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Transcription Factor IIA τ Is Associated with Undifferentiated Cells and Its Gene Expression Is Repressed in Primary Neurons at the Chromatin Level In Vivo

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ABSTRACT

The levels of General Transcription Factor (TF) IIA were examined during mammalian brain development and in rat embryo fibroblasts and transformed cell lines. The large TFIIA subunit paralogues $\alpha\beta$ and τ are largely produced in unsynchronized cell lines, yet only TFIIA $\alpha\beta$ is observed in a number of differentiated tissue extracts. Steady-state protein levels of the TFIIA τ, $\alpha\beta$, and γ subunits were significantly reduced when human embryonal (ec) and hepatic carcinoma cell lines were stimulated to differentiate with either all-*trans*-retinoic acid (ATRA) or sodium butyrate. ATRA-treated NT2-ec cells required replating to induce a neuronal phenotype and loss of detectable TFIIA τ and γ proteins. High levels of TFIIA τ, $\alpha\beta$, and γ and Sp factors were identified in extracts from human fetal and rat embryonic day-18 brains, but not in human and rat adult brain extracts. A high histone H3 Lys9/Lys4 methylation ratio was observed in the TFIIA τ promoter of primary hippocampal neurons from day-18 rat embryos, suggesting that repressive epigenetic marks of chromatin prevent TFIIA τ from being transcribed in neurons. We conclude that TFIIA τ is associated with undifferentiated cells during development, yet is down-regulated at the chromatin level upon cellular differentiation.

INTRODUCTION

TRANSCRIPTION FACTOR (TF) IIA mediates activator-dependent recruitment and assembly of the general transcription apparatus at core promoters (1,2). TFIIA stimulates TFIID [TATA-binding protein (TBP) + TBP associated factors-TAF$_{II}$s] binding to core promoters, thereby nucleating the formation of committed transcription preinitiation complexes (1,3,4). Human (hu) TFIIA consists of three conserved subunits (α, β, and γ) (4–6). The human TFIIA$\alpha\beta$ subunit shares 54% and 72% similarity to approximately 60-amino-acid stretches at the

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amino and carboxyl termini of the yeast large subunit, respectively (5,6). The TFIIA$\alpha\beta$ subunit is produced as a proprotein that is cleaved to α and β subunits in HeLa cells (5). Because both $\alpha\beta$ and the individual $\alpha + \beta$ subunits show similar transcriptional activity in vitro (4), the functional significance of TFIIA $\alpha\beta$ proteolysis is not well investigated. Several hydrophobic residues in the TFIIA β-sheet domain contact the TBP (7–11), while factors that bind to the TFIIA four-helix bundle domain have not yet been identified. Yeast strains (*toa2*) with defective TBP–TFIIA interactions show temperature-sensitive (ts) growth properties and reveal reduced levels of cell cycle-associated gene expression, but show normal levels of constitutive class II gene expression (10). These defective *toa2* strains also show defects in activator-dependent transcription levels from genes that contain multiple start sites (10). TFIIA and TAF1 interactions are critical for transcription start site selection from the *Drosophila Adh* promoter (12). TBP mutations in the TFIIA interaction region reveal reduced activator-dependent transcription levels in mammalian cell lines (13).

A human paralogue of the TFIIA$\alpha\beta$ subunit is referred to as the TFIIAτ subunit or ALF (TFIIA$\alpha\beta$-like factor) (14,15). TFIIAτ has 60-amino-acid amino- and carboxyterminal domains that show 94% similarity to the same domains in TFIIA$\alpha\beta$; however, the approximately 250-amino-acid nonconserved domain (NCD) is unique between the paralogues (14,15). TFIIAτ is expressed at high levels and translated in human NTera-2 (NT2) embryonal carcinoma (-ec) cells and male germ line tissue, yet TFIIAτ mRNA and protein levels were reduced in extracts prepared from several highly differentiated tissues (14,15). The TFIIA$\tau+\gamma$ subunit complex stimulates the formation of TBP–TFIIA–DNA and TBP–TFIIA–TFIIB–DNA complexes using the electrophoretic mobility shift assay (EMSA) (14,16). TFIIA$\tau+\gamma$ is a transcriptional co-activator for the GAL4-AH and -CTF activators in vitro (14). Because TFIIA$\tau+\gamma$ does not mediate the activity of the Epstein-Barr viral-encoded Zta transcription activator, it may be regarded as a selective co-activator of transcription (14). By contrast, TFIIA$\alpha\beta+\gamma$ functions as a general co-activator of transcription with Zta, Sp1, and additional transcriptional activators (4,8,17).

All-*trans*-retinoic acid (ATRA) induces NT2-ec cells to differentiate into neurons (18,19). NT2-ec cells are considered to be the malignant counterparts of embryonic stem cells because both cell types produce pluripotent markers TRA-1-60 and Oct-4, unlike neuronal precursors that do not produce these markers (20–22). Furthermore, NT2-ec cells differ from neuronal precursors in their capacity to be induced to form other cell types than those of the neural lineage (20–22). The induction of neuronal-specific gene expression in ATRA-treated NT2 cells is a stage-dependent process (23). The role of retinoic acid (RA) in the NT2 differentiation process is considered a model for the role of endogenous RA during embryonic neuronal development (24,25). ATRA similarly induces the differentiation of the human HepG2 hepatocyte line and the Caco-2 colon cell model (26–28). Also, certain retinoids, such as ATRA, have been employed as cancer chemoprevention in humans, but the frequent development of tumor cell resistance has substantially limited the clinical utility of these compounds as therapeutics (29). Because TFIIAτ is expressed in NT2-ec cells, we propose that it may have a regulatory role in brain development and contribute to maintenance of an undifferentiated cellular phenotype. In this study, we examined whether the TFIIA subunits were regulated in a development-specific manner in brain tissue and cultured neurons. Our results suggest that the TFIIA$\tau+\gamma$ complex reveals properties consistent with a general regulator of the undifferentiated cellular phenotype.

MATERIALS AND METHODS

Animal subjects

The embryonic day-18 (E18) and postnatal day-2 (P2) rat brains were surgically isolated as described elsewhere (30). Rat embryo fibroblasts (REF) were isolated, and extracts were prepared as described elsewhere (30). These studies were approved by the Institutional Animal Care and Use Committee from the Boston University School of Medicine, and all procedures described were performed in accordance with recommendations outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NRC, 1996) (see http://www.nap.edu/readingroom/books/labrats/contents.html).

Human tissue

Human 20- to 40-week-old aborted fetal brain, adult brain, ileum, liver, heart, lung, and testis extracts for western blot experiments were prepared by and obtained from BD Biosciences. This study was approved by the Institutional Review Board from the Boston University School of Medicine. Authors performed these studies after completing human subject protection training and certification as outlined by the National Institutes of Health and the Boston University School of Medicine.

Cell lines

The cell lines used included HeLa S3 (human cervical adenocarcinoma-ATCC), HepG2 (human hepatocellular carcinoma-ATCC), HEK293 (human 293 cells infected with adenovirus-ATCC), HT-29 (human colorectal adenocarcinoma-ATCC), Namalwa cells (human Burkitt's

lymphoma-ATCC), NTera2 (NT2) (human embryo teratocarcinoma-gift from P. Andrews), P19 (mouse embryo teratocarcinoma-ATCC), and Jurkat cells (human T-cell acute lymphoblastic leukemia cell line-Cell Signaling Technology).

Cell culture and differentiation

NT2-ec and P19 cells were maintained in Dulbecco's modified Eagle medium (DMEM), 10% fetal bovine serum (FBS), and 100 units penicillin/100 μg streptomycin (BioWhittaker) in 10% CO_2. NT2-ec cells were treated with 10 μM ATRA (Sigma) for up to 4 weeks at weekly intervals (18) and replated on P100 Δ plates (Nunc) coated with 100 μg/ml poly-D-lysine (Sigma) to purify neurons as described elsewhere (19). NT2-neurons were scraped from the Δ plates in ice-cold 135 mM phosphate-buffered saline (1 $\times$ PBS) for biochemical isolation. HepG2 cells (ATCC) were grown in DMEM, 10% FBS, and 100 units of penicillin/100 μg streptomycin (BioWhittaker) in 5% CO_2. The HepG2 culture medium was supplemented with ATRA at a final concentration of 10 μM as described elsewhere (26,28). HepG2 and HT-29 cells were grown in roller bottles and harvested at approximately 80% confluence. HeLa and Namalwa cells were grown in suspension as described (4,14).

