A Testis-specific Transcription Factor IIA (TFIIAγ) Stimulates TATA-binding Protein-DNA Binding and Transcription Activation*

(Received for publication, April 9, 1999, and in revised form, September 7, 1999)

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The general transcription factor IIA (TFIIA) stimulates RNA polymerase II-specific transcription by stabilizing the association of the TATA-binding protein (TBP) with promoter DNA, inhibiting repressors of TBP, and facilitating activator-dependent conformational changes in the preinitiation complex. TFIIA is encoded by two genes (αβ and γ) that are highly conserved between human and yeast. Here, we report the molecular cloning of a novel human gene that shares significant sequence similarity to the evolutionarily conserved amino- and carboxyl-terminal domains of TFIIAαβ. The TFIIA-related protein (TFIIAγ) was cloned from a testis-specific cDNA library, and its mRNA is expressed predominantly in testis tissue as determined by expressed sequence tag data base analysis and Northern blotting analysis. The TFIIA complex reconstituted with the testis-specific subunit, TFIIA (αγ), formed the TFIIA-TBP-TATA DNA (T-A) and TFIIA-TFIIB-TBP-TATA DNA (TAB) complexes indistinguishably from TFIIA (αβ+γ). TFIIA (αγ) supported basal and activated transcription for most activators in reactions reconstituted with TFIIA-depleted nuclear extracts. However, TFIIA (αγ) was reduced relative to TFIIA (αβ+γ) for stimulating transcription with at least one activator, suggesting that these two forms of TFIIA have activator specificity. These results suggest that TFIIAγ may be important for testis-specific transcription regulation.

The general transcription factors were initially identified as a set of accessory proteins required for accurate RNA polymerase II transcription initiation from the majority of viral and cellular promoters examined (reviewed in Refs. 1–3). The general factors can assemble into a stable preinitiation complex nucleated by general transcription factor IID (TFIID) binding the TATA box found at the −30 position of many viral and cellular promoters (4, 5). The general transcription factors TFIIA and TFIIB bind directly to the TATA-binding protein (TBP) of the TFIID multiprotein complex and stabilize its association with promoter DNA (6). TFIIA provides a docking site for polymerase II and additional general factors that associate tightly with polymerase II. TFIIA binds directly to TBP and at least one TBP-associated factor (TAF) in the TFIID complex (7). TFIIA has a regulatory function in preinitiation complex formation that can be modulated by a variety of positive and negative acting transcriptional modifiers. TFIIA binding to TBP can prevent the inhibitory effects of transcriptional repressors, Dr1/DRAP1, HMG1, DSP1, MOT1, and TAFII250 (8–14). TFIIA can also directly mediate a stimulatory effect of promoter-specific transcriptional activators on the binding of TFIID to the promoter (15–17). TFIIA induces a conformational reorganization of TAFs in the TFIID complex bound to DNA, which can be further stimulated by a subset of transcriptional activators and correlates with an increase in transcription activity (18–20). Inhibition of this conformational change may also be an important target for transcriptional repressors, pRb and RBP(CBF) (21, 22).

TFIIA can be isolated from metazoan cells as a heterotrimERIC complex encoded by two evolutionarily conserved genes (7, 23–28). TFIIAαβ encodes the two largest subunits of the trimeric form of human TFIIA (referred to as α and β), which are derived by a sequence-specific post-translational proteolytic cleavage of the αβ polypeptide (7, 24, 27). The small subunit (referred to as γ) is encoded by the TFIIA γ gene. In the budding yeast Saccharomyces cerevisiae, TFIIA can be isolated as a heterodimer, and the large subunit (TOA1) is not proteolytically cleaved (29, 30). The amino-terminal (α) and carboxy-terminal (β) domains of TFIIAαβ share significant sequence similarity with the amino- and carboxyl-terminal domains of yeast TOA1. The spacer regions between these two domains share little homology, and genetic experiments in yeast indicate that this region is not essential for viability (31). The conserved regions of TFIIAα, β, and γ are essential for viability in yeast (31).

