The DNA Glycosylase T:G Mismatch-specific Thymine DNA Glycosylase Represses Thyroid Transcription Factor-1-activated Transcription*

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The transcription factor thyroid transcription factor-1 (TTF-1) is a homeodomain-containing protein that belongs to the NK2 family of genes involved in organogenesis. TTF-1 is required for normal development of the forebrain, lung, and thyroid. In a search for factors that regulate TTF-1 transcriptional activity, we isolated three genes (T:G mismatch-specific thymine DNA glycosylase (TDG), homeodomain-interacting protein kinase 2 (HIPK2), and Ajuba), whose products can interact with TTF-1 in yeast and in mammalian cells. TDG is an enzyme involved in base excision repair. In the present paper, we show that TDG acts as a strong repressor of TTF-1 transcriptional activity in a dose-dependent manner, while HIPK2 and Ajuba display no effect on TTF-1 activity, at least under the tested conditions. TDG-mediated inhibition occurs specifically on TTF-1-responsive promoters in thyroid and non thyroid cells. TDG associates with TTF-1 in mammalian cells through the TTF-1 carboxyl-terminal activation domain and is independent of the homeodomain. These findings reveal a previously unsuspected role for the repair enzyme TDG as a transcriptional repressor and open new routes toward the understanding of the regulation of TTF-1 transcriptional activity.

The transcription factor thyroid transcription factor-1 (TTF-1); also named Nkx2.1 or T/EBP) belongs to the Nkx2 class of homeodomain-containing proteins (1, 2). Nkx proteins regulate regional specification, cell fate determination, and organ morphogenesis during embryonic development. Among the vertebrate NK2 factors, Nkx2.5 is required for proper heart formation (3, 4), and Nkx2.2 for development of the spinal cord and the pancreatic islet cells (5, 6), while TTF-1/Nkx2.1 is involved in the regulation of thyroid, lung, and ventral forebrain development (7, 8).

The Nkx2 proteins share high similarity in their homeodomain, a 60-amino acid region involved in DNA binding. The TTF-1 homeodomain was the first one shown to bind DNA elements containing a 5′-CAAG-3′ core sequence, instead of the canonical 5′-TAAT-3′ (1, 9). The Nkx2 proteins contain two other highly conserved regions, the tin domain (or NK decapetide) and the NK2-specific domain (10, 11). The short tin domain of unknown function is found at the N-terminal region of most NK2 proteins, while the NK2-specific domain is located in a C-terminal region separated from the homeodomain by a short amino acid stretch and is thought to mediate protein-protein interaction.

During development, TTF-1 is expressed in the thyroid anlage, in restricted areas of the developing brain and in the lung bronchial epithelium (12). It is required for proper development of these tissues, since TTF-1 null mice die at birth lacking lung parenchyma and the thyroid, and have severe defects of the ventral area of the forebrain (7, 8). During embryogenesis, TTF-1 is expressed at embryonic days 8.5–9.5 in the pharyngeal floor, a portion of which evacuates and becomes the thyroid diverticulum. At the same time, other transcription factors that are required for thyroid development start to be expressed (13–16). However, thyroid-specific gene expression is not turned on until embryonic day 15, suggesting that these transcription factors, although expressed early in development, are inactive or require additional factors to drive thyroid-specific gene expression. Besides TTF-1, other Nkx2 family members, Nkx2.5 and -2.8, are expressed very early in the pharynx (17, 18). Nkx2.8 expression is progressively lost in the pharyngeal floor, while Nkx2.5 expression becomes restricted to the thyroid primordium. The role of Nkx2.5 in thyroid development is unknown given that Nkx2.5 null embryos die before thyroid formation, from failure in the heart tube looping process (3, 4).

In addition to its role in development, TTF-1 is thought to regulate tissue-specific transcription in differentiated thyroid and lung cells. The expression of thyroid-specific genes, such as thyroglobulin, thyroperoxidase, thyrotropin receptor, and Na-I symporter is positively controlled by TTF-1 (19–23). In the lung, TTF-1 positively regulates the expression of surfactant proteins A, B, and C (24–26).

