 μg of DNA/3.5-cm dish. The induction shown is the average of six experiments, and the *error bars* reflect the standard deviation of each mean value. *Panel b*, transient transfection of K562 cells measuring the effect on luciferase induction with the different TCF11/p45 NF-E2 chimeric proteins (Fig. 6). The PBGD3.2Luc reporter and internal control (pRSVCAT) were co-transfected with either empty pcDNA3 (*first bar*), p45 NF-E2 (*second bar*), or one of the chimeric constructs (*third* through *sixth bars*). Luciferase activity was normalized to the CAT activity and is relative to the activity obtained with cotransfection of the empty vector. The cells were transfected by using DEMRIE-C transfection reagent and a total of 1 μg of DNA/2.0×10^6 cells. The induction shown is the average of six experiments, and the *error bars* reflect the standard deviation of each mean value.
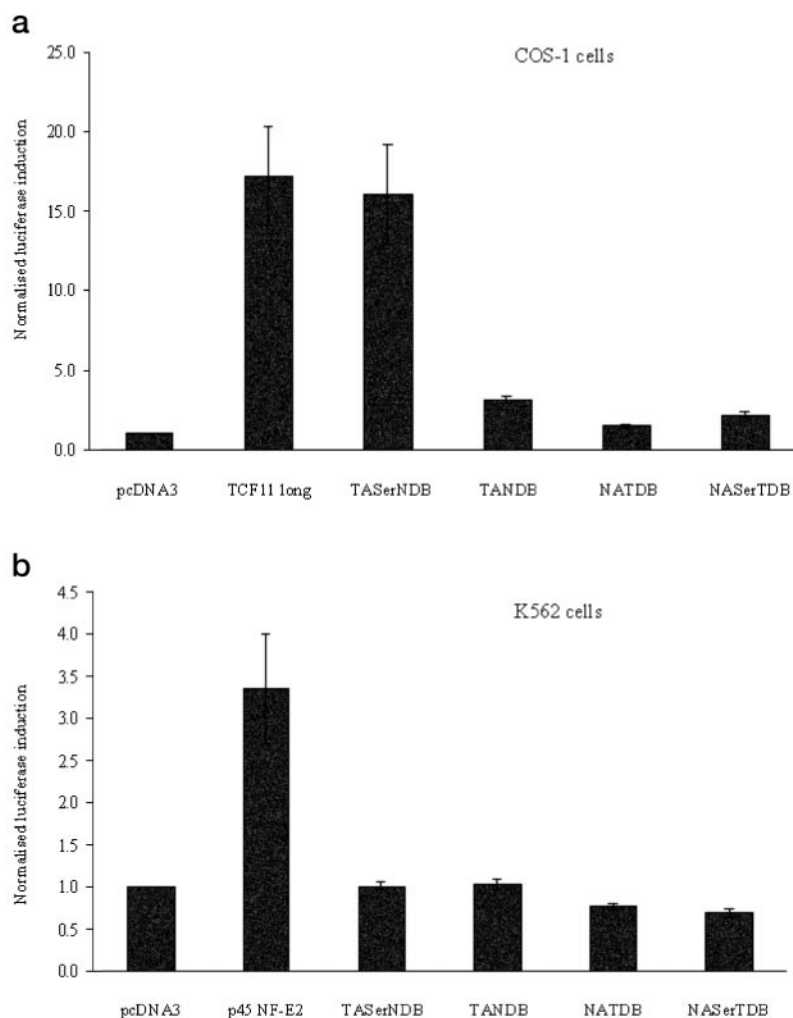
tion of luciferase reporter through the single NF-E2 site (Fig. 7b, *second* and *third bars*) despite previous findings that MafK may dimerize with TCF11 (13). This is in contrast to the results reported by Caterina *et al.* (11), where a fusion of the short form of TCF11 with the Gal4 DNA binding domain had transactivating ability in K562 cells, using a reporter construct with two Gal4 binding sites. Surprisingly, when both TCF11 and p45 NF-E2 are coexpressed, the level of luciferase induction did not change compared with expression of p45 NF-E2 alone (Fig. 7b, compare *fifth* and *sixth bars* with *fourth bar*). This implies that the two transcription factors do not compete for binding to the NF-E2 site in the reporter construct in K562 cells and that TCF11 does not appear to interfere with the activity of p45 NF-E2.

Transactivation Abilities of Fusion Products from TCF11 and p45 NF-E2 in COS-1 and K562 Cells—To investigate further the differences in activities of TCF11 and p45 NF-E2 in the two different cell types, fusion constructs between the transactivation domain of TCF11 and the DNA binding domain of p45 NF-E2 and vice versa were prepared and tested in COS-1 and K562 cells. Because there is no region in p45 NF-E2 similar to the serine-rich domain of TCF11, fusions were made both with and without this domain to investigate its possible role in the activity differences (Fig. 6).