Despite an absolute requirement for yeast viability, TFIIA may not be essential for the transcription of all promoters in yeast or metazoan genomes (29, 32). Early biochemical experiments revealed that TFIIA was not required for basal transcription reactions reconstituted with TBP and highly purified or recombinant general transcription factors (33). However, these highly purified systems do not respond robustly to transcriptional modulation by promoter-specific activators and repressors. Transcription activation reactions reconstituted with TFIID (TBP+TAF16) have shown a strong dependence on TFIIA (23, 26). However, transcription reactions reconstituted with yeast general transcription factors and the mediator-SRB complex does not have any apparent requirement for TFIIA (34, 35). Similarly, there was not a strong dependence on TFIIA in transcription reactions reconstituted with holo-RNA polymerase II that lacked TAF16 (36). Thus, TFIIA may have a specific function in TAF16-dependent transcription activation.

*This work was supported by an National Cancer Institute Howard Temin Award (to J. O.), by grants from the Leukemia Society of America and the National Institutes of Health Grant GM54687-02 (to F. M. L.), and by funds from the Wistar Cancer Center Core Facilities for DNA sequencing. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§The abbreviations used are: TFII, transcription factor II; TBP, TATA-binding protein; TAF, TBP-associated factor; EST, expressed sequence tag; PIPES, 1,4-piperazinediethanesulfonic acid; GST, glutathione S-transferase; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; TRP, TBP-related protein; VP16, virion protein 16; CTF, CAAT transcription factor; AH, amphiphatic helix; TRF, TBP-related factor.
Consistent with this model is the observation that TFIIA can overcome the inhibitory activity of the amino-terminal domain of TAFII250 and function as a high copy suppressor of a conditionally lethal mutation in the yeast TAFII250 homologue, yTAFII145 (13, 14).

The variable requirement for TFIIA in transcription activation reactions suggests that TFIIA functions at a subset of promoters in vivo and is likely to have activator and promoter specificity. Recent experiments with TFIIA mutants compromised for their ability to bind to TBP reveal promoter and activator-specific defects in vivo, consistent with the prediction that TFIIA may be rate-limiting at a subset of promoters (32). Although the precise determinants conferring this specificity are not clear, it appears that at least a subset of activators can bind directly to TFIIA subunits. In metazoan systems, the viral activators Zta and HTLV-I Tax and the carboxy-terminal activation domain of VP16 can all bind directly to TFIIA and stimulate the formation of a stable TFIIA-TFIID-promoter complex (15, 16, 26, 37). These data support a role for TFIIA as a coactivator for TAFII-specific transcription regulation.

Variant and tissue-specific general transcription factors and coactivators may be required for complex combinatorial gene regulation. The Drosophila TBP-related factor (TRF1) is expressed primarily in nervous system tissue and can substitute for TBP with a corresponding novel set of associated factors (38, 39). Similarly, a B cell-specific TAF II135 has been identified in human tissue, but the function of this subunit is unclear (40). We have searched an expressed sequence tag data base and found that a gene related to TFIIA αβ exists in human tissue. In this work we describe the isolation of the full-length gene encoding this TFIIAαβ-related protein and demonstrate that it can functionally substitute for TFIIAαβ in T-A formation and stimulation of basal transcription in vitro. Interestingly, we found that this TFIIAαβ-related gene is only detected in mRNA derived from testis. This testis-specific TFIIA (referred to as TFIIAγ) supported transcription activation for some but not all activators tested.

**MATERIALS AND METHODS**

**Isolation of a cDNA encoding TFIIA**—The Human Genome Sciences data base of over 2 million expressed sequence tags (ESTs) generated from over 800 human normal and disease-tissue-specific cDNA libraries was screened for altered forms of TFIIA subunits (αβ and γ) using the BLAST algorithm (41–43). Several ESTs with significant sequence similarity to the conserved amino- and carboxy-terminal domains of human TFIIAαβ were found in testis cDNA libraries. To obtain the complete open reading frame, a random primed testis cDNA library (CLONTECH) was screened using a fragment of the partial cDNA clone. Filters were hybridized with the probe in PIPES, pH 8.0, 1% SDS, 50% formamide, and 1 μg/ml of denatured salmon sperm DNA at 42 °C overnight and then washed at a final stringency of 0.2 x SSC, 0.1% SDS at 50 °C. Multiple independent cDNA clones were identified, and the longest clone was isolated. Sequence analysis revealed an open reading frame of 478 amino acids with a predicted molecular mass of 52.4 kDa and an isoelectric point of 4.78.