Cell proliferation assays

The CellTiter 96 Aqueous One Solution Cell Proliferation Assay is composed of a tertrazolium compound, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tertazolium, inner salt (MTS*) and an electron-coupling reagent, phenazine ethosulfate (PES). The assay was performed for 1 h at 37°C as described (Promega). The NT2 cell line conversion of MTS to a water-soluble formazan was detected by absorbance at 490 nm.

Whole cell, nuclear, and tissue extract preparations

Nuclear extracts (NE) were prepared as described elsewhere (4,31). The Jurkat cell NE was obtained from Cell Signaling Technology. The following protease inhibitors were used for the production of NE: 10 μg/ml aprotinin A, 5 μM leupeptin, 5 μM pepstatin, 10 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride (PMSF). NE was dialyzed against D100 buffer: 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), pH 7.9, 20% glycerol, 0.2 mM EDTA, 100 mM KCl, 7 mM β-mercaptoethanol, and 1 mM PMSF, clarified at 12,000 $\times$ *g*, flash frozen, and stored at -80°C. Rat E18 brain tissue extracts from either whole-cell or nuclear fractions were prepared using protease inhibitors (above) and showed similar levels for β-actin and TFIIAτ in Western blot assays (data not shown). Whole-cell extracts for cell lines and other tissues were prepared in lysis buffer: 50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol (DTT), 2% sodium dodecyl sulfate (SDS), and 10% glycerol, sonicated for 2 min, placed in a 90°C dry block for 10 min, and stored at -80°C until use. Protein concentrations of the samples were determined by Bradford assays (Promega) and 50 μg applied to 12% SDS-PAGE gels for the Western blot experiments. Equal protein amounts for sample loading were confirmed by either glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and/or β-actin levels in western assays.

Western assays and antibodies

Rabbit polyclonal antibodies were raised against the following MAP-peptides: TFIIAτ1 (175-HDESLSTSPHGALHQHVTDI-194), τ2 (58-RNSIQSLPFTLQLPHSLHQT-77), and $\alpha\beta$ (115-PQVIVPDSKLIQHMNASNMS-135), which correspond to unique TFIIA NCD sequences (Invitrogen) (14). The TFIIA peptide sequences showed poor identity to the NCBI Entrez Proteome using BLAST programs (not shown). Sites within the NCDs that showed identity to known proteolysis consensus motifs were not selected for antibody production (32; data not shown). Pure nitrocellulose was used for the Western blot experiments (Osmonics). The electrotransfer of proteins was performed at 2,000 mA-hr, and washes were performed sequentially using 300 mM NaCl 1$\times$ PBS, 300 mM NaCl, 1$\times$ PBS, and 0.05% Nonidet-40, and 300 mM NaCl 1 $\times$ PBS. The western blots were incubated with TFIIA antibodies (1:500) in 5% nonfat dried milk and 1$\times$PBS for 12 h at 4°C. TFIIAτ Ab1 was described elsewhere (14). The TFIIAγ rabbit polyclonal antibody was used at 1:250 as described elsewhere (8). The TRK-A and TBP rabbit polyclonal antibodies were used at 1:500 (Upstate Biotechnology). The RARβ and Sp antibodies from Santa Cruz Biotechnology were used as described elsewhere (28). The rabbit G3PDH antibody was used at 1:1,000 (ATCC). The goat anti-rabbit secondary antibody was used at 1:2,000 (Sigma). The mouse immunoglobulin G_{2A} (IgG_{2A}) against β-tubulin III was used at 1:1,000 (Upsate Biotechnology). The mouse monoclonal anti-human β-actin clone AC-15 was used at 1:1000 (Sigma). The goat anti-mouse IgG (Fc-specific) secondary antibody was used at 1:2000 (Sigma). The enhanced SuperSignal West Pico chemiluminescent substrate for horseradish peroxidase (HRP) was used for the detection of Western blot signals (Pierce). Broad molecular weight range and other prestained protein markers were used for the Western blot assays (New England Biolabs).

Northern assay

The Northern analysis was performed as described elsewhere (14).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were performed as described by Upstate Biotechnology. Briefly, human HEK293 cells or rat primary hippocampal neurons were maintained on 100-mm tissue culture dishes (1.33 brains per dish) and subjected to formaldehyde treatment to crosslink protein–DNA interactions in vivo. Primary hippocampal neurons (4×10^6 cells per assay) were derived from rat E18 brain tissues and grown as described in serum-free conditioned medium (33). Cortical cultures were maintained for 7 days in culture. Extracts were sonicated prior to immunoprecipitations. Anti-dimethyl-histone H3 (Lys9) or (Lys4) rabbit IgG antibodies (Cat. No. 07-212 and 07-030, respectively) were used (1:200; Upstate Biotechnology) to immunoprecipitate cross-linked protein–DNA complexes. Total input DNA and a sample lacking antibody as a control were included in all experiments to determine the relative efficiency of immunoprecipitations. Immunoprecipitated DNAs were isolated and dissolved in 1,000 μl of TE. A quantitative PCR assay was used to determine a linear amplification range for each experiment. A 10-μl sample of immunoprecipitated genomic DNAs was used as template for PCR reactions (*Pfu* Polymerase, Stratagene) to amplify either the *Megalin* (168-bp) or *TFIIA*τ (212-bp) promoter fragments. Primers: 5′-GTGTTCGAGTGCCCACTCCTC-3′ (hu Megalin-forward), 5′-GTGTTCAAGTGCCCACTCCTC-3′ (rat Megalin-forward), and 5′-GAGGCATCCCGTTTTCTACCC-3′ (human and rat Megalin-reverse). Primers 5′-CCGCCTCTCCGCCTTGACCCCC-3′ (human TFIIAτ-forward), 5′-TTCCTTACCACACACGTGCCAC-3′ (rat TFIIAτ-forward). 5′-TCCTTACCACCGGGTTGAGGCA-3′ (human TFIIAτ-reverse), and 5′-CGCCTCCTTACCACCAGGTTGA-3′ (rat TFIIAτ-reverse). ^{35}S-Labeled PCR products were resolved on 5% polyacrylamide gels, exposed to X-ray film (Kodak), and quantified using a Phosphorimager.

RESULTS

TFIIAτ and $\alpha\beta$ subunits are produced in unsynchronized mammalian cell lines

We compared the production of TFIIA$\alpha\beta$ and τ in exponentially growing, unsynchronized mammalian tumor cell lines. We initially examined whether these TFIIA large subunits were expressed in the mouse embryonal carcinoma cell line P19 because the embryonal line NTera-2 was reported to produce TFIIAτ (14). Second, we examined whether TFIIAτ was produced in the HepG2, HeLa, Namalwa, and Jurkat cell lines, all of which have been extensively used as models to characterize eukaryotic transcription mechanisms (14,28,34). TFIIAτ and $\alpha\beta$ were both observed in extracts prepared from HT-29, HepG2, NTera-2 (NT2), HeLa, and Namalwa cell lines (Fig. 1A). Also, TFIIAτ was detected in the P19 cell, but not in the Jurkat cell extract. By contrast, TFIIA$\alpha\beta$ was produced in Jurkat cells, but not in the P19 cell extract (Fig. 1A). β-Actin levels were similar amongst samples in the cell line panel (Fig. 1A). Jurkat cells are a mature and lineage committed T cell line (35); thus, the reduction of TFIIAτ levels in Jurkat cells may be dependent upon their commitment to certain differentiation processes. The NT2 and P19 embryonal carcinoma lines are undifferentiated and have been reported as models to study the regulation of neuronal differentiation in culture (19). We conclude that both $\alpha\beta$ and τ are produced in the vast majority of tumor cell lines tested (Fig. 1A).

