The TATA-binding Protein and Its Associated Factors Are Differentially Expressed in Adult Mouse Tissues*

(Received for publication, February 12, 1999, and in revised form, March 22, 1999)

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We have investigated the expression levels of the TATA-binding protein (TBP) and several TBP-associated factors (TAFII28) in differentiated adult mouse tissues. Immunoblots performed using monoclonal antibodies show that there are considerable variations in the levels of TBP and many TAFII proteins present in various tissues. Consequently, the relative levels of TAFII85s and TBP vary significantly from one tissue to another. TBP and several TAFII85s are overexpressed in both testis and small intestine, while in marked contrast, many of these proteins, including TBP itself, were substantially down-regulated in nervous tissues and in the heart. These tissues do, however, show a high expression level of the TBP-like factor, which thus may represent an alternative factor for the specialized transcription program in some differentiated tissues. While there are significant variations in the levels of TAFII28 protein, reverse transcription-coupled polymerase chain reaction shows similar expression of the TAFII28 mRNA in different tissues. The variations in TAFII28 protein levels therefore result from post-transcriptional regulatory events.

TFIID is a multiprotein complex, which together with TFIIA, TFIIB, TFIIE, TFIIF, and TFIIF assists RNA polymerase II to correctly initiate transcription (1). TFIID is composed of the TATA-binding protein (TBP), which specifically binds the TATA element, and a series of evolutionary conserved TBP-associated factors (TAFII85). TAFII85s have been shown to be involved in promoter recognition (2, 3) and to act as specific transcriptional coactivators in vitro and in transfected mammalian cells (Refs. 4–8 and references therein; for review, see Ref. 9). Genetic experiments in yeast have shown a variable expression of only a subset of promoters involved for example in cell cycle control, while others are more generally required (10–14). Recently, a subset of TAFII85s have been found in other complexes devoid of TBP, such as the PCAF-SAGA complex in humans and in yeast (15–17), and the TBP-free TAFII-containing complex (TFTC) (18). Despite the fact that TFTC does not contain TBP it can replace TFIID in both basal and activated transcription in vitro, suggesting that TBP may not always be an essential transcription factor in vivo.

While much has been learned about the function of TFIID in biochemical assays and in yeast, little is known concerning the expression of its constituent subunits in animal tissues. Previous studies on TBP (19) have demonstrated an overexpression of TBP mRNA and to a lesser extent of the TBP protein in testis. The mRNAs of several TAFII85s have been shown to be equally expressed in several rat tissues (20). However, the TAFII105 mRNA is widely expressed yet the corresponding protein shows cell specificity, being much more abundant in mature lymphoid B cells (8).

The above observations prompted us to investigate the expression of TBP and TAFII85 proteins rather than their mRNAs in a variety of adult murine tissues. Immunoblots performed with a series of monoclonal antibodies show that the relative expression levels of these TAFII85s, and TBP can vary extensively from tissue to tissue, suggesting that the transcription program in different tissues may have differential requirements for TFIID components. Furthermore, the levels of TBP and many TAFII85 is significantly reduced in extracts from the nervous system (brain, cerebellum, eye, spinal cord), kidney, and in the heart. Interestingly, several of these tissues show high expression levels of the previously described TBP-like factor (TLF) (18), raising the possibility that TLF may functionally substitute for TBP in certain tissues. In the case of TAFII28, whose mRNA is equivalently expressed in many tissues, the variations in TAFII28 protein must result from post-transcriptional events.

**MATERIALS AND METHODS**

Preparation of Murine Tissue Extracts—Four individual 6-week-old Black 6 mice were sacrificed and the tissues extracted and immediately frozen in liquid nitrogen. Protein extracts were made as described (21) by shearing the tissues in 2mL of buffer A (50 mM Tris-HCl (pH 7.9), 20% glycerol, 1 mM dithiothreitol, and 0.01% β-mercaptoethanol. The extracts were analyzed by SDS-PAGE and staining with Coomasie Blue to normalize each preparation.

Preparation of Cell Line Extracts—Cell extracts were prepared as described previously (5) by freeze-thawing 100 μl of buffer A (50 mM Tris-HCl (pH 7.9), 20% glycerol, 1 mM dithiothreitol, and 0.01% Nonidet P-40) containing 0.5 mM EDTA and 2.5 μg/ml leupeptin, pepstatin, aprotinin, antipain, and chymostatin. The proteins were quantified by Bradford test and the equivalent amounts were used for immunoblots.

**Antibody Preparation**—Monoclonal antibodies (mAbs) against TBP (3G3), TAFII55 (19TA), TAFII135 (20TA), TAFII100 (1TA), TAFII30 (4G2), TAFII20 (22TA), and TAFII18 (16TA) and mouse polyclonal sera against TAFII20 (22TA), and TAFII18 (16TA) were raised against purified Escherichia coli expressed GST-mTAFII28.