**Plasmids**—TFIIAγ cDNA was cloned into pBSKII (Stratagene) (pBS-TFIIAγ) as a BamHI-XhoI fragment with T3 promoter directing in vitro transcription and coupled translation. pGEX-5X-IIAγ was generated by Vent polymerase (New England Biolabs) amplification of the IIA open reading frame using EcoRI (5’) and XhoI (3’) and subcloning into the corresponding sites of pGEX-5X-3 (Amersham Pharmacia Biotech). pBSIIAαβ, pGEX2T-IIAαβ, and pGEX2T-IIAγ have been described previously (26). pGEX-TP is described previously (44). pGEX-TRP has been described elsewhere and consists of a ubiquitously expressed human TBP-related protein.2 Expression vectors and purification of GAL4-VP16, GAL4-αH, GAL4-CTF, and Zta proteins have been reported previously (26). The G, E4T4CAT, Z, E4T4CAT, and TATA/Inr reporter plasmids have been described previously (45, 46).

dTAFII110 was in vitro translated from p7β110 (47) (gift of R. Tjian). Protein Purification—Purification of TFIIAγ. A B.2L1 strain of Escherichia coli containing pGST-IIAγ was grown in LB at 37 °C. When an A600 of 0.45 was reached, GST-IIAγ expression was induced for 2 h at 29 °C by the addition of 1 mM isopropyl-thio-β-D-galactopyranoside. GST-IIAγ was purified similarly to the other GST fusion proteins described above. GST was cleaved from the IIA-α fusion by Factor Xa (Novagen) in 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM CaCl2, 7 mM β-mercaptoethanol (Sigma) for 18 h at 22 °C. Cleaved GST protein was purified from IIAγ in batch by an incubation with glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM CaCl2, 7 mM β-mercaptoethanol, 1 mM PMSF) (26). TFIIA (γ) was dialyzed against denaturant buffer at 22 °C, followed by a stepwise renaturation into D100 buffer (100 mM KCl, 20 mM HEPES-KOH, pH 7.9, 20% glycerol, 0.2 mM EDTA, 1 mM PMSF, 1 mM DTT) at 4 °C as described (48). Renatured TFIIA (γ) was purified away from Factor Xa by binding to nickel-nitrotriacetic acid agarose (Qiagen) in batch at 4 °C in D100, 5 mM imidazole, 7 mM β-mercaptoethanol, 1 mM PMSF for 2 h, washed twice in binding buffer, and then eluted in D100, 250 mM imidazole, 7 mM β-mercaptoethanol, 1 mM PMSF. TFIIA (γ) was analyzed against D100, 50 mM β-mercaptoethanol and 1 mM PMSF at 4 °C and was flash frozen in small volumes on dry ice.

E. coli DH5α cells transformed with pGEX-TBP, pGEX-TRP, pGEX-IIAαβ, pGEX-IIAγ, and pGEX-2T were grown in LB at 37 °C until an absorbance of 0.600 of 0.4 was reached. Isopropyl-thio-β-D-galactopyranoside was added to 1 mM, and cultures were induced at 28 °C for 4 h. Cells were harvested and washed once in 1 x phosphate-buffered saline and then sonicated in 20 mM Tris, pH 8.0, 5.0 mM EDTA, 1 mM DTT, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 mM PMSF. After sonication, 0.1% Nonidet 40 and 100 mM NaCl were added and incubated on ice for 15 min. The clarified lysates were mixed with glutathione-Sepharose (Amersham Pharmacia Biotech) for 2 h at 4 °C with gentle shaking. The beads were then washed twice in 20 mM HEPES, pH 7.9, 12% glycerol, 200 mM KCl, 0.1% Nonidet P-40, 1 mM DTT, and 1 mM PMSF, and the fusion proteins were eluted with 10 mM reduced glutathione in 50 mM Tris-Cl, pH 8.0, 1 mM DTT at room temperature for 10 min. Eluted material was dialyzed directly into D100 buffer and stored in small aliquots at −70 °C. Purification of GAL4-VP16, GAL4-CTF, GAL4-αH, and Zta has been described previously (48). Human TBP, TFIIA, and TFIIIB have been isolated from E. coli BL21 cells as described (48).