To test whether TFIIAτ is also associated with developing nonneuronal cells, we prepared lysates from rat day-18 embryo fibroblasts (E18 REF). TFIIAτ and $\alpha\beta$ are produced in normal human testis tissue and we observed TFIIAτ in the E18 REF whole-cell extract as well (14) (Fig. 1B). Because testis tissue has low β-actin levels (36), G3PDH levels were used to demonstrate comparable total protein levels between the testis and E18 REF lysates (Fig. 1B). We conclude that TFIIAτ is produced in normal developing cells as well as in unsynchronized tumor cell lines.

Because TFIIAτ mRNA expression was not detected in differentiated tissues (14,15), we compared the levels of TFIIAτ and $\alpha\beta$ in a panel of extracts prepared from a human tissue panel (Fig. 1C). Human ileum, liver, heart, lung, and brain whole-cell extracts were probed with either TFIIAτ or $\alpha\beta$ anti-serum (Fig. 1C). TFIIAτ was not detected in extracts from the human tissue panel (Fig. 1C). TFIIA$\alpha\beta$ was robustly detected in human ileum, liver, heart, and lung whole-cell extracts, but extremely low levels were detected in brain whole-cell extracts (Fig. 1C). The levels of β-actin were similar in human ileum, liver, lung, and brain whole-cell extracts, but not in heart extracts, which were previously shown to have extremely low β-actin levels (Fig. 1C) (36). We conclude that TFIIA$\alpha\beta$ is produced at high levels in many human tissues, except brain (Fig. 1C). Furthermore, our findings are consistent with previous reports that observed low TFIIAτ mRNA levels in a number of differentiated tissues (Fig. 1C) (14,15).

TFIIA subunit levels are significantly reduced in NT2-neuronal cells

Both ATRA treatment and replating on a poly-D-lysine-coated surface stimulates human NT2-ec cells to dif-

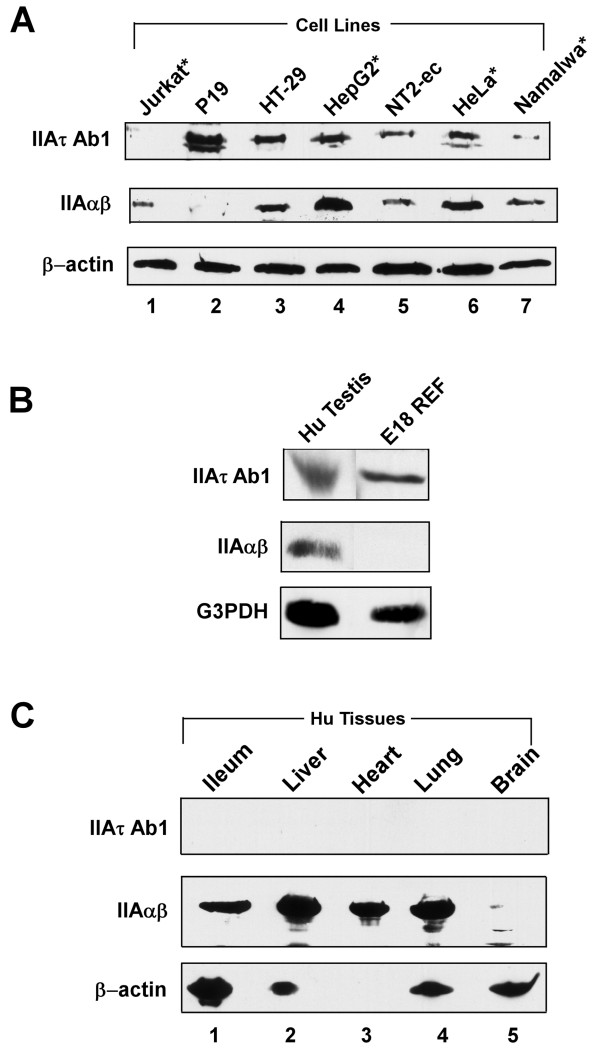

FIG. 1. The TFIIA$\alpha\beta$ and τ subunits were observed in proliferating mammalian cells lines. (**A**) Lane 1, Jurkat*; lane 2, P19; lane 3, HT-29; lane 4, HepG2*; lane 5, NT2-ec; lane 6, HeLa*; and lane 7, Namalwa*; whole-cell or NE extracts (*) were used in Western blot assays with antibodies against either TFIIAτ (Ab1) (52 kD) (*top panel*), IIA$\alpha\beta$ (42 kD) (*middle panel*), or β-actin (43 kD) (*bottom panel*). (**B**) Human (hu) testis tissue and day-18 (E18) rat embryo fibroblast (REF) extracts were used in Western blot assays with IIAτ (Ab1) (*top panel*) and IIA$\alpha\beta$ antibody (*middle panel*). G3PDH antibody was used in Western blots (37 kD) (*bottom panel*). Equal protein levels were loaded, 50 μg/sample. (**C**) Lane 1, Ileum; lane 2, liver; lane 3, heart; lane 4, lung; and lane 5, brain; whole-cell extracts were used in a Western blot assay with antibodies against either IIAτ (Ab1) (*top panel*), IIA$\alpha\beta$ (*middle panel*), or β-actin (*bottom panel*).

ferentiate into neurons (18). After several weeks of RA treatment and replating, NT2 neuronal cells become terminally differentiated (Fig. 2A,B) (19). In this study, we examined whether the levels of TFIIA subunits were modulated during ATRA-induced differentiation of NT2-ec cells to neurons.

We reported that yeast TFIIA mutants with substitutions at the interface with TBP reveal a temperature-sensitive growth phenotype and arrest at the G_2/M border of the cell cycle (10). As it is not fully understood whether TFIIA regulates mammalian cell growth control, we examined the levels of TFIIA subunits during the NT2 neuronal differentiation process. The treament of NT2-ec cells with ATRA and replating caused neuronal differentiation, as observed by light microscopy analysis (Fig. 2A,B) (19). TFIIAτ expression was comparatively analyzed in NT2-ec and neuronal cells. High levels of TFIIAτ mRNA were observed in NT2-ec cells (14), which decreased progressively in NT2 cells that had been treated with ATRA for increasing lengths of time (Fig. 2C). β-Actin mRNA levels were equivalent amongst these samples (Fig. 2C). We concluded that *TFIIAτ* expression is negatively regulated by ATRA treatment, which is required for NT2 cells to terminally differentiate to neurons (Fig. 2).

Changes in TFIIAτ levels during NT2 differentiation were further investigated by analyzing a Western blot that contained extracts from NT2-ec cells, NT2-ec cells treated with ATRA for 28 days, or NT2 neurons. Antibodies (τAb1) were made against a TFIIAτ-NCD peptide and specifically detect TFIIAτ (Fig. 3A and Materials and Methods). TFIIAτ was detected in untreated NT2-ec cells and cells treated for 28 days with ATRA (Fig. 3A, row 1, columns A and B). Importantly, steady-state levels of TFIIAτ were not observed in NT2 neurons (Fig. 3A, row 1, column C). Cell–cell signaling contacts

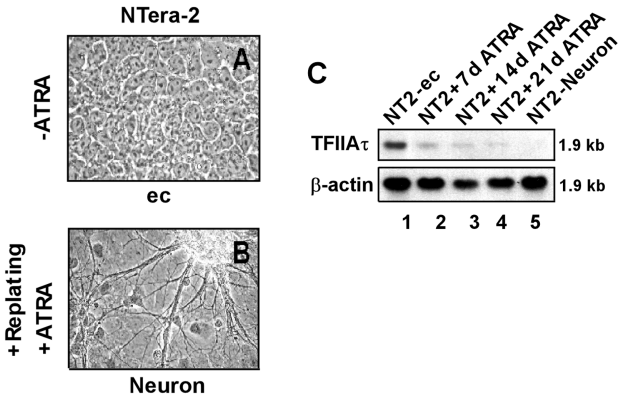

FIG. 2. TFIIAτ mRNA was expressed in NT2-ec, but not in NT2-neuronal cells. Human NTera2 (NT2) embryonal carcinoma (-ec) cells (**A**) and differentiated NT2 neurons (**B**) are shown. Cells are shown at 10× power using phase-contrast light microscopy (Nikon TMS) to view cells (compare **A** and **B**). (**B**) ATRA treatment (+) (21 days) and replating (+) are indicated. (**C**) NT2-ec and ATRA-induced NTera2 neuronal differentiation (7 days, 14 days, 21 days ATRA or neuron) is shown (*top*). TFIIAτ and β-actin mRNA levels were detected in a Northern blot assay using ^{32}P-labeled probes (*top and bottom panels*, respectively). Sizes are shown to the right in kilobases.

