Retinoic Acid Receptors Interact Physically and Functionally with the T:G Mismatch-specific Thymine-DNA Glycosylase*

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The pleiotropic effects of retinoids are mediated by nuclear receptors that are activated by 9-cis- or all-trans-retinoic acid to function as ligand-dependent transcription factors. In a yeast one-hybrid screen for proteins capable of interacting with native retinoid acid receptor (RAR), we have isolated the T:G mismatch-specific thymine-DNA glycosylase (TDG), which initiates the repair of T:G mismatches caused by spontaneous deamination of methylated cytosines. Here, we report that TDG can interact with RAR and the retinoid X receptor (RXR) in a ligand-independent manner, both in yeast and in vitro. Mapping of the binding sites revealed interaction with a region of the ligand binding domain harboring α-helix 1 in both RAR and RXR. In transient transfection experiments, TDG potentiated transactivation by RXR from a direct repeat element spaced by one nucleotide (DR1) and by RXR/RAR heterodimers from a direct repeat element spaced by five nucleotides (DR5). In vitro, TDG enhanced RXR and RXR/RAR binding to their response elements. These data indicate that TDG is not only a repair enzyme, but could also function in the control of transcription.

Retinoic acids exert their pleiotropic effects on vertebrate development and homeostasis by binding to nuclear receptors that function as ligand-dependent transcription factors regulating the expression of target genes. These receptors belong to a gene superfamily including the receptors for steroid hormones, thyroid hormone, vitamin D3, and a growing number of other ligands that function as ligand-dependent transcription factors. In a yeast one-hybrid screen for putative cofactors, we report here the isolation of a new splice variant of the T:G mismatch-specific thymine-DNA glycosylase, which is most likely encoded by the TDG gene. This protein is capable of interacting with native retinoic acid receptors (RARs) and the retinoid X receptor (RXR). The interaction of TDG with RAR and RXR is ligand independent, as shown by transcriptional assays using yeast and mammalian cells. The interaction of TDG with RAR and RXR is specific, as other nuclear receptors do not bind to TDG. The interaction of TDG with RAR and RXR is independent of the ligand binding domain (LBD), which functions as a ligand-dependent activation domain (AF-2). These data indicate that TDG is not only a repair enzyme, but could also function in the control of transcription.

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1 The abbreviations used are: RAR, retinoic acid receptor; RXR, retinoid X receptor; TDG, thymine-DNA glycosylase; t-RA, all-trans-retinoic acid; 9c-RA, 9-cis-retinoic acid; AAD, acidic activation domain; LBD, ligand binding domain; AP, abasic site; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair(s); tk, thymidine kinase; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay; CIP, calf thymus intestinal phosphatase.
have been implicated in various processes such as control of gene expression (26), chromatin structure (27), somatic X-chromosomal inactivation in females (28), timing of DNA replication (29), and genomic imprinting (30). Methylation of cytosines is essential in vertebrates (31). However, it also causes genome instability; although not more than 1% of bases are methylated cytosine in mammalian genomes, they probably cause one third of all transition mutations responsible for genetic diseases and cancer in humans (32–34). This is due to the much higher spontaneous deamination of 5-methylcytosine when compared with cytosine; deamination of 5-methylcytosine leads to T:G mismatches, whereas deamination of cytosine generates U:G mismatches in the DNA. The repair of U:G and T:G DNA mismatches is initiated by mismatch-specific DNA glycosylases (35), which excise in a first and rate-limiting step the mismatched base leaving an abasic site (AP) that is cleaved by an AP-endonuclease and deoxyphosphodiesterase to create a single nucleotide gap; this gap is then filled in by DNA polymerase-β and finally sealed by DNA ligase (36). TDG was first characterized as a 55 kDa protein purified from HeLa cells (37). Further analysis revealed that TDG was not only able to excise thymine from T:G, but also uracil from U:G DNA mismatches (38). The cloning of the human TDG cDNA showed that this glycosylase belongs to a new class of excision repair enzymes with no significant sequence similarity to the established DNA glycosylase gene family including the U-G mismatch-specific uracil-DNA glycosylase (UNG) (39). However, the gene for human TDG contains conserved regions with greater than 30% sequence identity with an E. coli gene (MUG) which was found later to encode an U-G mismatch-specific DNA glycosylase that, in contrast to the human TDG, cannot cut at T:G mismatches under physiological conditions (40). The recently published crystal structure of the E. coli MUG, nevertheless, revealed structural and functional homologies with UNG despite of their low sequence similarity (41). Interestingly, TDG was initially isolated and characterized in a yeast two-hybrid screening as a protein that can interact with the transcription factor c-Jun (42). This interaction, together with those with RAR and RXR described here points to a possible function of TDG in transcription and DNA repair.