**GST Interaction, Electrophoretic Mobility Shift, and in Vitro Transcription Assays**—pBS-TFIIAαβ, γ, and T were used as templates for T7 or T3 polymerase directed coupled in vitro transcription-translation reactions using rabbit reticulocyte lysate (Promega) supplemented with [35S]methionine. GST interaction assays were described previously (26). Electrophoresis mobility shift assays were described previously (26). In vitro transcription reactions with nickel-nitrotriacetic acid agarose depletion of TFIIA has been described previously (26).

**Northern Blotting**—Multiple tissue Northern blots (CLONTECH) containing 2 μg of poly(A)+ RNA isolated from 16 tissue types were hybridized with random primed 32P-labeled probes (26) described above. The G5E4TCAT, Z7E4TCAT, and TATA/Inr promoter regions were used as probes for nuclear run-on transcription. Blots were reprobed after stripping the membrane by boiling in 0.5% SDS for 30 min.

**Chromosome Mapping**—A panel of 24 monoclonal somatic cell hybrids were obtained from Quantum Biotechnology, and the G3 panel of 83 radiation hybrids was obtained from Research Genetics. The following oligonucleotides which span a 150-base pair region of the P110 locus were used for polymerase chain reaction (PCR) analysis on 100 ng of template: DNA, GTGACACTACAGCTGACTC (5 ’ primer), and CATGGATCCATGTCGAGAC (3 ’ primer). 35 cycles of polymerase chain reaction amplification (94 °C for 30 s, and 58 °C for 45 s, and 72 °C for 1 min) were performed on 100 ng of each hybrid in a 50-μl reaction. Analysis of the radiation hybrid data was performed using the server at the Stanford Human Genome Center.

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Antibodies and Western Blotting—A rabbit antiserum was raised against a synthetic peptide corresponding to nonconserved amino acids 275–294 of TFIIA\textit{b} (HDESLSTSPHGLHQVTHD) according to the manufacturer's protocol (Research Genetics, Inc.). A 1:1000 dilution of polyclonal serum (second bleed) was used to perform Western blotting results are consistent with the detection of all subunits.

**Results**

Isolation of a Human cDNA Encoding a TFIIA-related Protein—To determine whether a human gene encoding a protein with sequence similarity to the hTFIIA\textit{b} subunit exists, a data base of expressed sequence tags generated from multiple tissues was searched by the BLAST algorithm (41). Several ESTs that showed significant homology to the conserved amino- and carboxyl-terminal domains of hTFIIA\textit{b} were identified in testis-specific mRNA and used to probe a cDNA library derived from testis tissue. A full-length cDNA was isolated from testis-specific library encoding a 478-amino acid protein—termed TFIIA-\textit{ab} (hIIA-\textit{ab}), Drosophila TFIIA-L (dIIA-L), and yeast TFIIA large subunit (TOA-1). Alignments were generated using the ClustalW and BoxShade programs. B, schematic alignment of conserved amino- and carboxyl-terminal domains of human TFIIA\textit{ab} and \(\tau\) subunits. To sublocalize TFIIA\textit{ab} on chromosome 2, a panel of 83 radiation hybrids were used. In addition to the human genomic DNA, amplicons were observed in hybrids 1, 2, 44, 50, 51, 61, 66, and 68 (data not shown). Analysis of this data using the Stanford Human Genome Center RH server revealed linkage to the SHGC-34039 marker on chromosome 2. Superposition of this map with the cytogenetic map of human chromosome 2 allowed the assignment of TFIIA\textit{ab} to chromosomal band 2p15–16.

**Testis-specific Expression of TFIIA-\textit{ab}**—The cDNA encoding TFIIA-\textit{ab}–related protein was radiolabeled by random priming and used to probe mRNA derived from various human tissues by Northern blot analysis (Fig. 2). A specific hybridization with an RNA species of \(-1.9\) kilobases was detected in testis tissue but not in any other tissue examined, including spleen, thymus, ovary, prostate, lung, and brain (Fig. 2, upper panels). The blot was then stripped and hybridized with human TFIIA\textit{ab}–specific probe. A prominent RNA species migrating at \(-7\) kilobases was detected in spleen, thymus, prostate, testis, ovary, and small intestine. We did not detect significant expression of TFIIA\textit{ab} in colon or peripheral blood lymphocytes (Fig. 2, right panels). The blot was then stripped and hybridized with a \(\beta\)-actin–specific probe that reacted strongly with a \(-2.0\)-kilobase species in all tissues tested. Note that although TFIIA\textit{ab} and TFIIA\textit{b} were expressed highest in testis, \(\beta\)-actin expression was slightly decreased in this tissue. These Northern blotting results are consistent with the detection of all TFIIA-\textit{ab} ESTs in testis-derived tissue (data not shown), which further suggests that TFIIA-\textit{ab} is a testis-specific form of the large TFIIA subunit.