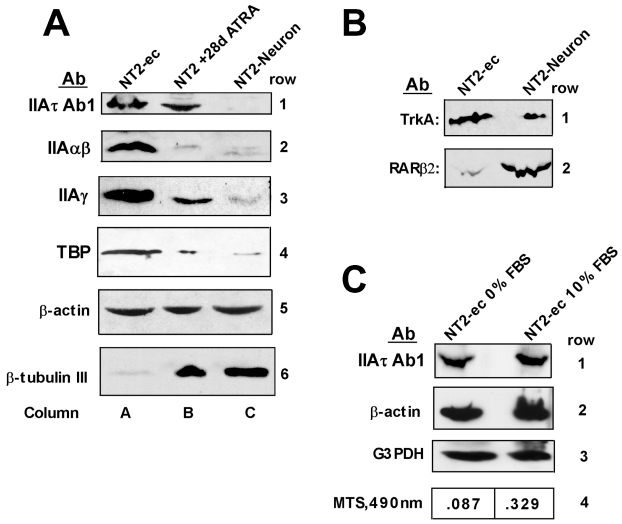

FIG. 3. TFIIA subunit levels are associated with undifferentiated NT2-ec cells. (**A**) TFIIAτ levels were detected using τAb1 and whole-cell lysates from NT2-ec (column A). NT2+28d ATRA (column B), and NT2-Neurons (NT2-N) (column C) (*top*). TFIIAτ peptide (amino acids 175–194) antibody 1 (τAb1) signal is shown (τ-52 kD) (row 1). TFIIA$\alpha\beta$ (42 kD) levels are shown using a polyclonal peptide (amino acids 115–135) antibody (row 2). TFIIAγ (12 kD) levels are shown using a polyclonal γ antibody (row 3). TBP (37 kD) levels were assayed using the amino-terminal antibody (Upstate) (row 4). β-actin (43 kD) levels were used as controls (row 5). β-tubulin III (50 kD) levels were used to indicate neuronal development (row 6). (**B**) Trk A (87 kD) (row 1) and RARβ2 (40 kD) (row 2) levels were examined in NT2-ec and NT2-neuron whole-cell extracts in a Western blot assay (above). (**C**) NT2-ec cells were treated with either 0% or 10% FBS for 3 days (above). Whole-cell extracts were made and used in Western blot assays with either IIAτ Ab1 (row 1), β-actin Ab (row 2), or G3PDH Ab (row 3) (*left*). MTS is converted by proliferating NT2-ec cells into a water-soluble formazan compound that was detected at 490 nm and is indicative of mitosis. Cells were treated with MTS reagent (Promega) and incubated at 37°C for 1 h and analyzed at 490 nm. OD 490 nm readings are shown (row 4).

during replating are critical for neuronal differentiation to occur (37,38). Thus, TFIIAτ might be degraded during the neuronal replating step. Low TFIIAτ mRNA levels after initial ATRA treatments and stable TFIIAτ levels after prolonged ATRA treatment (28 days) indicate that TFIIAτ is a long-lived protein (Figs. 2C and 3A). Other reported long-lived proteins include numerous sarcoplasmic reticulum proteins from skeletal muscle that have half-lives up to 2 weeks in length in vivo, including 53-kD glycoprotein and calsequestrin (39).

TFIIA$\alpha\beta$ levels were high in NT2-ec cell extracts, yet were reduced in extracts from cells treated for 28 days with ATRA and in NT2 neuronal extracts (Fig. 3A, row 2). Importantly, steady-state TFIIAτ protein levels were stable, even after 28 days of ATRA treatment, which was not observed with the $\alpha\beta$ subunit (Fig. 3). The levels of the small TFIIA subunit (γ) were high in NT2-ec cells, but substantially reduced in NT2-ec cells that had been treated for 28 days with ATRA and barely detectable in NT2 neuronal extracts, which is similar to our observations with the τ subunit (Fig. 3A, row 3). Because TBP–DNA binding is stimulated by TFIIA (4), we examined the levels of TBP during NT2 neuronal differentiation. We observed reduced TBP levels during NT2 differentiation into neurons (Fig. 3A, row 4), similar to reports that utilized F9 cells (40). The β-actin levels were comparable between the samples in the panel (Fig. 3A, row 5). β-Tubulin III levels correlate with NT2 neuronal development and increased with ATRA administration (Fig. 3A, row 6) (41,42). Interestingly, β-tubulin III levels were significantly increased by ATRA treatment for 28 days, yet prior to the replating step of neuronal differentiation (Fig. 3A, row 6). Furthermore, β-tubulin III levels in NT2-ec cells were induced by reduced serum exposure (2%) for 2 days (data not shown).

The TRK-A receptor is expressed in both NT2-ec and NT2 neuronal cells, as reported elsewhere for developing and differentiating neurons (Fig. 3B, row 1) (43). Additionally, RARβ2 receptor levels were stimulated by ATRA treatment of NT2 cells as reported elsewhere (Fig. 3B, row 2) (44,45). Thus, TFIIAτ mRNA and protein levels were inversely correlated to NT2 neuronal development. Furthermore, TFIIA$\alpha\beta$ and γ levels were inversely correlated to both ATRA treatment and NT2 neuronal development. We conclude that TFIIA subunits are associated with undifferentiated NT2-ec cells, not neurons.

The TFIIAτ subunit was observed in serum-starved NT2-ec cells in vitro

Although TFIIAτ appears to be associated with proliferating REF cells and tumor cell lines (Fig. 1), we tested whether TFIIAτ was more strictly associated with an undifferentiated phenotype. Use of a proliferation assay in combination with serum starvation of NT2-ec cells was used to determine the correlation of TFIIAτ levels with a proliferative or undifferentiated cellular phenotype. Because undifferentiated NT2-ec cells treated with ATRA for 28 days show high TFIIAτ levels, we tested whether serum-deprived NT2-ec cells might also show high TFIIAτ levels. NT2-ec cells were plated at 30% confluence and maintained in either standard 10% FBS DMEM or in 0% FBS DMEM for 3 days. A Western blot analysis and a chemically based proliferation assay were performed simultaneously with cell samples to correlate TFIIAτ protein levels with the extent of proliferation in the cell culture samples. The MTS assay is a nonisotopic proliferation assay that is more sensitive and precise compared to radiolabeled thymidine incorporation (46). We

determined that NT2-ec cells grown in 10% FBS for 3 days showed a four-fold higher reduction of the MTS tertazolium compound in comparison to NT2-ec cells maintained in DMEM alone (Fig. 3C, row 4). An increase in MTS reduction correlates with an increased cellular proliferation capacity. A four-fold increase of the OD 490-nm value between the samples is a nearly 16-fold difference in NT2 cell number (Fig. 3C, data not shown). Serum deprived NT2-ec cells adhered to the tissue culture plates (Δ Nunc) with a similar morphology to 10% FBS-fed NT2-ec cells (data not shown). We did not observe apoptosis, which is correlated with cell-surface detachment from a different plate surface, as reported elsewhere (47; data not shown).

Whole-cell extracts were prepared from 0% FBS-treated and 10% FBS-treated NT2-ec cells, which were assayed in a Western blot with either TFIIAτ Ab1, β-actin, or G3PDH antibodies (Fig. 3C). Interestingly, NT2-ec cells that have been treated with 0% FBS or 10% FBS showed similar levels of TFIIAτ, as well as the controls β-actin and G3PDH (Fig. 3C). Comparison of NT2 cultures grown in either 2% FBS and 10% FBS showed identical MTS reduction levels, and the corresponding extracts revealed similar TFIIAτ and control levels in western blots (data not shown). Thus, 3 days of serum deprivation, not serum reduction, caused decreased MTS activity in NT2-ec cells. Because the 0% FBS- and 10% FBS-treated NT2-ec extracts revealed similar TFIIAτ levels, we conclude that TFIIAτ is most closely associated with an undifferentiated cellular phenotype (Fig. 3).