**EXPERIMENTAL PROCEDURES**

Cloning of mTDG Isoforms—The Saccharomyces cerevisiae reporter strain BP-G5 (MATa ura3-D1 his3-d200 leu2-D1 tryp1::DR5 RARE-URA3) was derived from the strain PL1 (MATa ura3-D1 his3-d200 leu2-D1 tryp1::ERE-URA3) (43) by substituting the ERE by one copy of a DR5 RARE (AGG TGACggagAGG TCA). The one-hybrid screening was performed as described in Ref. 10 using S. cerevisiae strain BP-G5 expressing full-length hRARα from YEp90, and a VP16-tagged mouse embryo cDNA library (10). Library plasmids from positive clones were isolated and sequenced leading to the isolation of a TDG cDNA encoding the TDG isoform. A cDNA fragment (286–2567 bp) was used as a probe to screen a mouse embryo cDNA library (10 days post coitum) for additional TDG cDNAs. A full-length mTDGα cDNA was cloned by PCR using a 5'-primer encoding the missing 8 amino acids (ATG GAC GCG CCA GCA AGC) and the mTDGα cDNA as a template.

Plasmid Constructs—For the expression of full-length or various truncated mTDGα, the pSG5 vector (44) was used for mammalian cells, the pET15b vector (Novagen) for His-tagged proteins in Esherichia coli, and the pPER vector2 for expression from Vaccinia virus. Reporter gene constructs and expression vectors for RAR and RXR were described as described (47–49); Gal4-c-jun fusion proteins were expressed from a pG4polyII vector, and other plasmid cDNAs used as described (46). Further details on all constructs used in this study are available on request.

Northern Blots and RT-PCR—Northern blot analyses were performed using a mouse multi-tissue mRNA blot (CLONTECH) and a 32P-labeled fragment of the mTDGα cDNA (286–2567 bp) as described by the manufacturer. RNA preparation form P19 cells and RT-PCRs were performed using standard protocols using 50 μg of protein from whole cell extracts separated on 10% SDS gels. For dephosphorylation experiments, 25 μg of a whole cell extract from MCF7 cells were incubated for 30 min at 37 °C with 20 units of calf thymus intestinal phosphatase (CIP) in a buffer provided by the manufacturer (Boehringer Mannheim). To avoid nonspecific degradation, leupeptin, pepstatin, aprotinin, antipain, and chymostatin were added to the reactions at a final concentration of 2.5 μg/ml, and vanadate was used at a concentration of 50 μM.

Two-hybrid System and Transient Transfections—For the mapping of the interaction domains in yeast, the LexA system was applied using the vectors pBTM116 (49) for the cloning of the LexA fusions and pASV3 (50) for the cloning of the VP16 fusions. β-Galactosidase assays on individual L40 transformants were carried out as described (46). The expression of all LexA and VP16 fusion proteins was checked by Western blotting using anti-LexA or anti-VP16-specific antibodies (data not shown). Transient transfections in COS cells as well as CAT and β-galactosidase assays were performed as described in Ref. 51. All transfections employed duplicate samples, and were repeated at least twice. Representative experiments are shown in the figures.

**RESULTS**

Isolation and Characterization of a New Splice Variant of TDG—Previous studies in yeast have shown that RAR cannot efficiently induce transcription from a DR5 element without heterodimerization with RXR (54–56). In a search for additional partners allowing RAR to transactivate from such an element, the yeast strain BP-G5 harboring one DR5 element within the URA3 promoter was designed for a functional one-hybrid screening with full-length RARα as a bait and a mouse embryo cDNA library fused to the acidic activation domain of the viral VP16 protein. Positive clones from this screening were expected not only to interact with RAR, but also to stabilize its binding to the DR5 element, thus allowing efficient RAR-mediated activation of the selection marker. Out of 30 positive clones obtained from the screening, 25 were identified as RXXs. From the five remaining clones, three encoded for another nuclear receptor, COUP-TFI (57), and two for TDG, which was initially cloned as a c-Jun-interacting protein (42). The 2859-bp TDG cDNA clone obtained from the yeast screening (denoted b isoform, Fig. 1, A and B) differs between positions 1 and 58 from the previously published sequences of TDG, here called TDG isoform (a) (39). Due to these differences, the mouse TDGα cDNA lacks the codon for the starting methionine of the TDG isoform 2 and encodes for a truncated form of the enzyme, missing the first 25 amino acids (Fig. 1B). Inasmuch as, in the TDGα cDNA, methyl-CpG dinucleotide was found in front of the first methionine (Fig. 1A), a mouse embryo (10 days post coitum) cDNA library was screened for longer mTDG cDNAs. However, this screening did not result in the cloning of further 5'-extended TDGα cDNA.