The activity of eukaryotic transcription factors can be regulated by various mechanisms, including phosphorylation, acetylation, and interaction with transcriptional modulators. We have previously shown that TTF-1 is phosphorylated on seven serine residues, three of which serve as substrates for the
mitogen-activated protein kinase kinase extracellular signal-regulated kinase (27, 28). Expression of oncogenic Ras in thyroid cell results in TTF-1 inactivation and loss of expression of several thyroid-specific genes. Ras inhibitory effect is at least partially mediated in an extracellular signal-regulated kinase-dependent manner (27, 29). To identify other regulators of TTF-1, we performed a yeast two-hybrid screening using the full-length TTF-1 cDNA fused to the GAL4 DNA binding domain. We found that T-G mismatch-specific thymine DNA glycosylase (TDG), an enzyme involved in repair of methylated DNA (30–32), interacts with TTF-1 and strongly represses its transcriptional activity. TDG binds to the activation domain at the TTF-1 C terminus and suppresses TTF-1-activated transcription in thyroid and nonthyroid cells.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid System—All experiments were performed in the yeast reporter MaV203. The cDNA library was synthesized from rat FRTL-5 cell poly(A)+ RNA plasmid by Life Technologies and cloned into the pC86GALAD vector. Screening of the library was performed essentially following instructions for the ProQuest two-hybrid system (Life Technologies) and has been previously described (33). Briefly, the GAL4 DNA binding domain/TTF-1 fusion was constructed in pPC97GAL4DB, and proper expression of the fusion protein in yeast was confirmed. Subsequently, yeasts carrying the pC97GAL4DB-TTF-1 plasmid were transformed with the pC86AD-cDNA library and plated onto plates lacking histidine in the presence of 3AT (aminotriazole; 10 mM). Approximately 1.2 × 106 individual clones were plated, and about 200 grew on the selective medium. Resistant colonies were grown on a master plate and then replica-plated onto selection plates to determine their ability to induce three independent reporters (HIS3, URA3, and lacZ). Seventy-five independent clones were isolated after this first screening. DNA was isolated from each positive clone and sequenced to identify the inserts. Independent pC86AD clones were retransformed into yeast and tested for interaction with a fresh TTF-1 clone. Yeast strains expressing GAL4DB-Rb, GAL4AD-E2F, GAL4DB-Fos, GAL4AD-Jun, GAL4DB-DP1, GAL4AD-E2F1, and full-length GAL4 were obtained from M. Vidal.

DNA Constructs—For the yeast two-hybrid screening, the coding sequence of rat TTF-1 was fused in frame with the GAL4AD binding domain in the pC97GAL4DB plasmid. T-G, a plasmid in which a luciferase reporter gene is controlled by a 400-bp thyroglobulin promoter, has been previously described (21). C5-CAT is a reporter construct in which the chloramphenicol acetyltransferase is under the control of a promoter containing five binding sites for TTF-1 (34). Ajuba full-length coding sequence was generated by 5′-rapid amplification of cDNA ends polymerase chain reaction (Life Technologies). The coding sequence was then inserted in frame with the FLAG epitope (Stratagene) and then inserted in frame with the FLAG epitope in pFLAG-CMV2 (Sigma) to create mammalian expression plasmids, pRC-TTF-1, GAL4-TTF-1, TTF-1 deletion mutants, and G5-CAT were previously described (35).

Cells and Transient Transfection Assays—Rat thyroid follicular FRTL-5 cells were maintained in Coon’s modified Ham’s F-12 medium (Sigma) supplemented with 5% calf serum (Life Technologies) and six growth factors including thyrotropin (1 milliunit/ml) and insulin (10 μg/ml) as previously described (28, 36). Human COS-7 cells and HeLa were obtained from ATCC and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Life Technologies, Inc.). Transient transfections in FRTL-5 and HeLa cells were carried out by calcium phosphate-DNA precipitation (34). Briefly, FRTL-5 cells were plated at a density of 5 × 106 cells/ml, and 48 h later C5-CAT or Tg-CAT (2.5 μg) reporter plasmids were transfected in the presence of different expression vectors as indicated in the figure legends. Cells were collected 72 h after transfection. HeLa were plated the day before transfection at 8 × 106/ml. After transfection, cells were incubated for 48 h and then collected. Almost confluent COS-7 cells were transiently transfected using the LipofectAMINE 2000 kit following the manufacturer’s instructions (Life Technologies). 4 μg of DNA was used for a 60-mm dish (5 × 106 cells). Cells were collected 48 h after transfection.