TFIIA subunits levels are developmentally regulated in human brain

TFIIAτ was shown to have restricted levels of mRNA expression amongst a panel of differentiated tissues in a slot blot and Northern analysis (14,15). Because EST analysis readily detects low levels of gene expression, National Center for Biotechnology Information (NCBI) expressed sequence tag (EST) databases were queried to identify

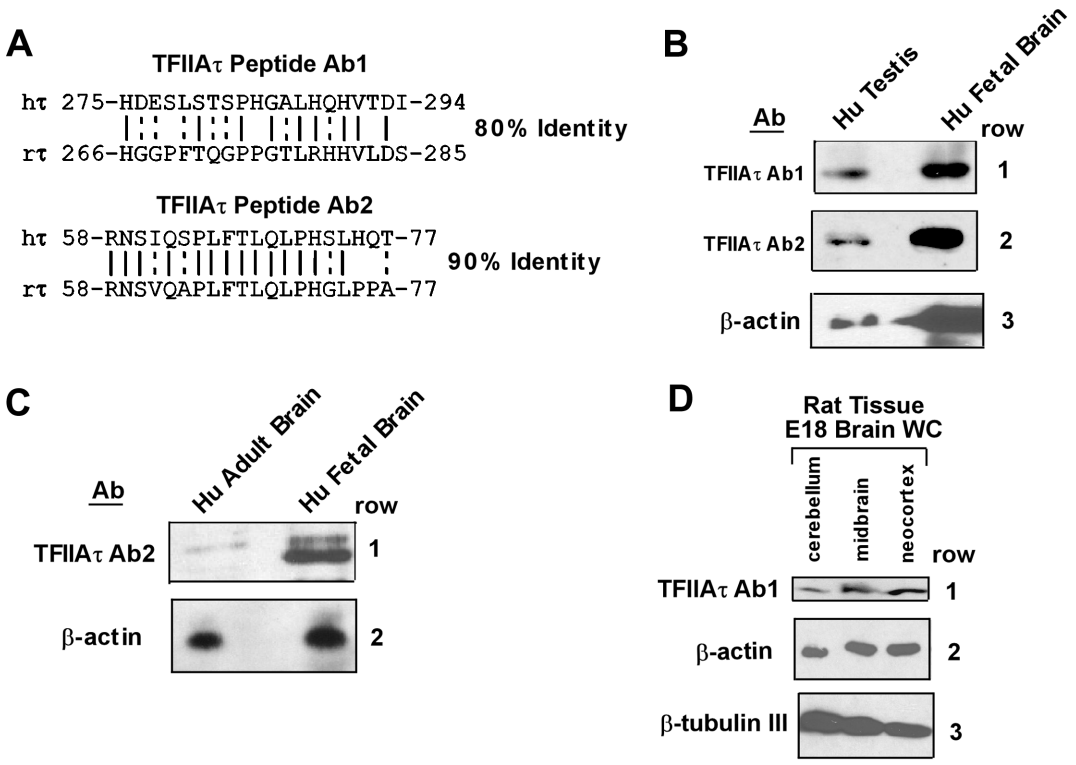

FIG. 4. TFIIA subunits were observed during prenatal mammalian brain development. (**A**) Human (h) and rat (r) sequence identity was compared at TFIIAτ peptide sequences 1 (80%) and 2 (90%), which were used to generate τAb1 and 2, respectively. Percent identity is indicated (*right*). A solid line indicates an amino acid match and a hatched lined indicates a semi-conservative substitution. (**B**) Western blots were performed with human tissue homogenates using TFIIAτ Ab1 (row 1) and Ab2 (row 2) and β-actin antibody (row 3). Adult testis (left) and pooled human fetal brain sample (20–40 weeks) (right) whole cell tissue extracts were assayed. Note that adult human testis showed low β-actin levels (88). (**C**) TFIIAτ was assayed in human adult brain lysates (*left*) and fetal brain samples (*right*) using τAb2 (row 1). β-Actin levels are shown (row 2). (**D**) TFIIAτ Ab1 (row 1) was used in Western assays with day-18 embryonic rat brain whole-cell extracts (E18 Brain WC) from cerebellum (*left*), midbrain (*middle*), and neocortex (*right*) (*top*). β-actin (row 2) and β-tubulin III (row 3) levels are shown.

whether TFIIAτ expression was previously detected in neuronal tissues. TFIIAτ mRNA was identified in a human multiple sclerosis library (dbEST 452883), brain medulla libraries (dbEST 9649316 and 11125138), and a pituitary gland library (dbEST 16708515). Thus, we examined the developmental production of TFIIAτ in aborted human fetal brain and normal rat embryonic brain tissues.

The human TFIIAτ-NCD sequences (peptides 1 and 2) used to generate antibodies were aligned to the corresponding rat TFIIAτ sequences (Entrez accession number AAH82104) (Fig. 4A). On the basis of these sequence identities (48), we predicted that TFIIAτ Ab1 and 2 were likely to show reactivity to rat TFIIAτ (Fig. 4A). TFIIAτ is produced in human testicular embryonal tumor cells (NT2), which can be differentiated into neurons (14); thus, we tested whether human 20- to 40-week-old aborted fetal brain samples also showed production of TFIIAτ. Human testis tissue and second-trimester brain tissue were assayed in Western blots using either τAb1 or τAb2 (see Fig. 4A). TFIIAτ was detected in both human testis and fetal brain whole-cell extracts by both antibodies as a 52-kD band, which is the predicted molecular weight of τ (Fig. 4B, rows 1 and 2; data not shown). Using human extracts, the τAb1 and τAb2 antisera showed comparable detection of TFIIAτ in the Western blot assay (data not shown). Remarkably, human TFIIAτ levels in fetal brain tissues were nearly as abundant as previously observed in testis tissue (Fig. 4B). Importantly, this is the first observation of TFIIAτ production in human tissues other than testis. Note that the β-actin levels were considerably reduced in human testis tissue extracts relative to fetal brain extracts as previously described (35) (Fig. 4B). Our Western blot results with NT2 neurons and previous work indicated that adult brain tissue has low TFIIAτ levels (Fig. 3) (14). In this study, steady-state levels of TFIIAτ were at very low levels in human adult brain extracts, but were observed at much higher levels in fetal brain extracts (Fig. 4C) (14).

To determine whether TFIIAτ production was restricted to a particular region of embryonic brain, whole-cell extracts were prepared from several regions of E18 rat brains and were assayed for TFIIAτ. The rat brain samples were examined in Western blot assays with τAb1 (Fig. 4D; data not shown). Rat embryonic cerebellum, midbrain, and neocortical samples showed similar levels of TFIIAτ (Fig. 4D). All embryonic samples showed comparable levels of β-actin and β-tubulin type III (Fig. 4D). Thus, different regions of developing brain showed specific production of TFIIAτ.