To determine whether TFIIA-\textit{ab} protein was expressed in testis tissue, polyclonal rabbit antiserum was raised against a 20-amino acid peptide derived from the nonconserved domain of TFIIA-\textit{ab}. Serum derived from the peptide-immunized rabbits
was reactive to recombinant TFIIAγ, whereas preimmune serum demonstrated no reactivity toward recombinant TFIIAγ (Fig. 3A). The TFIIAγ-specific antiserum was tested for its ability to recognize a specific polypeptide in testis tissue by Western blotting analysis of protein extract derived from human testis or from HeLa cell lines (Fig. 3B). A ∼52-kDa polypeptide was reactive with the peptide-immunized rabbit serum in testis tissue but not in HeLa extract (Fig. 3B). The preimmune serum did not detect any proteins of similar mobility in testis or HeLa extracts. We next tested whether TFIIAγ protein was expressed in a testis-derived teratocarcinoma cell line, NTERA-2 (Fig. 3C). We found that NTERA-2 expressed a ∼52-kDa protein that was reactive to the TFIIAγ-specific antiserum, whereas extracts derived from HeLa (cervical carcinoma) and HepG2 (hepatic) cell lines had much lower or undetectable levels of expression. Again, the preimmune serum did not recognize any polypeptides of similar mobility in the NTERA-2 cell line extracts. These results demonstrate that TFIIAγ is expressed as a 52-kDa protein and that its expression is significantly enriched in testis-derived tissue.

**TFIIAγ Stimulates TBP-DNA Binding—**TFIIAγ was expressed and purified from E. coli and then renatured with the TFIIAγ subunit. TFIIAγ (∧+γ) was compared with TFIIA (αβ+γ) for the ability to form the T-A complex with human TBP and the adenovirus E4 TATA box oligonucleotide probe (Fig. 4A). Addition of 10–20 ng of recombinant human TBP does not form a very stable complex with the E4 TATA under these conditions of electrophoretic mobility shift assay (Fig. 4A, lanes 2 and 3). However, addition of TFIIA (αβ+γ) to TBP results in a stable TATA bound complex with slower mobility (Fig. 4A, lane 4). Addition of TFIIIB to the T-A complex further alters the mobility, indicating that TFIIA can efficiently bind to the T-A complex (Fig. 4A, lane 5). We next tested whether TFIIAγ (∧+γ) could form a T-A complex similar to TFIIA (αβ+γ). Indeed, TFIIAγ (∧+γ) formed a stable T-A complex at similar concentration ranges but slower mobility than TFIIA (αβ+γ) (Fig. 4A, lane 6). Addition of TFIIIB altered the mobility of the T-A complex formed with TFIIAγ (Fig. 4A, lane 7), further indicating that TFIIAγ can support T-A-B complex formation.

**TFIIAγ Can Bind dTAFII110 in Vitro—**TFIIAαβ has been shown to interact with several polypeptides in vitro, including TFIIAγ, TBP, a TBP-related protein (TRP), and dTAFII110 (7, 26). We next determined whether TFIIAγ could similarly interact with these polypeptides using GST pull-down assays. TFIIAαβ and TFIIAγ were translated in vitro and translated in the presence of [35S]methionine and compared for their ability to bind to GST, GST-TBP, GST-TRP, and GST-IIAγ (Fig. 4B). As reported previously, TFIIAαβ bound to GST-TBP, GST-TRP, and GST-IIAγ (top panel). Under identical binding conditions, we found that TFIIAγ bound to GST-IIAγ but did not demonstrate any specific binding for GST-TBP or GST-TRP (Fig. 4B, middle panel). Luciferase control did not bind significantly to GST, GST-TBP, GST-TRP, or GST-IIAγ (bottom panel). Thus, although TFIIAγ (∧+γ) could form a stable T-A complex with TBP in electrophoretic mobility shift assay (Fig. 4A), it appears that some TBP-specific contacts made by TFIIAαβ may be absent in TFIIAγ.