Transactivation Assay—FRTL-5 and HeLa cell extracts were lysed in lysis buffer (10 mM HEPES, pH 7.9, 400 mM NaCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, 0.5 mM phenylmethylsulfonyl fluoride). Luciferase and chloramphenicol acetyltransferase (CAT) activities were measured as described (34, 37). CAT activity was measured by incubation with 5 mM chloramphenicol and 0.1 μCi of [3H]acetyl coenzyme A (1.4 Ci/mmol; 50 μCi/ml). Reactions were performed in the presence of water-insoluble scintillation fluid (Econofluor-2; Packard Bioscience) at 37 °C, and radioactivity released was counted after 5 h. Luciferase activity was measured in the presence of 0.2 mM d-luciferin (Sigma) in a Lumat LB 9501 luminometer (Berthold). Approximately one-tenth of the total volume was employed for each assay.

Repression of TTF-1 Transcriptional Activity by TDG—Yeast Two-hybrid System—Yeast cells (MaV203) transformed with GAL4DBTTF-1 and GAL4AD interaction constructs were assayed as described in Fig. 1. Seventy-five positive clones were identified after the first screening, of which 25 were sequenced to identify the inserts. The number of clones found to correspond to each gene is indicated. +++, strong positive interaction; ++, intermediate interaction; +, weak interaction; −, no interaction.

| GAL4AD plasmid | Number of clones | Strength of interaction |
|---------------|----------------|------------------------|
| UBC9          | 20             | +++                    |
| PIAS3         | 17             | +                      |
| Ajuba         | 10             | +                      |
| TDG           | 4              | +                      |
| HIPK2         | 2              | +                      |
| PIAS1         | 1              | +                      |
| Hex           | 1              | −                      |

RESULTS

Isolation of TTF-1 Interactors by Yeast Two-hybrid Analysis—in a yeast two-hybrid screen of a rat cDNA library, 65 positive clones were isolated, of which 55 clones corresponded to sequences homologous to known human or mouse genes. Of these, 20 were homologous to mouse ubiquitin-conjugating enzyme (UBC9) (38), 17 to mouse and human protein inhibitor of activated STAT 3 (PIAS3) (39), one to the highly related protein (PIAS1 (40), 10 to mouse Ajuba (a LIM domain-containing protein) (41), four to human and mouse TGF (32), two to mouse and human homeodomain-interacting protein kinase 2 (HIPK2) (42), and one to the transcription factor Hex (43) (Table I). The longest clone of each gene was entirely sequenced and found to correspond to the rat homologue of the above mentioned genes.

Interaction between TTF-1 and the isolated clones was confirmed by transformation in yeast and test using three independent reporters (HIS3, URA3, and lacZ), in the presence of GAL4 DNA binding domain alone or of TTF-1-GAL4 fusion protein. As shown in Fig. 1, the strongest interaction was detected with Ajuba, UBC9, PIAS3, and the related gene PIAS1. TDG interacted also quite strongly, while interaction between TTF-1 and HIPK2 was weak. Other potential interactors such as the Hex gene and the clones Y66, Y120, and Y137, corresponding to unknown genes, scored positive in the context of one promoter but not in the context of the others and were excluded from further analysis. Thus, rat genes corresponding
to TDG, UBC9, Ajuba, HIPK2, PIAS1, and PIAS3 displayed the ability to interact with TTF-1 in yeast, while the other clones selected during the first screening, including all of the unknown genes (Y66, Y129, and Y137 and others not shown) were isolated in a two-hybrid screening as TTF-1 interactors. These clones were transformed in yeast with either the pPC97GAL4DB empty vector (interact.) or in the presence of pPC97GAL4DB-TTF-1 (TTF-1/interact.). As negative control, pPC97GAL4DB-TTF-1 (TTF-1) was transformed with the empty vector pPC86GAL4AD. Selection of positive clones was performed with three reporter genes driven by independent promoters (HIS3, URA3, and lacZ). A, two-hybrid-dependent induction of HIS3 reporter gene allowed cell growth in medium lacking His and supplemented with 3-aminotriazole (3AT). B, two-hybrid-dependent induction of URA3 resulted in conversion of 5-fluoroorotic acid (5-FOA) to 5-fluorouracil, which is toxic (negative selection). C, induction of the lacZ gene results in a blue color when assayed with X-Gal. Interaction was considered to be positive if clones grew on 3AT, were inhibited in 5-fluoroorotic acid, and turned blue with X-Gal in the presence of TTF-1 but not in its absence. Interaction between the empty vectors pPC86GAL4DB and pPC97GAL4AD was used as negative control (ctr). Interaction between the retinoblastoma gene product DB-Rb and AD-E2F (Rb/H11001/E2F) was used as an example of weak interaction in yeast. The transcription factors DB-Fos and AD-Jun (jun/H11001/fos), DB-DP1 and AD-E2F1 (E2F/H11001/DP), and the full-length GAL4 (GAL4) are shown as positive controls.