The expression of the mouse TDGα and TDGβ mRNAs was confirmed by RT-PCR analysis performed with isoform-specific primers encoding the missing 8 amino acids (ATG GAC GCG CCA GCA AGC) and the mTDGα cDNA as a template.
A monoclonal anti-TDG antibody was used to analyze TDG protein levels in various monkey, mouse, and human cell lines (Fig. 2B). TDG was detected in all tested cell lines with the lowest amount in F9 teratocarcinoma cells. In all extracts, two protein species of ~63 kDa were revealed which comigrated with E. coli-expressed mTDG(32–421), thus suggesting that the two species could represent TDGα and TDGβ isoforms (see below). The mouse TDG was slightly larger than monkey and human TDG. In addition to the ~63-kDa species, the antibody revealed in some extracts two slower migrating forms of the protein with an apparent molecular mass of ~90 kDa. The relative level of these species varied in different extract preparations from the same cell line, suggesting that they may represented modified forms of the enzyme. As TDG contains a number of putative kinase sites, MCF7 cell extracts were treated with CIP in the presence and absence of vanadate as a phosphatase inhibitor. In the absence of the phosphatase inhibitor, CIP converted the majority of the upper species into forms migrating with an apparent molecular mass of ~63 kDa (Fig. 2C).

To further characterize the two TDG species of ~63 kDa, the mouse TDGα cDNA was cloned by PCR and expressed in transfected COS cells along with mTDGβ cDNA. Extracts from transfected COS cells were analyzed by Western blotting together with an extract from P19 cells. Overexpressed mTDGα comigrated in this experiment with the upper species from P19 cells whereas mTDGβ migrated at the level of the lower species (Fig. 2D). Similar comigrations were also observed for the larger protein species recognized by the TDG antibody. Since P19 cells were shown to contain transcripts for the TDGα and TDGβ isoforms (see above), it is likely that the two TDG protein species found in extracts of various cells correspond to TDG isoforms a and b. In all cell lines and tissues analyzed by Western blotting, these two TDG isoforms were found in similar amounts (Fig. 2B and data not shown).

**TDG Interacts with RAR and RXR in Yeast and in Vitro—** Full-length mTDGα was fused to the VP16 acidic activation domain (denoted AAD) and assayed for interaction with full-length RARα and RXRα fused to LexA in the yeast strain L40 which contains a lacZ reporter gene driven by eight LexA binding sites (49). Interestingly, while a very weak (~5-fold), but reproducible, increase in β-galactosidase activity was obtained by co-expressing AAD-TDGα with unfused LexA, 33% and 75-fold enhancements were observed by co-expressing AAD-TDGα with LexA-RXRA and LexA-RXRB, respectively, in the absence of ligand (Fig. 3A). Addition of 9c-RA to yeast expressing LexA-RXRA and AAD-TDGα or t-RXα to yeast expressing LexA-RXRB and AAD-TDGα diminished by a factor of ~2 and 3, respectively, the -fold enhancement mediated by AAD-TDGα over the AAD control (Fig. 3A), indicating that TDGα may interact preferentially with the unliganded forms of RXRα and RXRβ in yeast. As a representative of the other conserved gene family of DNA glycosylases (see Introduction), the human uracil-specific UG mismatch-specific DNA glycosylase (hUNG1) (58, 59) was tested for its ability to interact with RXRα and RXRβ in yeast. Similarly to AAD-TDGα, co-expression of AAD-UNG1 with unfused LexA resulted in a weak activation of the reporter gene (Fig. 3A). However, no further stimulation was observed by co-expressing AAD-UNG1 with LexA-RXRB or LexA-RXRA in either the presence or absence of ligand (Fig. 3A), indicating that, in contrast to TDGα, UNG1 does not interact with RXRα and RXRβ.