TFIIAγ was compared with TFIIAαβ for its ability to bind to dTAFII110 (Fig. 4C). In vitro translated [35S]methionine-labeled dTAFII110 was tested for its ability to bind GST, GST-IIAαβ, or GST-IIAγ. As shown previously, dTAFII110 bound GST-IIAαβ but did not bind significantly to GST alone (Fig. 4C, upper panel). Interestingly, dTAFII110 also bound GST-IIAγ more abundantly than it bound to GST-αβ (Fig. 4C, lane 4). Luciferase control protein did not bind significantly to GST, GST-IIAαβ, or GST-IIAγ. These results indicate that TFIIAγ
shares with TFIIAαβ the ability to bind dTAF110 and suggests that TFIIAα will be capable of interacting with TFIID subunits to stimulate transcription.

**TFIIAα** Can Stimulate Basal and Activated Transcription—

HeLa nuclear extracts were depleted of TFIIA activity by incubation with nickel-nitritriacetic acid beads, which bind specifically to the stretch of seven histidines present in the nonconserved region of TFIIAαβ. These extracts have been used previously to demonstrate the activity of recombinant TFIIA (αβγ) in transcription reactions with various activators (26). TFIIA-depleted extracts were shown to be defective for basal transcription with a promoter (TATA/Inr) containing the strong core promoter elements derived from the adenovirus major late promoter TATA box and the terminal deoxynucleotidyl transferase promoter initiator (Fig. 5A). Depleted extracts (Fig. 5A, lane 1) in the absence of exogenous TFIIA were significantly reduced for transcription compared with undepleted extracts (Fig. 5A, lane 4). Addition of recombinant TFIIA reconstituted with TFIIA (αβγ) stimulated transcription from the basal promoter in depleted extracts (Fig. 5A, lane 2). Similarly, addition of TFIIAα (αγ) to TFIIA-depleted extracts stimulated transcription from the TATA/Inr promoter (Fig. 5A, lane 3), indicating that TFIIAα functions in basal transcription.

TFIIA-depleted extracts have also been used to demonstrate a stimulatory effect by TFIIA on transcription regulated by various activators (26). Transcription stimulated by the activators is defective in TFIIA-depleted extracts with the activation domains fused to the GAL4 DNA-binding domain (Fig. 5B). In the absence of exogenous TFIIA, transcription was significantly reduced (Fig. 5B, lanes 1, 4, and 7). Addition of TFIIA reconstituted with TFIIA (αβγ) stimulated transcription in the presence of GAL4-VP16 (Fig. 5B, lane 2), GAL4-CTF (Fig. 5B, lane 5), and GAL4-AH (Fig. 5B, lane 8). Addition of recombinant TFIIAα (αγ) subunits also stimulated transcription from GAL4-VP16 (Fig. 5B, lane 3), GAL4-CTF (Fig. 5B, lane 6), and GAL4-AH (Fig. 5B, lane 9). These results suggest that TFIIAα can support transcription activated by several activators with efficiency similar to that of TFIIA αβ.

We next compared the ability of TFIIA (αβγ) and TFIIAα (αγ) to stimulate transcription with the Epstein-Barr virus lytic activator, Zta. Zta can form a stable complex with TFIIA (αβγ) and TFIIID and may interact with TFIIA in a manner different than the activators described in Fig. 5B. In TFIIA-depleted extracts, Zta did not stimulate transcription in the absence of exogenous TFIIA (Fig. 5C, lane 1). Addition of TFIIA (αβγ) strongly stimulated Zta activated transcription (Fig. 5C, lane 2). Interestingly, addition of TFIIAα (αγ) and TFIIA (αγ) were carefully normalized for T-A complex formation and for transcription stimulation by GAL4-AH (data not shown). At no concentration of TFIIA (αγ) was Zta transcriptional activation restored to levels detected with TFIIA (αβγ). These results indicate that TFIIAα is defective for Zta-mediated transcription and suggest that TFIIAα may have specificity for a subset of transcriptional activators.