**RNA Preparation and RT-PCR**—RNA from tissue samples was prepared as described previously (26). RT-PCR was performed on 1 μg of total RNA using the following primers 5′-GGACAAGAAGGAGAA-3′ and 5′-CCTTTGTTGCTTTTAGTTGGGAT-3′ specific to different exons of mTAFII28 generating a 360-base pair fragment. Samples were denatured for 3 min at 94 °C and annealed for 10 min at 50 °C. A mix of avian myeloblastosis virus reverse transcriptase and Taq polymerase was added and incubated for another 20 min at the same temperature. 30 cycles of PCR were then performed. After 15, 23, and 30 cycles an aliquot of each sample was removed and electrophoresed, transferred to a hybond membrane, and hybridized with a 32P-

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† Supported by fellowships from the Association pour la Recherche contre le Cancer and a TMR grant from the European Union.

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§ The abbreviations used are: TF, transcription factor; TBP, TATA-binding protein; TAFII, TBP-associated factor; TLF, TBP-like factor; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; RT-PCR, reverse transcription-polymerase chain reaction; HPRT, hypoxanthine guanine phosphoribosyltransferase.
labeled TAFII28-specific oligonucleotide probe. As a control a 200-base pair fragment of the hypoxanthine guanine phosphoribosyltransferase (HPRT) gene was amplified in the same reactions and detected by hybridization using an HPRT-specific oligonucleotide probe. Amplification with no avian myeloblastosis virus reverse transcriptase was also performed as a negative control.

RESULTS AND DISCUSSION

Variations in TAFII Protein Content of Adult Murine Tissues—To investigate the levels of TFIIID components in adult murine tissues immunoblots were performed using mAbs against a selection of TAFIIs, which are either TFIIID-specific (TAFII28, TAFII18) or are present in other TAFII-containing complexes (TAFII135, TAFII100, TAFII55, TAFII30, TAFII20). Six-week-old mice were sacrificed, dissected, and their organs were immediately frozen in liquid nitrogen. Equivalent amounts of the proteins extracted from each tissue (see “Materials and Methods” and Fig. 1) were used to make several replica immunoblots along with extracts from human HeLa and murine F9 cells as controls. All the antibodies used detected both the human TAFII8s and their murine counterparts. Analogous results to those shown below were observed in blots from independently prepared extracts (data not shown).

TAFII135 and TAFII100 could be detected in all tissues, with the exception of the spinal cord where TAFII100 was seen only very weakly, while TAFII135 was undetectable (Fig. 2, see lane 17). Both TAFII135 and TAFII100 were strongly overexpressed in the testis where, and for the sake of clarity, a 5-fold shorter exposure is shown (lane 15, Fig. 2). An exposure time comparable with that shown in the other lanes resulted in a saturated black signal (data not shown). Varying levels of TAFII55 could also be detected in all tissues with overexpression in the testis being less dramatic than for TAFII135 and TAFII100 (note that for TAFII55 the same exposure time is shown in all tissues).

Although these TAFII8s are widely expressed, their relative expression levels vary from tissue to tissue. For example, equivalent signals for TAFII135 and TAFII100 are seen in the liver, lung, and adrenal gland (Fig. 2, lanes 9–11, respectively), while the signal for TAFII100 is stronger than that for TAFII135 in the pituitary and the small intestine (lanes 3 and 4, respectively). The opposite relationship is observed in the eye, tongue, and spleen (lanes 2, 7, and 8, respectively). Similarly, the ratio of TAFII55 and TAFII100 signals changes when one compares the pituitary or the liver, where the signal for TAFII100 is the stronger, with the heart and lung, where the opposite is seen (lanes 3, 9, 6, and 10, respectively). Therefore, not only do the expression levels of a given TAFII vary from tissue to tissue, but the relative abundance of TAFII8s also varies.
as in the testis. In contrast, many TAFIIs were down-regulated to the point of being undetectable in tissues such as brain, heart, kidney, and spinal cord. This is also the case in the kidney with the exception of TAFII30, which is as abundant as in intestine. Comparison of the signals observed in the brain, kidney, and lung with those obtained with serial dilutions of the small intestine extract showed that the levels of TAFII135 and TAFII100 were 5-fold lower in the brain and kidney than in intestine, while those in the lung were around 3-fold lower (data not shown). Note that the levels of these TAFIIs are even lower in the spinal cord and heart than in the brain or kidney. Similar titrations showed that TAFII55 levels were 10-fold lower in the brain and kidney than in the testis, while the levels in the lung were 2–3-fold lower (data not shown). This suggests that the distinct transcriptional programs of each tissue show differing requirements for a given TAFII.