In COS-1 cells only the fusion between the transactivating domain of TCF11, including the serine-rich stretch, and the DNA binding domain of p45 NF-E2 (TASerNDB) was able to induce the reporter to the same level as TCF11 long form (Fig. 8a, *third bar*). These results showed that the DNA binding and

zipper domain of TCF11 could be replaced by the corresponding part of p45 NF-E2. However, a similar construct without the serine-rich stretch (TANDB) did not induce the reporter to the same extent (Fig. 8a, *fourth bar*). To test if this difference in transactivation ability was caused by changes in their dimerization potential we compared their ability to associate with TCF11. We showed that TANDB, which lacks the serine-rich domain, had a reduced ability to dimerize with MBP-TCF11-A compared with TASerNDB (Fig. 9c, compare *eighth lane* with *seventh lane*). However, even the latter chimeric protein showed reduced dimerization compared with TCF11 long form (Fig. 9c, compare *seventh lane* with *sixth lane*). On the other hand, both of these chimeric proteins showed the same dimerization ability with MBP-MafG as TCF11 long form (Fig. 9d, compare *seventh* and *eighth lanes* with *sixth lane*).

The reciprocal constructs, with the transactivating domain of p45 NF-E2 and the DNA binding and dimerization domains of TCF11, showed that the putative transactivating domain of p45 NF-E2 (30, 31) was unable to induce the reporter. No change was seen when the serine-rich domain from TCF11 was included (Fig. 8a, *fifth* and *sixth bars*). These two fusion proteins showed no change in the dimerization ability compared with TCF11 long form (Fig. 9, c and d, compare *ninth* and *tenth lanes* with *sixth lane*). Analysis of cell extracts showed that all of the chimeric proteins were expressed (Fig. 9b, *fourth* through *seventh lanes*). In K562 cells only p45 NF-E2 was active, and none of the fusion constructs was able to reproduce this transactivation pattern (Fig. 8b, compare *third* through *sixth bars* with *second bar*).
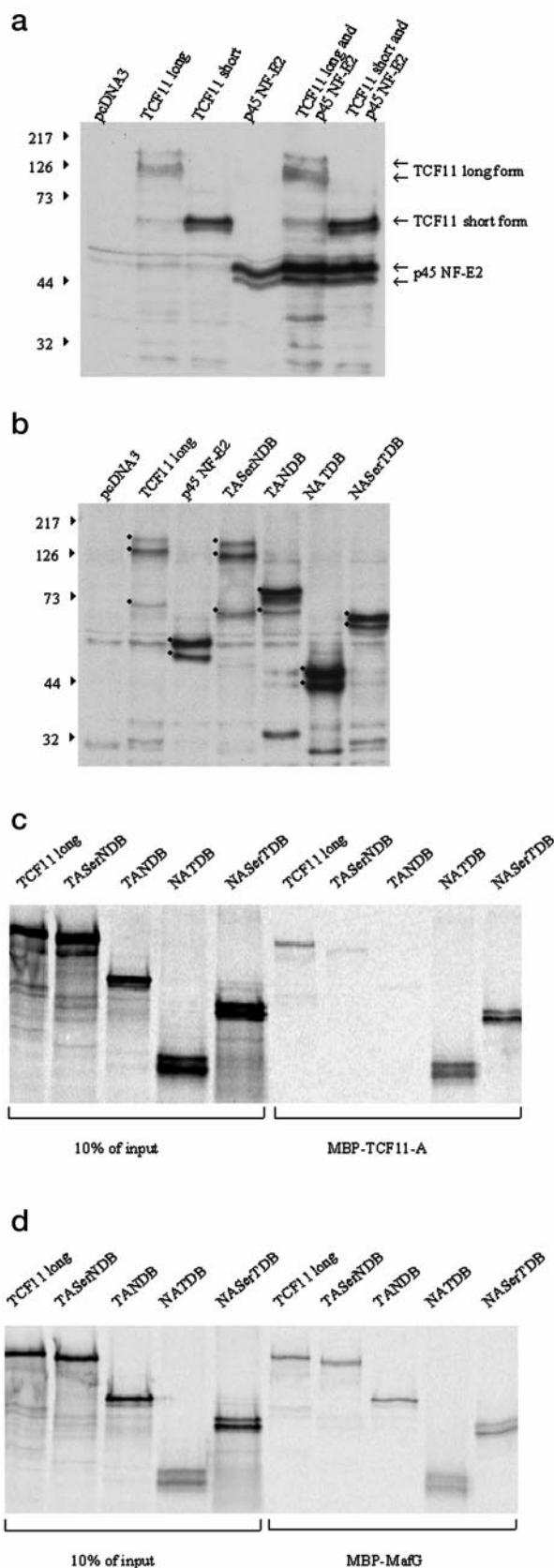
FIG. 9. *Panel a*, detection of the different translation products following single and cotransfection with TCF11 and p45 NF-E2 expression vectors using Western blot analysis. Whole cell extracts were prepared from COS-1 cells transiently transfected with TCF11 long and short form and p45 NF-E2, each alone or together. After SDS-PAGE and transfer to a nitrocellulose membrane, immunodetection with a polyclonal antibody against the C terminus of TCF11 (12) was performed.