TDG was then tested for a direct binding to RAR and RXR in vitro. GST pull-down assays using Vaccinia virus-expressed TDGα(32–421) (Fig. 3B) or in vitro translated full-length TDGα (data not shown) and E. coli-expressed GST-RXRA or RXRB fusion proteins revealed a ligand-independent interaction between TDG and the receptors.

To map the interaction domains of the nuclear receptors and TDG, a deletion analysis of each protein was performed using the yeast two-hybrid system. Deletion derivatives of RXRα were expressed as fusion proteins with LexA and tested for interaction with AAD-TDGα(32–421) (Fig. 4A). Similarly to AAD-TDGα, AAD-TDGβ(32–421) interacted with RXRα in both the presence and absence of 9c-RA (Fig. 3A and 4A). A region between residues 227 and 263 harboring the α-helix 1 (H1) of the LBD of RXRα was found to be sufficient for mediating an interaction with TDG (Fig. 4A, see LexA-RXRA[227–263] and AAD-TDGβ(32–421)). Interaction with the whole LBD of RXRα (amino acids 206–467) exhibited a 3-fold increase in the presence of 9c-RA (Fig. 4A). In view of this ligand-dependent enhancement, the effects of point mutations in the core motif of the AF-2 activation domain (AF-2 AD/helix 12 of the LBD) were studied. Out of the three mutations tested (FL to AA for aa 455 and 456, ML to AA for aa 459 and 460, and E to Q for aa 461), only amino acid changes in positions 455 and 456 reduced significantly the interaction with TDG (Fig. 4A). This indicates that not all of the conserved residues of the AF-2 AD core, which are important for transactivation by the ligand-dependent AF-2 (11), are required for the binding of TDG to the LBD of RXRα.

Similarly, deletion derivatives of RXRβ were examined to localize the region(s) required for interaction with TDG in yeast. No significant increase in reporter activity was observed with the N-terminal A/B region of RXRβ (Fig. 4B; LexA- RARβ[1–88]). In contrast, a ~10-fold activation was detected in the presence of the LexA-RARβ[80–211] fusion protein containing the DNA-binding domain (region C) and D region of the receptor (Fig. 4, B and C). Co-expression of a LexA fusion containing the C region only (LexA-RARβ[80–155]) with AAD-TDGβ(32–421) resulted in a modest (~10-fold) activation of the reporter gene (Fig. 4B), whereas a C-terminal fragment of the D region encompassing the α-helix 1 of the LBD (amino acids 170–211) interacted strongly with TDG (Fig. 4B). Thus, as observed with RXRα, the helix 1 of the LBD of RXRβ is sufficient for mediating an interaction with TDG. However, in contrast to the RXRα LBD, the whole LBD of RXRβ (amino acids 154–462) showed no ligand-dependent interaction with TDG (Fig. 4B).

To map the receptor interacting domain in TDG, various deletion mutants of TDG were fused to the VP16 AAD and assayed for interaction with LexA-RXRA[DE] in yeast. These experiments revealed a central region of TDG between amino acids 122 and 346 as sufficient for the interaction with RXRα (Fig. 4C) and RXRβ (data not shown). Further deletions in that region abolished interactions with the receptors.

Since TDG was initially cloned as a c-Jun interacting protein...

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**Fig. 1. Cloning of mouse TDG isoforms.** A, nucleotide and amino acid sequences of the 2859-bp mTDGb cDNA clone. The first ATG codon is underlined. The deduced amino acid sequence in front of the first methionine is shown in lowercase. B, alignment of mouse TDGα (mTDGα; (39)), mouse TDGb (mTDGβ; this study), and human TDGα (hTDGα; (39)). Dots represents gaps used to align the sequences.
we used the yeast two-hybrid assay to investigate the interaction of the C-terminal part of c-Jun (amino acids 247–331) with the different TDGa deletions. The same central region of TDG from position 122 to 346 was found to be required for the binding to c-Jun (data not shown).

TDG Enhanced Transactivation by RXR and RXR/RAR—To investigate whether the interactions between TDG and either RAR or RXR could affect the transactivation mediated by RXR homodimers or RXR/RAR heterodimers, transient transfection experiments with increasing amounts of TDGa(32–421) were performed in COS cells. Ligand-induced transactivation by RXRα from a single DR1 element in a DR1(G)-tk-CAT reporter gene enhanced up to 4-fold by addition of TDGa(32–421) (Fig. 2).