Fig. 2. Association of TTF-1 with the interactors in mammalian cells. A, COS-7 cells expressing equivalent amounts of TTF-1 and FLAG-tagged TDG were lysed, and proteins were immunoprecipitated with anti-FLAG antibody. Used as negative controls were cells that expressed TTF-1 alone (TTF), TDG alone (TDG), or an unrelated expression vector (CTR). Samples were loaded on 10% SDS-PAGE gels and analyzed for the presence of TTF-1 bound to TDG by immunoblotting with anti-TTF-1-specific antibody (Flag ip TTF blot, top panel). The amount of FLAG TDG present in the immunoprecipitate is shown in the middle panel (FLAG ip FLAG blot). The presence of TTF-1 in the total cell extracts was detected by immunoblotting with anti-TTF-1-specific antibody (TTF blot, bottom panel). B, association between TTF-1 and FLAG-tagged HIPK2 was measured as described for A. Samples were run on a 7.5% SDS-PAGE to visualize HIPK2. HIPK2 expression was low, possibly due to protein instability. For this reason, the immunoprecipitated samples were detected using a sensitive immunodetection substrate (SuperSignal West Femto substrate; Pierce). C, association between TTF-1 and FLAG-tagged Ajuba was measured as described for A. Samples were run on a 10% SDS-PAGE gel. This set of experiments was performed twice with similar results.

Fig. 3. TDG represses TTF-1-activated transcription in thyroid cells. Transient expression of FLAG-TDG in FRTL-5 cells inhibited in a dose-dependent manner the activity of the endogenous TTF-1 as measured on the C5-responsive (A) and thyroglobulin-responsive (Tg, B) promoters. In contrast, Ajuba (C) and HIPK2 (D) had no effect on TTF-1 function. The indicated amounts of FLAG-TDG, FLAG-Ajuba, or FLAG-HIPK2 were co-transfected with a CAT reporter construct containing either the thyroglobulin or the C5 promoters (2.5 μg). The amount of total DNA was held constant by including pCMV βGAL expression vector. Seventy-two hours after transfection, CAT activity was determined as described under "Experimental Procedures." All values were normalized for co-transfected luciferase activity and are presented as the percentage of CAT activity in the absence of the interactor. Error bars indicate the S.E. of four independent experiments.
known genes, did not.

Association of the Interactors with TTF-1 in Mammalian Cells—The TTF-1 interactors were further characterized for their ability to associate with TTF-1 in mammalian cells. Their coding sequences, lacking the first methionine, were fused at the N terminus to the FLAG epitope and cloned into a mammalian expression vector. Individual constructs were co-transfected with an equal amount of a TTF-1 expression vector in COS-7 cells. After 48 h, cells were harvested and lysed under mild conditions, and then immunoprecipitation was performed with anti-FLAG-specific antibody. Total cell extracts and immunoprecipitates were then run on SDS-PAGE gels under the appropriate conditions, and immunoblotting analysis was performed using either anti-FLAG or anti-TTF-1 antibody. Immunoprecipitation of TDG and Ajuba resulted in a readily detectable amount of TTF-1 expression vector in COS-7 cells. Although HIPK2 immunoprecipitation was not very efficient due to its low levels of expression, a low but significant amount of TTF-1 was detected in HIPK2 immunoprecipitation (Fig. 2B). TTF-1 association with these proteins was specific, since no association could be detected with FLAG-UBC9 and FLAG-PIAS3, although both proteins were efficiently produced. PIAS1 was not tested in mammalian cells. On the basis of these results, TDG, HIPK2, and Ajuba were considered as potential regulators of TTF-1 activity.