**DISCUSSION**

Tissue-specific gene expression is thought to occur largely through the combinatorial effects of tissue- and promoter-specific activators and repressors (49). The general transcription factors are thought to be essentially the same in all tissue types and may respond in a limited number of mechanisms to the synergistic signals generated by enhancers and promoters (1, 2). Variation of the composition of general transcription factors has been observed in vitro for several promoters and has been implicated by some genetic experiments in yeast S. cerevisiae (50–54). The identification of tissue-specific general transcription factors and coactivators suggests that tissue-specific gene expression may also be controlled by variation in the components of the preinitiation complex. The most provocative example is the identification of a neural tissue-specific form of TBP that has been isolated from Drosophila (38, 39). However, a
similar neural-specific form of TBP has not yet been identified in human tissue.

In this work we describe the first example of a human tissue-specific general transcription factor. TFIIAα was discovered by analysis of a database of expressed sequence tags derived from various human tissues. TFIIAα-specific ESTs were found in testis tissue and Northern blot analysis detected TFIIAα mRNA only in testis. Antibodies raised against a TFIIAα-specific peptide reacted to a 52-kDa protein found in testis-derived tissue and cell line extract. TFIIAα protein complexed with TFIIAγ to form a functional TFIIA heterodimer that could stimulate TBP-TATA binding and support TFIIA assembly in electrophoretic mobility shift assays. TFIIA (αβ+γ) stimulated transcription in vitro from the GAL4-AH, VP16, and CTF transcription activators, with similar activity to that of TFIIA (αβ+γ). However, for at least one transcriptional activator, Zta, we found that TFIIA (αβ) was reduced for activity relative to TFIIA (αβ+γ). Thus, TFIIAα may confer activator specificity on the general transcriptional machinery by providing novel interacting surface for activation domain targets. Thus, TFIIAα appears to be a tissue-specific subunit of a general transcription factor that may have overlapping, as well as unique features relative to the ubiquitously expressed TFIIAαβ.

Several genes have been identified that are closely related to general transcription factor polypeptides. A TFIIIB-related polypeptide (BRF), identified originally as a high copy suppressor of a TFIIA mutation, has been found to be an essential component of the RNA polymerase III general transcription factor TFIIIB (55). TRF1 has been identified from a Drosophila genetic screen for neurological phenotypes (38). TRF1 has subsequently been found to function homologously to TBP but is expressed predominantly in neural tissue (39). TRF1 can substitute for TBP in TATA binding and transcription reactions reconstituted with purified remaining general transcription factors. Like TBP, TRF1 associates with a novel set of associated factors, which are likely to confer promoter and activator specificity to TRF1 (39). A mouse TBP-like protein has been cloned (56). Unlike Drosophila TRF1, TRP-like protein is expressed in most tissue examined and does not bind TATA-DNA. We have also identified a human TRP that is highly related to TRP-like protein.2 TRP does not bind to TATA-DNA but does interact with general transcription factors TFIIA and TFIIIB. Unlike, TBP and TRP, TRP inhibits transcription in vitro and in vivo, presumably by sequestering essential general transcription factors, like TFIIA and TFIIIB.3 The isolation of a testis-specific form of TFIIAβ raised the question of whether TFIIAα was the counterpart of an altered form of TBP, perhaps TRP. However, TFIIAα does not bind TRP in solution (Fig. 4), and we have been unable to determine any association or stimulation of TRP-DNA binding activity by TFIIAα (data not shown).

The designation of TFIIA as a general transcription factor may be a misnomer, TFIIA is not required for reconstitution of basal transcription with highly purified essential general factors (33). TFIIA may better be characterized as a global but not universal transcriptional coactivator and derepressor. Several tissue-specific coactivators have been previously characterized. These include, among others, a B cell-specific form of TAF1135 (40) and OCA-B, a B cell-specific coactivator for the octamer-binding protein 1 (57). More recently, a testis-specific coactivator (ACT) has been identified for the testis-specific isoform of the cyclic AMP response element modulator (CREMβ) (58, 59). Interestingly, both the promoter-specific factor CREMα and the CREM-specific coactivator ACT have expression restricted to testis tissue. Testis-specific gene expression may require specialized coactivators and global regulators, like ACT and TFIIAα, to function in the protamine-rich environment unique to post-meiotic testis tissue. Future work can address the role of TFIIAα in germ line-specific transcription regulation.

Acknowledgment—We thank Stephan Przyborski for providing the NTERA-2 cell line. References
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