Expression of mTDG in various tissues and cell lines. A, an approximately 3-kilobase RNA transcript is revealed in various mouse tissues by Northern blot analyses. B, Western blot detection of two TDG species with an apparent molecular mass of ~63 kDa (arrow) of whole cell extracts from COS, CV1, F9, HeLa, JEG3, MCF7, and P19 cells as indicated. In addition, larger forms of TDG were detected with an apparent molecular mass of ~90 kDa (arrow). In the last lane, 25 ng of E. coli-expressed mTDGa(32–421) were loaded as a control. C, dephosphorylation of TDG. 25 µg of a whole cell extract from MCF7 cells were incubated for 30 min with 20 units of CIP in the presence or absence of 50 µM vanadate (inhibitor). After the reaction the different forms of TDG were revealed by Western blotting. D, TDGa and TDGb are present in P19 cells. COS cells were transfected with 1 µg of pSG5-mTDGa or pSG5-mTDGb, and whole cell extracts were prepared 24 h after transfection. These extracts from transfected COS cells and a whole cell extract from mouse P19 cells were analyzed by Western blotting. The two upper species, P-TDGa and P-TDGb, correspond to the phosphorylated forms.

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Fig. 3. TDG interaction with RXR and RAR. A, TDG, but not UNG1, interacts with RXR and RAR in a yeast two-hybrid system. Plasmids expressing either “unfused” LexA or LexA fused to mRXRα or hRARα1 were co-transformed in the yeast reporter strain L40 with either the VP16 AAD or the VP16-AAD linked to mTDGa or hUNG1. Transformants were grown in liquid medium in the presence or absence of 500 nM ligand (9c-RA for RXR and t-RA for RAR) for about five generation times. β-Galactosidase activities determined on each cell-free extracts were expressed in nanomoles of substrate/min/mg of protein. The values (~20%) are the averages of at least three independent transformants. B, TDG interacts with RXR and RAR in vitro. About 5 µg of GST-mRXRα or GST-hRARα1 were bound to a glutathione-Sepharose matrix and incubated with 100 ng of Vaccinia virus expressed mTDGa(32–421) in the presence or absence of 1 µM 9c-RA (for RXR) or t-RA (for RAR). Proteins bound to the beads were eluted and analyzed by Western blotting.

(42), we used the yeast two-hybrid assay to investigate the interaction of the C-terminal part of c-Jun (amino acids 247–331) with the different TDGa deletions. The same central region of TDG from position 122 to 346 was found to be required for the binding to c-Jun (data not shown).

TDG Enhanced Transactivation by RXR and RXR/RAR—To investigate whether the interactions between TDG and either RAR or RXR could affect the transactivation mediated by RXR homodimers or RXR/RAR heterodimers, transient transfection experiments with increasing amounts of TDGa(32–421) were performed in COS cells. Ligand-induced transactivation by RXRα from a single DR1 element in a DR1(G)-tk-CAT reporter gene enhanced up to 4-fold by addition of TDGa(32–421) (Fig. 2).
In similar transfection assays using a DR5(G)-tk-CAT reporter gene cotransfected with RARα and RXRα expression vectors, t-RA induced activation from one DR5 element increased by 2–3-fold (Fig. 5B). Control experiments using a reporter gene construct lacking a RARE indicated that overexpression of TDG had no effect on transcription from the tk promoter (data not shown). The truncated TDGa(32–307), which could not bind to RXRα (see Fig. 4C), did not enhance transactivation from a DR1(G)-tk-CAT reporter gene (Fig. 5C). Similarly, the N-terminally truncated mutant TDGa(122–421) was unable to increase transactivation by RXRα, although it could still interact with RXR in yeast (see Figs. 4C and 5C). This observation points to a requirement of the N terminus of TDG for enhancement of receptor-mediated transcription.

yeast and mammalian cells did not reveal any transactivation domain in the N terminus or any other part of TDG (data not shown).

As TDG is known to interact also with c-Jun (Ref. 42 and see above), we investigated in transient transfection assays whether it could be involved in transrepression between the retinoic acid receptors and AP1 (62–64). However, overexpression of TDGa(32–421) in COS and HeLa cells did not relieve the transrepression exerted by RAR/RXR on the activation of the stromelysin or collagenase promoter by AP1, nor the transrepression exerted by AP1 on RXR/RAR-mediated transactivation from promoter regions containing RAREs (data not shown).