The regulation of TFIIAτ during development of rat brain

The regulation of TFIIAτ during development of the rat brain was examined using the τAb2 antibody (Fig. 5). TFIIAτ was produced at high levels in rat E18 brain and in human fetal brain, whereas low levels of TFIIAτ were observed in brain samples isolated from postnatal day-2 (P2) rats (Fig. 5, row 1). TFIIAτ was not detected in adult brain extracts (Fig. 5, row 1). Using rat extracts, the τAb2 antisera showed better detection in the Western blot assay compared to τAb1 (data not shown). Importantly, TFIIAγ levels were also reduced during postnatal brain development, similar to our observations with τ (Fig. 5, row 2). Although the levels of TFIIA$\alpha\beta$ were substantially reduced in adult rat brain, high levels were observed in both rat E18 brain NE and P2 extracts (Fig. 5, row 3 and column B). Nevertheless, these observations with $\alpha\beta$ are largely consistent with observations for τ and γ (Fig. 5). The levels of β-tubulin III were significantly higher in the rat P2 and adult brain samples compared to the human fetal and rat embryonic brain samples (Fig. 5, row 4). In the developing mammalian brain, β-tubulin III levels are inversely correlated with cellular proliferation and are associated with neuronal differentiation (49). The rat embryo and human fetal samples showed comparable levels of β-tubulin III (Fig. 5, row 4, columns A and D). G3PDH was observed in rat E18 brain, P2 brain, and adult brain and human fetal brain lysates; however, rat E18 and P2 brain samples showed reduced levels of

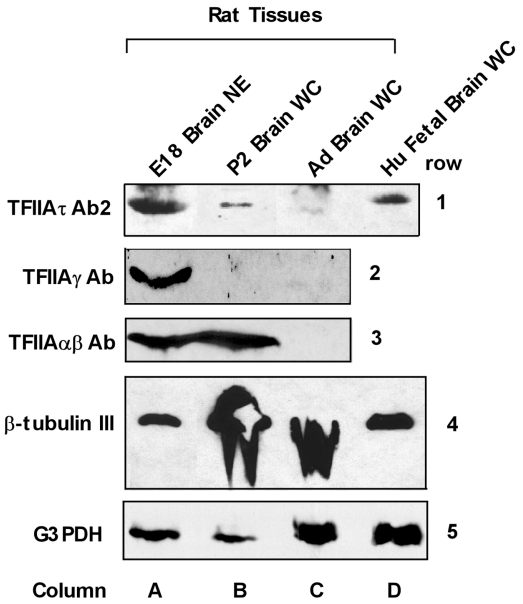

FIG. 5. TFIIA subunit levels were reduced in postnatal rat brain during development. TFIIAτ levels were assayed using day-18 embryonic rat brain nuclear extract (E18 Brain NE) (column A), postnatal day-2 (P2) rat brain whole cell extract (WC) (column B), rat adult brain whole cell (WC) extract (column C), and human fetal brain whole cell lysate (WC) (column D). IIAτ Ab2 (row 1), IIAγ Ab (row 2), IIA$\alpha\beta$ Ab (row 3), β-tubulin III Ab (row 4), and G3PDH antibody were used in Western blots (row 5).

G3PDH (Fig. 5, row 5). We conclude that the levels of TFIIA subunits during rat brain development were consistent with the corresponding TFIIA profiles observed during NT2 differentiation and in human aborted fetal brain tissues (Figs. 3–5). These data further support the hypothesis that TFIIA could function as a negative regulator of neuronal differentiation processes.

The TFIIAτ promoter is repressed in rat primary neuronal cultures at the chromatin level in vivo

Because ATRA-treated NT2-ec cells and neurons showed reduced TFIIAτ mRNA levels (Fig. 2), we examined the methylation status of the chromatin surrounding the *TFIIAτ* promoter in rat primary hippocampal cells in vivo. A higher histone H3 Lys4 methylation compared to Lys9 methylation in a promoter region is indicative of high gene expression levels, whereas a high ratio of Lys9 relative to Lys4 methylation indicates a form of repressive chromatin causing gene silencing (50). We tested the *TFIIAτ* and *Megalin* (MEG) promoters in ChIP analysis in human HEK293 (HEK) cells and rat E18 primary hippocampal cells (Fig. 6). Antibodies specific to methylated Lys residues at either the Lys4 or Lys9 positions of histone H3 were used for these experiments. Both the *Megalin* and *TFIIAτ* promoters showed high Lys4 methylation levels in the HEK293 cell line, which is a transformed cell line (Fig. 6, left panel). These observations are entirely consistent with high TFIIAτ levels observed in proliferating cells (Figs. 1–3). *Megalin* showed high Lys4 and low Lys9 methylation levels in rat primary neurons, consistent with its critical role in forebrain development (51). The *TFIIAτ* promoter showed a high ratio of histone H3 Lys9 to Lys4 methylation (0.78) in rat primary neurons, indicative of a repressed promoter in vivo (Fig. 6) (50). We conclude from these observations that *TFIIAτ* expression in neurons is repressed by epigenetic changes in chromatin. This is the first observation of epigenetic regulation of TFIIA.

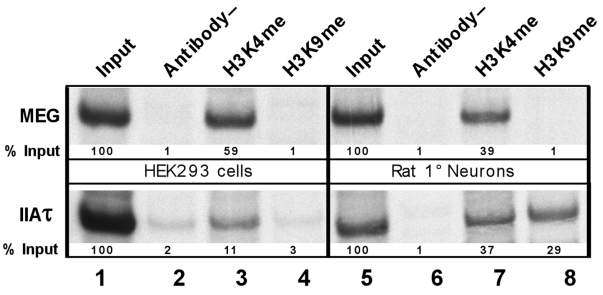

FIG. 6. TFIIAτ mRNA expression was repressed in rat primary hippocampal neurons at the chromatin level. ChIP assays were performed on the TFIIAτ (IIAτ) promoter region (*bottom panel*) and the control Megalin (MEG) promoter region (*top panel*). ChIP assays were performed in human HEK293 cells (*left panels*) and primary hippocampal cells from E18 rats (*right panels*). Input is shown (100%) (lanes 1 and 5). Antibody minus (−) is shown (lanes 2 and 6). Histone H3 (H3) Lys4 (K4) methylation (me) is shown (lanes 3 and 7). Histone H3 (H3) Lys9 (K9) methylation (me) is shown (lanes 4 and 8). Phosphorimager analysis of the immunoprecipitated promoter PCR amplicons are shown below each sample as % input.

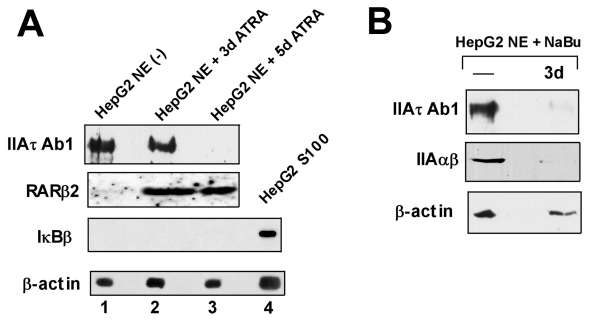

FIG. 7. Reduced TFIIAτ levels were observed in HepG2 cells treated with either ATRA or NaBu. (**A**) Nuclear extracts prepared from treated or untreated cells and were assayed with TFIIAτ Ab1. Western blot analysis of HepG2 Ne (−) (untreated) (lane 1), HepG2 NE+3d ATRA (lane 2), and HepG2 NE+5d ATRA (lane 3) is shown above. Cytoplasmic HepG2 S100 extract (lane 4) is shown. Treated HepG2 cells used 10 μM ATRA (3d and 5d) (*top*). The RARβ2 (38 kD) (*top*), IκBβ (49 kD) (*middle*), and β-actin (*bottom*) antibodies are shown on the left. (**B**) Nuclear extracts prepared from NaBu-treated 3 days (3d) (*right*) or untreated (−) (*left*). HepG2 cells were assayed with either TFIIAτ Ab1 (*top*), IIA$\alpha\beta$ (*middle*), or β-actin (*bottom*) antibodies. NaBu (10 μM) was used for HepG2 cell treatments.

ATRA- and sodium butyrate-treated HepG2 cells showed reduced levels of TFIIA subunits

Because ATRA treatment reduced TFIIA subunit levels in the NT2 embryonal tumor line (Fig. 3), we tested wheter another human tumor cell line showed altered TFIIA levels when differentiated with either ATRA or sodium butyrate. We examined TFIIAτ levels in the human HepG2 cell line that was induced to differentiate into hepatocytes, as reported elsewhere (26,28,52). Differentiated HepG2 cells display canalicular-like structures and reduced α-fetoprotein (AFP) levels (53). Nuclear extracts were prepared from ATRA-treated HepG2 cells (3 days and 5 days) and untreated cultures (−). Nuclear extract samples were assayed using TFIIAτ Ab1 in Western blot analysis (Fig. 7). High production of TFIIAτ protein was observed in untreated and 3-day ATRA-treated HepG2 NE samples, but the levels of τ were substantially reduced after 5 days of ATRA-treatment (Fig. 7A). Thus, we have demonstrated that TFIIAτ levels are reduced in various ATRA-treated cell types. TFIIA$\alpha\beta$

levels are also reduced with ATRA treatment in HepG2 cells (28). Although previous work with untreated whole-cell HepG2 extracts did not detect high levels of TFIIAτ (14), our NE preparations significantly enriched the levels of TFIIAτ in this cell line (Fig. 7A). The levels of RARβ2 are increased with ATRA treatment as reported elsewhere (Fig. 7A) (44,54). The cytoplasmic factor IκBβ was detected in HepG2 S100 extracts, but not in NE samples, as reported elsewhere (55) (Fig. 7A). β-actin levels were comparable among samples (Fig. 7A).