TDG Acts as a Strong Repressor of TTF-1-mediated Transcription—TDG was previously reported to interact in yeast two-hybrid assays with c-Jun and with the retinoic acid receptors RAR and RXR and to weakly enhance RAR and RXR transcriptional activity (44, 45). HIPK2 is a nuclear kinase that was reported to interact with NK2 family members (42). Ajuba was recently cloned as an interactor of GRB2 and was found to shuttle between the cytoplasm and the nucleus (41, 46). The ability of these proteins to interfere with TTF-1 transcriptional activity was evaluated in thyroid and nonthyroid cells. Cells were transiently transfected with an expression vector for TDG, Ajuba, or HIPK2 in the presence of either a natural TTF-1-responsive promoter (thyroglobulin) or an artificial one (C5), driving the expression of CAT. These promoters display strong basal activity in FRTL-5 cells and are activated specifically by exogenous expression of TTF-1 in nonthyroid cell lines (21, 34). In thyroid cells, TDG expression strongly repressed C5-CAT in a dose-dependent manner with a maximum inhibition of ~75% (Fig. 3A). The activity of the thyroglobulin promoter was similarly inhibited by TDG expression (Fig. 3B), while TDG had no effect on the CMV-Luc expression plasmid used as control (data not shown). In contrast, exogenous expression of either HIPK2 or Ajuba had no effect on TTF-1 activity as measured on C5 and thyroglobulin promoters (Fig. 3, C and D, and data not shown).

To confirm and further extend these results, HeLa cells that do not express TTF-1 were transiently transfected with the TTF-1-responsive construct C5-CAT in the presence of either HIPK2 or Ajuba had no effect on TTF-1 activity (data not shown). Exogenous expression of either HIPK2 or Ajuba had no effect on TTF-1 activity (data not shown), while TDG expression resulted in strong suppression of TTF-1 activity in a dose-dependent manner (Fig. 4A). Since TDG is expressed ubiquitously (Ref. 45 and data not shown), the endogenous protein may be sufficient to elicit an effect in the presence of low TTF-1 concentrations. Under these conditions, the addition of TDG could be inhibitory due to a nonspecific “squelching” effect rather than a biologically relevant function. To test this hypothesis, TTF-1 activity was measured in a wide variety of concentrations. In HeLa cells, TTF-1 could efficiently activate C5-CAT even at very low doses (0.05–0.1 μg), under which conditions TDG expression strongly repressed TTF-1 activity. In the presence of high doses of TTF-1 and low concentration of the TDG vector, TTF-1 transcriptional activity was still strongly inhibited by TDG expression in a dose-dependent manner (Fig. 4B). Similar suppression of TTF-1 activity was obtained by TDG expression in NIH3T3 fibroblasts at all tested concentrations (data not shown). Thus, taken together, these data suggest that TDG associates with TTF-1 in mammalian cells, where it acts as a specific repressor of TTF-1 transcriptional activity, while Ajuba and HIPK2 are able to associate with TTF-1 but have no apparent effect on its activity at least under the tested conditions.

Rat TDG Gene—All four TDG clones identified in the two-hybrid screening included the entire coding sequence. The longest TDG clone was 2883 bp long and included 124 nucleotides of 5′-untranslated region, and 1598 nucleotides of 3′-untranslated region. The open reading frame of 1158 base pairs encoded a protein of 386 amino acid residues with a predicted molecular mass of 42,454 Da (data not shown). The amino acid alignment had 88% identity with human TDG and 90% identity with mouse TDGb, while mouse and human sequences shared