Thus the opposite activities of TCF11 and p45 NF-E2 in different cell types cannot be entirely determined by the transactivation domain or the DNA binding and dimerization domains alone but require the correct combination of the bZIP and the transactivation domains.

DISCUSSION

TCF11 is a bZIP transcription factor of the CNC subfamily, as is p45 NF-E2. Whereas p45 NF-E2 has an expression pattern restricted to hematopoietic cells (3, 24), TCF11 is widely expressed (1). Both proteins can bind to and transactivate through the same DNA sequence and form heterodimers with the small Maf proteins like MafK and MafG (12, 13, 16, 18). We show here that despite their similarity, these proteins have different cell type-specific activities that are not determined by specific transactivation domains alone but by the combination of transactivation domain and bZIP domain. This indicates cooperation or interplay between the domains and interaction with cell-specific environmental factors.

Tcf11 transcripts are translated as two major products, one from the initial ATG and another from an internal ATG cluster. The existence of a poor Kozak sequence around the initial methionine may be important to allow initiation *in vivo* to occur also from the internal methionine residues, leading to production of both the long and short protein forms. We have demonstrated that both protein forms are present in extracts from transiently transfected cells (Figs. 2*b* and 4*a*), and we confirm that mutating all four potential internal start codons abolishes production of the smaller protein. Transfection with the 5'-incomplete clone that can only produce the shorter form showed a clearly higher protein expression. This might be caused by differences in the stability of the transcripts or the proteins and/or less efficient translation initiation from the initial ATG. Interestingly, the protein amount produced from the initial ATG did not change appreciably when the internal ATGs were mutated, indicating that there is not a competitive relationship between initial and internal initiation.

No transactivation activity was observed when only the shorter version of TCF11 was produced, whereas protein products from the originally isolated cDNA clones (1) (including the longer forms) caused transactivation in COS-1, HeLa, and HepG2 cell lines. An interesting question arising from this observation is why are two isoforms with the same dimerization and DNA binding domains but with different activities produced? We suggest that the non-optimal translation initiation sequence around the first methionine leading to the pos-

This antibody recognizes both TCF11 and p45 NF-E2 proteins, which have similar C termini. The different translation products are indicated by an *arrow* (←). The *arrowheads* on the *left* (▶) indicate the position of size standards with the size of each shown in kDa. *Panel b*, detection of the different chimeric translation products after transfection using Western blot analysis. Whole cell extracts were prepared from COS-1 cells transiently transfected with the different constructs. After SDS-PAGE and transfer to a nitrocellulose membrane, immunodetection with a polyclonal antibody against the C terminus of TCF11 (12) revealed the different translation products as indicated (●). The *arrowheads* on the *left* (▶) indicate the positions of size standards with the size of each shown in kDa. *Panel c*, *in vitro* dimerization between MBP-TCF11-A (12) and the different *in vitro* translated chimeric (TCF11/p45 NF-E2) proteins were tested in the MBP pulldown assay (*seventh* through *tenth lanes*). Dimerization between MBP-TCF11-A and TCF11 long form is used as a positive control of the assay (*sixth lane*). 10% of the input of ^{35}S-labeled proteins is shown in the *first* through *fifth lanes*. *Panel d*, *in vitro* dimerization between MBP-MafG (12) and the different *in vitro* translated chimeric (TCF11/p45 NF-E2) proteins was tested in the MBP pulldown assay (*seventh* through *tenth lanes*). Dimerization between MBP-MafG and TCF11 long form is used as a positive control of the assay (*sixth lane*). 10% of the input of ^{35}S-labeled proteins are shown in the *first five lanes*.

sibility of internal initiation is likely to be an important regulatory mechanism for keeping the positive transactivating form of TCF11 at a desired, limited level. In addition, the shorter protein may influence, directly or indirectly, the functioning of the more active longer protein. This hypothesis was supported with the observation that a mutated construct incapable of internal translation initiation showed enhanced reporter induction in transient transfections. In addition, this observed elevated induction was reduced to approximately the same level as wild type when the mutated construct was cotransfected with an incomplete clone that only produces the short form (Fig. 4*c*). The use of alternative translation initiation to generate protein isoforms with distinct transcriptional properties has been reported previously for other genes, like the Wilms' tumor (32) gene and Egr3 (33).