Partially Complementary Expression of TBP and TLF in Mouse Tissues—The same extracts were also tested for the expression of TBF and TAFII135. TBP is strongly expressed in the testis (Fig. 3A, lane 15) and in the small intestine and the pituitary (lanes 8 and 9, respectively). It is interesting that one of the highest levels of TBP is found in the pituitary, since many TAFIIs are under expressed in this extract. Intermediate expression levels were detected in the adrenal, lung, liver, spleen, and tongue (lanes 2–6, respectively). Strikingly, only very low levels of TBP could be detected in the brain and cerebellum (lanes 12 and 13, respectively), and TBP was virtually undetectable in the heart, eye, kidney, and spinal cord (lanes 7, 10, 16, and 17, respectively; note that since comparable exposures of two different blots are presented, the adrenal gland was included in both to allow comparison of the left and right panels). In these experiments, TBP was detectable in brain, cerebellum, heart, eye, kidney, and spinal cord only when very long saturating exposures of the blots were made (data not shown), while the non-saturating exposures shown in Fig. 3A highlight the differences in expression levels. Titration experiments using serial dilutions of the testis and small intestine extracts showed that TBP levels were 3–5-fold lower in the small intestine than in the testis, 5–6-fold lower in the lung, and more than 10-fold lower in the brain and kidney (data not shown). These results reveal a considerable variation in TBP expression levels among the different tissues.

The above result is rather unexpected considering the important role which TBP is thought to play in transcription. This prompted us to look at the expression of TLF, a factor highly related to the TBP core domain (18) and which consequently may be able to functionally substitute for TBP. The highest levels of TLF were detected in the adrenal, small intestine, brain, and spinal cord (Fig. 3A, lanes 2, 8, 13, and 17, respectively). TLF was also present in the liver, tongue, heart, pituitary, eye, cerebellum, and kidney (lanes 4, 6, 7, 9, 10, 12, and 16, respectively), but was undetectable in the lung and spleen (lanes 3 and 5, respectively). TLF was expressed in the testis, but in contrast to the other factors examined, it was under, rather than overexpressed, in this tissue (compare the contrasting levels of TBP and TLF in testis, lane 15, with brain or spinal cord in lanes 13 and 17, respectively, and the expression of TBP and TLF in the pituitary and eye, lanes 9 and 10, respectively). The fact that TLF expression can be readily detected in the eye, heart, spinal cord, and kidney (note also that TAFII30 is readily detectable in the kidney extract) extracts shows that there is no intrinsic defect in these extracts which would explain the observed low levels of TAFII135 and TBP. The presence of TLF in these extracts rather underlines the real differences which exist in the expression levels of TBP and TAFII135.

TLF was also present in the extracts from several cultured cell lines, being readily detected in total cell extracts from pluripotent murine F9 embryonal carcinoma cells (Fig. 3A, lane 1, and Fig. 3B, lane 2) and embryonic stem cells (Fig. 3B, lane 5) or from differentiated 3T3 fibroblasts and simian COS cells (lanes 3 and 4, respectively), but much more weakly in HeLa cells (lane 1).

Previous studies on TBP protein expression have been limited to only a few tissues and have employed polyclonal antisera. Here we have used a very sensitive monoclonal antibody against TBP that reveals unexpected and very significant variations in TBP expression. As described previously (19), TBP is overexpressed in the testis. This, however, is not unique since high expression was also observed in the small intestine and the pituitary. In contrast, TBP like many TAFIIs, was strongly down-regulated in the nervous tissues, eye, kidney, and in the heart.

In many of the tissues with low TBP expression, especially those of the nervous system, prominent levels of TLF were observed. Nevertheless, TLF expression was not limited only to nervous tissues or to tissues with low TBP levels, since it was also abundantly expressed in the adrenal and the small intestine extracts. Immunohistochemistry will help determine whether TBP and TLF are overexpressed in the same cell populations in these organs. Similarly, it will be interesting to determine which cells within the nervous system express TLF. The available antibody does not yet permit such studies.

In yeast and in mammalian cells, TAFII135 are essential for cell cycle progression and they regulate the expression of cell cycle

\(^{2}\) J.-C. Dantonel, J.-M. Wurtz, O. Poch, D. Moras, L. Tora, submitted for publication.
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The nature of this post-transcriptional regulation is at present unknown. It is possible that the efficiency of translation of the mRNAs varies in different tissues. Alternatively, it is interesting to note that when a given TAF\textsubscript{II} is depleted in yeast, the integrity of the TFIIID complex is compromised and the levels of other TAF\textsubscript{II}s are also strongly reduced (12, 35, 36). This suggests that many TAF\textsubscript{II} proteins accumulate only when they are stably associated in the TFIIID complex, otherwise they are be rapidly degraded. Therefore, the levels of one TAF\textsubscript{II} may indirectly control those of others, if it becomes limiting for TFIIID complex assembly. In the nervous tissues, it may even be the low levels of TBP itself that are limiting for TFIIID assembly. Further knowledge of how the different TAF\textsubscript{II}-containing complexes are assembled inside cells and what the limiting factors in this process are will help in understanding the mechanisms which regulate TAF\textsubscript{II} expression.

Acknowledgments—We thank P. Chambon for support; Y. G. Gaengloff and M. Abrink for critical reading of the manuscript; Y. Lutz and the monoclonal antibody facility; G. Cristina for help with the mice; the staff of cell culture, animal, and oligonucleotide facilities; G. Gaengloff and M. Abrink for critical reading of the manuscript; and B. Boulay, J. M. Lafontaine, R. Buchert, and C. Werlé for illustrations.

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