Butyrate is a product of colonic bacterial flora that induces hepatic differentiation and reduced levels of AFP (56). We tested whether TFIIA subunit levels were also modulated by sodium butyrate (NaBu) treatment of HepG2 cells. TFIIAτ and $\alpha\beta$ showed significantly reduced levels after 3 days of NaBu treatment, whereas β-actin levels were slightly reduced (Fig. 7B). We conclude that TFIIA subunit levels are inversely correlated to cellular differentiation regardless of the chemical inducing agent used or cell type assayed. At high concentration or long exposure periods, NaBu induces apoptosis (57). We tested genomic DNA isolated from untreated HepG2 cultures, 3-day NaBu-treated HepG2 cultures, and day-5 ATRA-treated HepG2 cultures and determined that these DNAs showed high molecular weight sizes with no ladder-like degradation indicative of apoptosis (58; data not shown). Furthermore, a ladder-like ubiquitination smear of increasing molecular weight, which is indicative of apoptosis, was not detected in Western blots with these extracts and anti-ubiquitin antibody (58; data not shown).

Sp transcription factors are regulated in a developmental manner similar to TFIIAτ

TFIIA is a co-activator of Sp1-dependent transcriptional activity in vitro (17). Sp factors activate *dynamin I* expression and mediate the neuronal differentiation of the N1E-115 cell line (59). We examined whether Sp levels were influenced by ATRA treatment during NT2 neuronal differentiation. Sp1, 3, and 4 levels were inversely correlated to ATRA treatment and NT2 neuronal differentiation, similar to our observation with TFIIA subunit levels (Fig. 8A, rows 1–3). Reduced levels of both Sp1 and 3 were revealed in NT2 cells treated for 28 days with ATRA (Fig. 8A, rows 1 and 2). We observed that Sp2 levels were significantly increased with the development of the NT2 neuronal phenotype (Fig. 8A, row 4). Array experiments also identified an increase in Sp2 mRNA levels during NT2 neurogenesis (60). We conclude that Sp1, 3, and 4 are associated with proliferation, whereas Sp2 is produced in mature neurons.

The levels of Sp factors were also assayed at different periods of rat brain development. Sp 1, 3, and 4 levels were identified in rat E18 brain extracts using Western analysis, whereas Sp3 was also produced in rat P2 brain

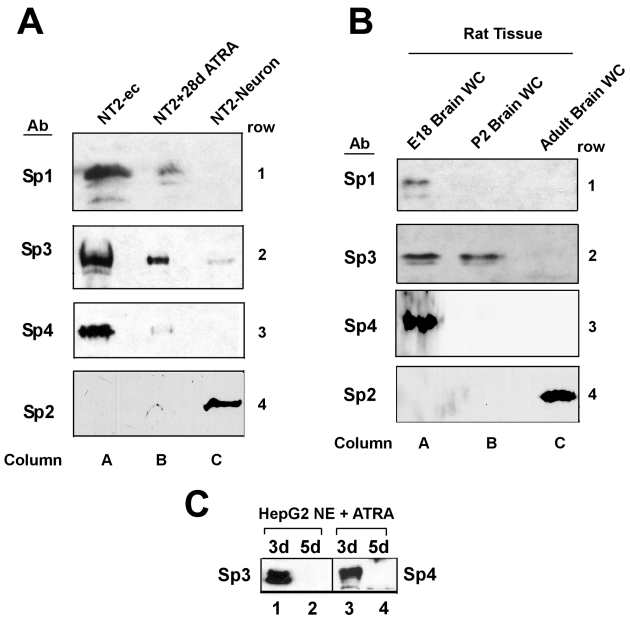

FIG. 8. Sp-factor levels in NT2 cells and rat brain tissue during development. (**A**) Sp1 (100 kD) (row 1), Sp3 (70 kD) (row 2), Sp4 (100 kD) (row 3), and Sp2 (125 kD) (row 4) levels were determined using NT2-ec (column A), NT2+28d ATRA (column B), and NT2-neuron (column C) whole-cell extracts in a Western assay (*top*). Sp antibodies are indicated (*left*). (**B**) Sp1 (100 kD) (row 1), Sp3 (70 kD) (row 2), Sp4 (100 kD) (row 3), and Sp2 (125 kD) (row 4) levels were determined in a Western assay using either day-18 embryonic rat brain whole-cell extract (E18 Brain WC) (column A), postnatal day-2 rat brain whole-cell lysates (P2 Brain WC) (column B), and adult rat brain whole cell lysates (Adult Brain WC) (column C). Sp1, 3, 4, and 2 antibodies are indicated (*left*). (**C**) The levels of Sp proteins in HepG2 cells treated with ATRA. Western blot analysis of the following nuclear extract was performed: HepG2 NE+3d ATRA (lanes 1 and 3) and HepG2 NE+5d ATRA (lanes 2 and 4) above ATRA (10 μM) was used to treat HepG2 cells. Sp3 (lanes 1 and 2) and Sp4 antibodies (lanes 3 and 4) were used to identify Sp3 and Sp4, respectively.

extracts (Fig. 8B). Sp2 levels were identified in adult brain extracts, but not in rat E18, nor in P2 brain extracts (Fig. 8B, row 4). We conclude that developmental regulation of several Sp factors in rat brain extracts is highly consistent with the profile observed during NT2 differentiation and with the TFIIA subunits (Fig. 8).

Sp1, 2, 3, and 4 were identified in HeLa and Namalwa nuclear extracts using EMSA and western assays (unpublished observations). The Sp transcription factor family is also induced to maximal levels in HepG2 cells after 3 days of ATRA treatment (28). We examined whether the Sp factors showed degradation or reduced levels after longer ATRA treatments (5 days) similar to our observations with TFIIAτ in HepG2 cells (Fig. 7A). Interestingly, Sp3 and 4 revealed significantly reduced

levels when HepG2 cells were treated with ATRA for 5 days (Fig. 8C). We conclude that TFIIA subunits and Sp1, 3, and 4 show similar regulation during cellular differentiation and development.

DISCUSSION

TFIIA subunits positively regulate an undifferentiated phenotype

The interaction of TBP with TFIIA promotes cell cycle progression through the G_2/M phase in yeast (10). The yeast *TFIIAγ Y69F/W76F* strain arrests at the G_2–M border and shows temperature-sensitive growth properties (10). *TFIIAγ Y69F/W76F* shows reduced expression of cell cycle-specific genes in vivo in addition to reduced activator-dependent transcription from multiple genes that use several start sites (10). Heterozygous deletion of TBP delays mitosis in the avian DT40 cell line (61). Furthermore, a Ras-mediated increase in TBP expression is required for anchorage-independent cell growth and tumor formation in athymic mice (62). Therefore, reports show that the interaction of TBP with TFIIA influences cellular proliferation. The TFIIA$\alpha\beta$ and τ subunits are expressed in unsynchronized tumor cell lines, yet only $\alpha\beta$ is expressed in differentiated tissue, suggesting that the loss of τ is concurrent with the development of differentiation processes (Fig. 1). Jurkat cells are committed to the T cell lineage and are phenotypically mature (35,63). Importantly, we demonstrated a lack of TFIIAτ production in Jurkat cells (Fig. 1), which further supports the association of TFIIAτ with an undifferentiated state.