Fig. 4. TDG represses TTF-1-dependent transcription in HeLa cells. A, the C5-CAT reporter gene (2.5 μg) was transiently transfected in the absence (●) or in the presence of a low dose of TTF-1 (0.05 μg), with increasing concentrations of TDG as indicated. The amount of total DNA was held constant by including pCMV βGal expression vector. Under these conditions, almost complete repression was obtained in the presence of 0.5 μg of TDG. B, TDG represses TTF-1-dependent transcription in the presence of high doses of TTF-1. The C5-CAT reporter gene (2.5 μg) was transiently transfected in the absence (●) or in the presence of high doses of TTF-1. TTF-1 activity was measured in the absence (white bars) or in the presence of 0.1 μg of TDG (gray bars) or of 0.5 μg of TDG (striped bars). All values were normalized for co-transfected luciferase activity and are presented as -fold induction of CAT activity in the absence of TTF-1 and TDG. Error bars indicate the S.E. of four independent experiments.
86% identity (data not shown). The DNA glycosylase domain comprised amino acids 98–245 of the rat sequence and contained a single substitution Asn to Ser at position 146 of the rat sequence. No other recognizable domain could be identified in the TDG sequence.

**TDG Binds to the Carboxyl-terminal Activation Domain of TTF-1**—To map the TTF-1 domains responsible for the interaction with TDG, previously characterized TTF-1 deletion mutants (Fig. 5A) (35) were transiently transfected in COS-7 cells either in the presence or in the absence of FLAG-TDG and immunoprecipitated with anti-FLAG monoclonal antibody. A TTF-1 deletion mutant lacking the entire C-terminal region (Δ14) was unable to associate with TDG, while a deletion of the first 51 amino acids at the N-terminal (Δ1) could efficiently bind to TDG (Fig. 5B). The Δ35 mutant lacking most of the N-terminal domain and part of the C-terminal region (amino acids 221–295), was able to associate with TDG to the same extent of the wild type (Fig. 5B). These data suggest that TDG binds to either the homeodomain and its N-terminal proximal region (amino acids 96–221), or to the C-terminal activation domain (amino acids 295–372). To assess whether the C-terminal domain was sufficient by itself to bind to TDG in the absence of the homeodomain, COS-7 cells were transfected with plasmids encoding for the TTF-1 activation domains fused to the GAL4 DNA binding domain, in the presence or in the absence of FLAG-TDG. Immunoprecipitation experiments using anti-FLAG monoclonal antibody revealed that TDG could associate specifically with the C-terminal activation domain (amino acids 295–372) fused to GAL4, but not with the N-terminal domain (Fig. 5D).

TTF-1 deletion mutants were tested for their ability to activate C5-CAT transcription in the presence and in the absence
of TDG. Similarly to the wild type TTF-1, the Δ1 mutant was strongly inhibited by TDG (Fig. 5C). In contrast, the Δ14 mutant that was unable to associate to TDG was unaffected by TDG expression. Interestingly, the Δ35 mutant that was sufficient to bind to TDG was unaffected by TDG, suggesting that TTF-1 regions that are not involved in binding to TDG are required for TDG-dependent inhibition.

Taken together, these data demonstrate that TDG associates in mammalian cells with TTF-1 specifically through a C-terminal domain between amino acids 295 and 372, corresponding to the activation domain. The activation domain is by itself necessary and sufficient for TDG binding, while TDG-mediated repression requires a more extended C-terminal region.

**DISCUSSION**

Tissue-specific gene expression is spatially and temporally regulated. TTF-1 is a critical component of the transcriptional machinery controlling the expression of thyroid- and lung-specific genes, and thus its activity is likely to be finely regulated during embryonic development and in adult life. In an attempt to isolate TTF-1 regulators, we have identified six genes (Ajuba, HIPK2, PIAS1 and -3, TDG, and UBC9), whose product can bind to TTF-1 in yeast. Among these interactors, the T:G mismatch-specific thymine glycosylase TDG displays a strong ability to repress TTF-1-activated transcription. TDG is an enzyme involved in repair of methylated DNA (30–32). Spon-

Taneous deamination of 5-methylcytosine generates T:G mismatches whose repair is initiated by TDG. In mammalian cells, there are only two known thymine glycosylases, TDG and MBD4 (47), which display no apparent amino acid sequence similarity, although they share the ability to bind methylated CpG and a very similar catalytic specificity in vitro (47, 48). DNA repair has recently revealed important links to transcription. For instance, proteins involved in nucleotide excision repair are components of the basal transcription factor TFIH (49, 50). TDG has been previously suggested to be involved in transcription regulation because of its ability to interact in yeast with other transcription factors such as c-Jun, the retinoic acid receptor, and p73 (44, 45, 51). TDG function in the regulation of Jun and p73 has not been explored, while TDG repression occurs only in the presence of the entire activation domain. Association of TDG but is not significantly inhibited. Interestingly, this repression is not significantly inhibited. Interestingly, this region comprises the NK2-specific domain that has been proposed to control the transactivation properties of the NK2 family members (10, 11).