**TDG Enhanced the Binding of RXR and RXR/RAR to DNA in Vitro**—EMSAs were performed to investigate whether TDG

**Fig. 4. Mapping of the TDG-nuclear receptor interaction domains.** A, residues 227–263 of the RXRα LBD harboring helix 1 (H1) are sufficient for interaction with TDG, and integrity of the AF-2 AD core (helix 12/H12) is not required for the ligand-dependent interaction of the RXRα LBD with TDG. Plasmids expressing RXRα or deletion mutants of RXRα fused to LexA were cotransformed into yeast L40 with AAD or AAD-TDGα(32–421). Transformants were grown in the presence or absence of 500 nM 9c-RA, β-Galactosidase activity is expressed as in Fig. 3A.

B, residues 170–211 of the RARα LBD harboring helix 1 (H1) are sufficient for interaction with TDG. L40 transformants expressing the indicated LexA and AAD fusion proteins were grown in the presence or absence of 500 nM t-RA, β-Galactosidase activity is expressed as in Fig. 5C. In panels A–C, all fusion proteins were expressed in yeast as assayed by Western blotting (data not shown).
could enhance the binding of RXR or RXR/RAR to their response elements. Liganded RXRoΔAB was studied for its binding to a DR1 element (Fig. 6B) as well as liganded RXRoΔAB/

Fig. 5. TDG enhances transactivation by RXR and RXR/RAR.

A, TDG stimulates the 9c-RA-induced transactivation by RXR. COS cells were transfected with 1 μg of DR1(G)-tk-CAT reporter gene along with 1 μg of pSG5-mRXRα and increasing amounts of pSG5-mTDGa(32–421) as indicated. Twelve hours after transfection, 500 nM 9c-RA was added for 24 h when indicated before measuring CAT activity in whole cell extracts. All values were standardized for β-galactosidase activity. B, TDG stimulates the t-RA-induced transactivation by RXR/RAR. COS cells were transfected with 1 μg of DR5(G)-tk-CAT reporter gene plasmid, 1 μg of each pSG5-mRXRα and pSG5-hRARα and treated for 24 h with 500 nM t-RA when indicated. C, effect of TDG deletions on the RXR-mediated transactivation. Transfection experiment was performed as in A with 2 μg of mTDGa(32–421), mTDGa(32–307), or mTDGa(122–421).

could enhance the binding of RXR or RXR/RAR to their response elements. Liganded RXRoΔAB was studied for its binding to a DR1 element (Fig. 6B) as well as liganded RXRoΔAB/

Fig. 6. TDG increases DNA binding by RXR homodimers and RXR/RAR heterodimers in vitro. For electrophoresis mobility shift assays, 50 ng of purified RXRoΔAB or RXRoΔAB/RARαΔAB heterodimer were incubated with increasing amounts of Vaccinia virus expressed mTDGa(32–421) (50, 100, 150, or 200 ng) and 5 ng of 32P-labeled oligonucleotides representing a DR1 or DR5 binding site. Protein-DNA complexes (closed arrowheads) and free oligonucleotides (open arrowheads) were separated on a 6% polyacrylamide gel. A, RXR/RAR binding to a DR1 element; B, RXR binding to a DR1 element; C, RXR/RAR binding to a DR5 element.

RARoΔAB heterodimers for their binding to DR1 and DR5 elements in the presence of increasing amounts of Vaccinia virus-expressed TDGa(32–421) (Fig. 6, A and C). Although no TDG-receptor complex could be identified in these experiments, in all cases the addition of TDGa(32–421) led to an approximately 3-fold increase in DNA-binding by RXRoΔAB and RXRoΔAB/RARαΔAB. Under similar EMSA conditions, TDGa(32–421) could not bind to DR1 or DR5 response elements on its own.

DISCUSSION

In a screening for factors supporting RAR to function as a ligand-dependent transcription factor in yeast, we have identified TDG as a protein that can interact with RAR and RXR in yeast as well as in vitro. In a yeast two-hybrid screening, TDG was previously cloned as a c-Jun interacting protein (42), and later identified as a T:G and U:G mismatch-specific DNA repair enzyme (39).