Several transcriptional factors influence an undifferentiated phenotype. MASH1 and 2 are the mammalian homologs of *Drosophila* neural determination factors ACHAETE-SCUTE. MASH-2 is produced in undifferentiated embryonal carcinoma cells, but not in neurons, whereas MASH-1 has the opposite expression profile (64). The embryonic stem cell undifferentiated state is sustained by transcription factors NANOG and STAT-3 (65,66). Undifferentiated embryonic cell transcription factor-1 (UTF-1) is expressed in germ-line tissues, but not in adult tissues and interacts with TBP (67). Thus, we examined whether the general transcription factor TFIIA revealed a similar expression profile to these promoter interacting transcription factors. In this study, we demonstrated that TFIIA subunit levels were strictly associated with an undifferentiated cellular phenotype. Seminal reports indicate that p53 levels during stem cell differentiation processes are highly similar to TFIIAτ in this report (68,69).

RA is a vitamin A derivative and an endogenous regulator that is essential for inducing normal patterning and neurogenesis during brain development (24,25). ATRA-treated NT2 cells undergo growth arrest and terminal differentiation into post-mitotic neurons after replating on a poly-D-lysine-coated surface (18,19). TFIIAτ mRNA and protein levels were inversely correlated to the development of the neuronal phenotype in NT2 cells (Fig. 2C and 3A). TFIIAτ and γ subunit levels were dramatically reduced when ATRA-treated NT2 cells were plated on a poly-D-lysine surface that promoted neuronal formation (Fig. 3A). Thus, loss of TFIIAτ levels was strictly coincident with the cell–cell contacts that are necessary for neuronal formation on poly-D-lysine-coated plates (37,38). Furthermore, TFIIAτ levels were observed even with serum deprivation in NT2-ec cells (Fig. 3C). The levels of TBP were reduced in ATRA-treated NT2 cells as well (Fig. 3A). Nuclear protease activity reduces the DNA-binding activity of several transcription factors in ATRA-treated F9 embryonic stem cells (70). We conclude that TBP and TFIIA levels are reduced during the establishment of neuronal differentiation processes. Interestingly, a large number of neuronal-specific genes, including *serotonin 1a receptor, telencephalin,* and *GABAβ1,* contain strong initiators or TATA-less core promoter regions (33,71–73). Whether neuronal genes have a reduced need for TBP-dependent transcription is not known. Furthermore, TBP-independent transcription systems have been described in vitro (74).

Negative neuronal regulators show an inverse association with neuronal differentiation (75). Surprisingly, several negative neuronal regulators show a similar mRNA expression and proteomic profile to TFIIAτ, including ATRA-regulated nuclear matrix-associated protein (RAMP) (76), the repressor element-1 silencing transcription factor (REST) (77), and MUSASHI-2 (78). In this report, we demonstrated TFIIA subunits as the first transcription co-activator to have an expression profile similar to negative neuronal regulators.

Both ATRA and NaBu stimulate HepG2 cell differentiation into hepatocytes (Fig. 7), however NaBu inhibits ATRA-induced differentiation of F9 cells indicating that these compounds affect cellular differentiation and apoptosis by different mechanisms (79). NaBu promotes histone hyperacetylation in embryonal carcinoma cells by inhibiting histone deacetylases (80,81). By contrast, ATRA mediates F9 cell differentiation by increasing RAR-β expression, even in the absence of new protein synthesis (82). Furthermore, expression of neuronal markers in NT2-ec cells is induced by ectopic expression of RAR-β and γ (83).

TFIIAτ expression is regulated during neuronal development at the chromatin level in vivo

TFIIAτ mRNA was identified in human brain EST libraries (medulla and pituitary). Furthermore, TFIIAτ protein levels were high in human fetal and rat embryonic

brain tissues, as shown with Western blot analyses (Figs. 4 and 5). The levels of these TFIIA subunits were substantially reduced in adult mammalian brains (14; Figs. 4 and 5), which was similar to our observations with NT2 neurons (Fig. 3). A high Lys9/Lys4 ratio of methylation in histone H3 was observed for a panel of gene promoters that are known to be epigenetically marked for repressed state of expression (50). Importantly, *TFIIAτ* also shows high methylation at the Lys9 position relative to Lys4 of histone H3 within the 200-bp promoter region (Fig. 6). We conclude that *TFIIAτ* gene expression is repressed in primary neurons as a consequence of the high Lys9/Lys4 methylation ratio in histone H3. By contrast, our ChIP analysis of TFIIAτ showed that proliferating HEK293 cells had high levels of Lys4 and low levels of Lys9 methylation of histone H3, which is also observed in the chromatin of highly expressed genes (50). Because apoptosis of neurons occurs when cyclin-dependent kinase genes are ectopically expressed (84), we predict that ectopic expression of TFIIAτ may induce apoptosis in neurons as well.

TFIIAτ and $\alpha\beta$ and TBP were increased to maximal levels during postnatal day 2 of liver proliferation and development (data not shown) where a second mitotic wave of proliferation occurs (85). TFIIAτ was produced in the human HepG2 nuclear fraction and showed reduced levels after exposure to differentiation agents (Fig. 7A,B). The Sp transcription factor family also showed differentiation-dependent reduction in HepG2 cells like our observations with the TFIIA subunits (Fig. 8C). We conclude from these observations that the NT2 and HepG2 cellular differentiation models are representative of the regulatory processes observed in developing brain and liver with TFIIA.

Several Sp factors are similarly regulated in rat brain development and in NT2-ec cells

Remarkably, Sp1, 3, and 4 levels were high in NT2-ec cells, embryonic rat brain, and mitotic HepG2 cells similar to observations with the TFIIA subunits (28, Fig. 7). The levels of Sp1, 3, and 4 and TFIIAτ showed an inverse correlation to the differentiation phenotype (Figs. 3, 7, and 8). Sp1 DNA-binding activity is reduced by cysteine protease activity in differentiated F9 cells (70). Furthermore, Sp1-dependent reporter gene expression is expressed preferentially in G_2 during early mouse embryogenesis (86). Therefore, Sp1, 3, and 4 and TFIIA subunits levels are associated with early developmental processes and an undifferentiated cellular phenotype.

Sp2 showed dramatically different regulation compared with TFIIA subunits and other Sp family members (Fig. 8). Thus, Sp2 revealed a positive correlation to neuronal differentiation and development (Fig. 8A,B). We propose that Sp2-dependent transcriptional stimulation would not require TFIIA as a limiting co-activator to direct neuronal-specific transcription. Interestingly, a large number of forebrain-specific genes (including *NMDA-R1, NMDA-NR2C,* and *telencephalin*) contain canonical Sp sites in their 5′ upstream regulatory regions in addition to TATA-less core promoters (73,87,88). Transgenic studies have yet to define which promoter elements direct forebrain specific gene expression.

TFIIAτ is a candidate oncofetal protein

We showed that developing tissues produced TFIIAτ (Figs. 1, 4, and 5). Therefore, we propose that TFIIAτ regulates gene expression programs to maintain cells in an undifferentiated state. Because TFIIAτ is expressed in embryonic brain, a panel of tumor cell lines, and REF cells, we propose that τ is a candidate oncofetal protein (Figs. 1, 4, and 5). Oncofetal proteins are produced in fetal tissues with significantly reduced expression in adult tissues (89). AFP is produced in rat fetal yolk sac and liver during early development and production stops 4 weeks after birth (90). Reduced AFP expression is also observed in HepG2 and a number of tumor cell lines treated with ATRA, similar to our observations with the TFIIA subunits (53; Fig. 7). AFP expression is induced with partial hepatectomy and proliferative disease (90,91). Because members of the steroid/thyroid receptor superfamily are oncofetal proteins (92), we hypothesize that TFIIA may co-activate transcription processes for this superfamily (45).

TFIIAτ demonstrated a profile in higher eukaryotic cells that strictly correlated with an undifferentiated phenotype. Considering that p53 and TFIIAτ share similar protein profiles during cellular differentiation processes, it is interesting to note that TFIIAτ is induced in several diseases of the colon, while p53 has been characterized as a critical factor in inflammatory diseases of the colon (93; data not shown). Thus, TFIIAτ is being assessed as a biomarker of diseases that undergo tissue dedifferentiation.

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