Only expression of the long TCF11 protein provides the combination of the two domains which we demonstrate to be essential for full transactivation: the N-terminal, acidic domain, and the serine-rich stretch (Figs. 2*a* and 5*a*). The ability of an acid-rich region to act as a transactivation signal has been reported for several transcription factors (for review, see Ref. 34). The serine-rich region has several potential phosphorylation sites, which may be important for TCF11 to retain full activity in the cell systems analyzed. This region is present both in the long and short forms and is therefore necessary but not sufficient to effect transactivation in COS-1, HeLa, and HepG2 cells. In K562 cells however, even the full-length protein with both domains does not transactivate, whereas p45 NF-E2 shows an opposite pattern, being active in K562 cells and not in COS-1 and HeLa cells. Coexpression of TCF11 and p45 NF-E2 did not essentially change the transactivation pattern, indicating that TCF11 and p45 NF-E2 factors did not compete directly with each other for partners and/or DNA in our assay (Fig. 7, *a* and *b*).

The domain swapping experiments involving the transactivation and bZIP domains of p45 NF-E2 and TCF11 shed some more light on the cell specific activities of the two proteins. The fusion joining the transactivation domains of TCF11 and the bZIP domain of p45 NF-E2 (TASerNDB) was active in COS-1 cells. However, the similar chimeric protein without the serine-rich domain (TANDB) was not active. Our deletion analysis similarly showed that the serine-rich region is necessary for full transactivation by TCF11, but the domain swapping experiment also shows that the TANDB chimera dimerizes less efficiently with MBP-TCF11. The results indicate that the serine stretch, in addition to being an activation domain, is important for dimerization. It might be that the serine-rich domain alters the dimerization characteristics of the chimeric protein, and it might promote homodimerization, which was shown previously not to be favored for p45 NF-E2 in its natural context (13). This experiment demonstrates that in this fusion context, the bZIP domain of p45 NF-E2 can dimerize and bind DNA in COS-1 cells. On the other hand, the N-terminal part of p45 NF-E2 was inactive when fused to the bZIP of TCF11, with or without the serine-rich domain, although both proteins showed dimerization with MBP-TCF11-A. The results of these experiments indicate that the inactivity of NASerTDB and NATDB in COS-1 cells is related to an inability to transactivate rather than an inability to dimerize and bind to DNA. Surprisingly, the N-terminal 83 amino acids of p45 NF-E2 in this context cannot reiterate the activity of the heterodimer p45NF-E2/MafG in cotransfections (Fig. 3) (13).

In K562 cells none of the fusion proteins was able to transactivate the reporter (Fig. 8*b*). The N-terminal part of TCF11 most likely either lacked interacting cofactor(s) or was not modified in a way necessary for transactivation, for example by phosphorylation. TCF11 may be phosphorylated both by casein kinase II and mitogen-activated protein kinase Erk2 (10). We have found that mutating a casein kinase II site in TCF11 increases the protein's ability to induce the luciferase reporter (data not shown), confirming the complex regulation of this transcription factor. However, more surprising was the lack of transactivation when the N-terminal 83 amino acids of p45 NF-E2 were fused to TCF11 CNC-bZIP because the p45 NF-E2 domain has been shown previously to transactivate in K562 cells when coupled to the GAL4 DNA binding domain (30). However, it has been shown that removal of amino acids 176–209 in p45 NF-E2 reduced the protein's activity in the MEL cell line CB3 (19). It is possible that this domain is also required to obtain a transcriptionally active chimeric protein together with the DNA binding and dimerization domains of TCF11. Alternatively, the inactivity of this fusion might indicate that p45 NF-E2 is dependent on strong DNA binding to activate transcription and that sufficiently strong binding is not achieved. The strength of binding may become critical in the presence of more strongly binding complexes, for example native NF-E2, which is present in K562 cells and is known to bind DNA very efficiently (35). Extending this idea, it is possible that the TCF11 CNC-bZIP domain is unable to bind DNA in K562 cells. This could be caused by the lack of a specific dimerization partner or by dimerization with a partner which results in a complex with low binding affinity for the NF-E2 site within the reporter plasmid or by incorrect modification of TCF11 which might prevent binding to DNA.

From this we conclude that TCF11 and p45 NF-E2 need interplay between their respective transactivation and DNA binding/dimerization domains in addition to interaction with cell-specific partners and/or cofactors to gain full activity as a transcription factor.

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