A new TDG isoform-specific cDNA (called b) has been cloned, which differs in the 5′ sequences from the previously published mouse and human cDNAs (denoted isoform a) (39). These cDNAs are likely to correspond to alternative splicing variants of the same transcript. Although Northern blots analysis detected only one RNA species in all mouse tissues tested, transcripts corresponding to the TDG isoforms a and b could be detected in P19 embryonal carcinoma cells. Using monoclonal anti-TDG antibodies, two major TDG species were revealed in whole cell extracts from various mouse, monkey, and human cell lines as well as mouse tissues. These two forms of TDG are probably encoded by the two different transcripts found in P19 cells. Alternatively, these two species could be translated from TDGa mRNA using two different starting methionines as discussed for the human enzyme (39). The cloning of additional splice variants from mouse suggests that similar transcripts could also exist in humans. However, the cloning of additional splice variants from mouse suggests that similar transcripts could also exist in humans. Although the first 25 amino acids of the TDGa isoform, which are lacking in TDGb, are dispensable for both the binding to RAR or RXR and the enzymatic activity (40), the functional significance of the two isoforms is unknown. In addition to the two TDGa and TDGb
isofoms, Western blot analyses and phosphatase treatment of cell extracts indicated the existence of phosphorylated forms of the TDG isofoms. The significance of this modification is also unknown, although it does not appear to be required for in vitro interactions between TDG and RAR or RXR.

In the present study, we have shown that TDG can interact with RAR and RXR, both in yeast and in vitro in a ligand-independent manner. Mapping of the binding sites revealed interaction with a region in the RAR and RXR LBD harboring the α-helix 1 and a central region of TDG between amino acids 122 and 346. An alignment of hTDGα with the related UG-specific DNA glycosylase from E. coli (MUG) has shown that the TDG region from amino acid 122 to 346 is the most conserved one and that it also contains the enzymatic center of the glycosylase (40). Interestingly, the same region of TDG appears to be required for its binding to c-Jun and its homodimerization in yeast, suggesting that it could participate in various protein-protein interactions.

Transfection of TDG in COS cells increased RXR- and RXR/RAR-mediated transactivation from reporter genes containing cognate response elements for the receptors. The observation that the truncated TDGα(32–307) could not bind to the receptors and failed also to enhance RXR-induced transcription suggests that the interaction between TDG and the receptors could be of functional importance. To investigate the basis for the increased transactivation by RXR and RXR in the presence of TDG in transient transfection experiments, TDG was fused to various DNA-binding domains as those of the yeast transcription factor GAL4, the estrogen receptor, or the E. coli regulator LexA. Using these fusion proteins in yeast or in transfected COS cells with cognate reporter genes did not reveal any transcriptional activation domain in TDG (data not shown). However, TDG was shown to enhance the DNA binding of either RXR homodimers or RXR/RAR heterodimers to cognate DR elements, arguing that TDG can enhance RXR- and RXR/RAR-mediated transactivation by stabilizing their binding to DNA. In this context, it is interesting to note that the N-terminal part of TDG (amino acids 70–122) is rich in basic amino acids sharing in part similarity with a HMG box motif (HMG-I/Y-family). This TDG region is required for its T:G-specific, but not for its U:G-specific DNA glycosylase activity, suggesting that it contributes to the enzyme selectivity by contacting DNA (40). Interestingly, HMG-1 was found to stabilize binding of the progesterone receptor to its target sequences in EMSA (66, 67) and to enhance transactivation by estrogen receptor at the level of DNA binding (68). In the case of the progesterone receptor, a model was proposed in which HMG-1 first bends the DNA, and dissociates from DNA after formation of a stable receptor-DNA complex (66). The N-terminal region of TDG may act similarly to support receptor-DNA binding. Accordingly, a truncated mTDGα(122–421) lacking the basic region could not enhance the RXR-mediated transactivation in transient transfection assays, even though it could still interact with the receptors. During the last years, an increasing number of putative coactivators was cloned which still interact with the receptors. During the last years, an increasing number of putative coactivators was cloned which still interact with the receptors.

DNA repair and transcription are linked by a number of factors participating in both processes (69) such as the c-Jun and p53 activating AP endonuclease Ref-1 (70, 71) or the basic transcription/repair factor TFIH (72). The fact that TDG can interact with different transcription factors like c-Jun, RAR, RXR, or other nuclear receptors and a transcription-related protein like TIF1α suggests that TDG could be another DNA repair enzyme which functions also in transcription. The use of DNA repair factors like TDG in transcription could therefore be one mechanisms of how to maintain the integrity of transcribed regions.

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S. Um, unpublished data.