The mechanisms through which TDG represses TTF-1 activity are unclear. Although TTF-1 binding to DNA in vitro is not inhibited by TDG,2 in vivo TDG could sequester TTF-1 in a different nuclear compartment, preventing binding to target promoters. Alternatively, TDG could work as a repressor by recruiting a histone deacetylase. However, this possibility is unlikely, since TDG-mediated repression was not alleviated by the histone deacetylase tricostatin A, and exogenous expres-

2 C. Missiro and R. Di Lauro, unpublished data.

TDG strongly represses TTF-1-activated transcription in several cell lines; however, the functional significance of this repression in vivo is unclear. TTF-1 is expressed early in mouse development (embryonic day 8.5), although thyroid-specific gene expression is not turned on until later (embryonic day 15) (12). It has been postulated that during this time TTF-1 may be kept inactive by a repressor. TDG is highly expressed during early mouse embryogenesis (52) and may prevent TTF-1-activated transcription.

As a result of our search for TTF-1-interacting proteins, we have isolated also Ajuba and HIPK2. Ajuba was the only isolated TTF-1 interactor that was reported to be mainly cytoplasmic, although it contains a nuclear export signal, and it has been suggested to shuttle between the cytoplasm and the nucleus (41). Ajuba belongs to the Zyxin family of LIM proteins, and it has recently been shown to induce growth inhibition and spontaneous endodermal differentiation in embryonal cells (46). LIM domains mediate strong protein-protein interactions, and this feature may allow nonspecific association upon overexpression. TTF-1 was exclusively located in the nucleus even upon overexpression in COS-7 cells, while Ajuba was detected primarily in the cytoplasm, and no colocalization could be detected (data not shown). Thus, the physiological significance of the interaction between TTF-1 and Ajuba remains unclear.

HIPK2 was the first member of a family of HIPKs identified for their ability to bind to NK2 and NK3 and to enhance their repressor activities (42). Subsequently, HIPKs were reported to interact also with other transcription factors, such as the androgen receptor, the glucocorticoid receptor, and p73 (51, 53, 54). However, the ability of HIPKs to associate with these transcription factors in mammalian cells could not be shown. In the present study, we report for the first time the interaction between HIPK2 and a member of the family of the homeodomain-containing proteins. HIPK2 protein was difficult to detect even by exogenous expression in COS-7 cells. Given that its C terminus contains a PEST sequence, the protein may be very unstable. For these reasons, it is likely that association with other proteins in mammalian cells might have been difficult to obtain. Under our conditions, HIPK2 displays no effect on TTF-1 transcriptional activity in either thyroid or HeLa cells; thus, the role of this interaction remains to be established.

The extent to which the molecules isolated in this study contribute to TTF-1 regulation in vivo remains to be elucidated. However, the identification and characterization of their interaction and, in particular, of TDG-mediated repression in cultured cells sets the molecular basis to dissect their functional role in TTF-1 regulation and in thyroid gland and lung physiology.

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**REFERENCES**

1. Guazzi, S., Price, M., De Felice, M., Damante, G., Mattei, M. G., and Di Lauro, R. (1990) EMBO J. 9, 3631–3639
2. Minno, K., Gonzalez, P. J., and Kimura, S. (1991) Mol. Cell. Biol. 11, 4927–4933
3. Biben, C., and Harvey, R. P. (1997) Genes Dev. 11, 1307–1309
4. Lyons, I., Parsons, L. M., Hartley, L., Li, K., Andrews, J. E., Robb, L., and Harvey, R. P. (1995) Genes Dev. 9, 1654–1666
5. Briscoe, J., Sussel, L., Serup, P., Hartigan-O'Connor, D., Jessell, T. M., Rubenstein, J. L., and Ericson, J. (1999) Nature 398, 622–627
6. Sussel, L., Kalamara, J., Hartigan-O'Connor, D. J., Menues, J. J., Pedersen,
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