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Induction of *Drosophila* RNA Polymerase III Gene Expression by the Phorbol Ester 12-*O*-Tetradecanoylphorbol-13-Acetate (TPA) Is Mediated by Transcription Factor IIIB

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We have previously found that the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces specific transcription of tRNA and 5S RNA genes in *Drosophila* Schneider S-2 cells (M. Garber, S. Panchanathan, R. F. Fan, and D. L. Johnson, J. Biol. Chem. 266:20598–20601, 1991). Having derived cellular extracts from TPA-treated cells, that are capable of reproducing this stimulation in vitro, we have examined the mechanism for this regulatory event. Using conditions that limit reinitiation and produce single rounds of transcription from active gene complexes, we find that the number of functional transcription complexes is increased in extracts prepared from TPA-induced cells. We have analyzed the activities of the transcription factors TFIIIB and TFIIIC derived from extracts prepared from TPA-induced and noninduced cells. Examination of the relative activities of TFIIIC showed that both its ability to reconstitute transcription with TFIIIB and RNA polymerase III and its ability to stably bind to the DNA template are unchanged. However, the activity of TFIIIB derived from the TPA-induced cells is substantially increased compared with that derived from the noninduced cells. The differences in TFIIIB activity account for the differences in the overall transcriptional activities observed in the unfractionated extracts. Western blot analysis of the TATA-binding protein subunit of TFIIIB revealed that there is an increase in the amount of this polypeptide present in the induced cell extracts and TFIIIB fraction. Together, these results indicate that the TPA response in *Drosophila* cells stimulates specific transcription of RNA polymerase III genes by increasing the activity of the limiting transcription component, TFIIIB, and thereby increasing the number of functional transcription complexes.

RNA polymerase (pol) III is responsible for the transcription of a variety of small cellular RNAs including tRNA, 5S RNA, and U6 RNA and virus-associated RNAs such as VA1 and VA2 from adenovirus (for reviews, see references 20, 23, 47, and 74). The tRNA, 5S RNA, and VA RNA genes contain intragenic control regions, and 5′-flanking sequences of these genes may serve to modulate transcription efficiency. In the insect systems, however, 5′-flanking sequences are essential for transcription of these genes (14, 60). Fractionation of cell extracts has shown that two factors, TFIIIB and TFIIIC, in addition to RNA pol III, are required for the transcription of the tRNA and VA RNA class of genes, whereas the 5S RNA genes additionally require TFIIIA (57). Another class of RNA pol III genes including U6 RNA and 7S K RNA contains promoter elements exclusively in the 5′-flanking regions of these genes (for a review, see reference 37). Unlike the other RNA pol III genes, these genes appear to require a unique set of transcription factors in addition to TFIIIB and RNA pol III, including TFIID (40, 44, 59). Recent studies have demonstrated that the TATA-binding protein (TBP), a transcription factor previously thought to be RNA pol II specific, is also required for the transcription of all types of RNA pol III genes and RNA pol I genes (10, 11, 56, 71). In the case of RNA pol III transcription, TBP appears to be an integral part of the TFIIIB complex (33, 41, 62, 70).

Examination of the mechanism for transcription has shown that RNA pol III genes form stable transcription complexes that remain associated for many rounds of transcription initiation (38, 55). In the case of tRNA genes, this complex involves the direct interaction of TFIIIC with the template and the subsequent association of TFIIIB with this complex, which serves to stabilize TFIIIC-DNA interactions (30, 38). Although the sequestration of TFIIIB onto these complexes is directed by TFIIIC, DNase I footprint assays have revealed that yeast TFIIIB directly contacts the DNA in the 5′-flanking region of the gene (34). Under high salt or heparin concentrations, yeast TFIIIC can be dissociated from these complexes, and the resultant TFIIIB-DNA complexes are capable of correctly positioning RNA pol III for repeated cycles of initiation (32). Although the basic function of the transcription components appears to be conserved in all systems so far examined, there appears to be some species specificity of the RNA pol III transcription machinery. Although *Xenopus* and mammalian TFIIIB and TFIIIC components can be functionally exchanged in vitro (53), the *Drosophila* and mammalian components cannot be exchanged to produce heterologous active transcription complexes (28).

Analysis of the subunit structure of TFIIIC has revealed that this component consists of a complex set of polypeptides. For *Saccharomyces cerevisiae*, the use of antibodies (19, 48), photo-cross-linking reagents (3), and Southwestern (DNA-protein) blotting analysis (29) has identified several polypeptides that copurify with TFIIIC and specifically interact with the DNA template. Two of the DNA-binding subunits of TFIIIC with sizes of 95 kDa (61) and 135 kDa (39) have been cloned. In the human HeLa system, TFIIIC can be fractionated into two components, C1 and C2 (76) and C2

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has also been shown to contain a complex set of subunits (77). Two components derived from the *Bombyx* system, TFIIIC and TFIIID, may represent the C1 and C2 equivalents of the human system (46).

Initial purification of TFIIIB from yeast (35) and human HeLa (68) cells revealed that TFIIIB activity was contained on a polypeptide with a molecular size of 60 kDa. More recently, yeast TFIIIB activity was shown to be separable into two fractions, containing polypeptides of 70 and 90 kDa (3). The relationship between the 60- and 70-kDa polypeptides is not yet clear. A large body of evidence now supports the involvement of the TBP as part of the TFIIIB complex (74). The gene encoding the 70-kDa protein of yeast TFIIIB has been independently cloned by using two different genetic approaches. By its overexpression in yeast cells, the gene was isolated on the basis of its ability to suppress a temperature-sensitive mutation within the TBP gene (5, 10) and its ability to suppress a tRNA gene A block promoter mutation (43). In systems other than yeasts, however, the protein subunits complexed to TBP to form TFIIIB have not yet been defined.

The signaling pathway involving the activation of protein kinase C by tumor promoters such as TPA has been extensively studied by using mammalian cells (45). This response involves the induction of specific cellular and viral genes including proto-oncogenes, growth factors, various proteases, and RNA and DNA tumor virus genes (for a review, see reference 31). The analysis of the transcriptional response of many of the genes to phorbol esters revealed that a common DNA sequence element, TPA-responsive element, was required (1) and that this element was the recognition sequence for the transcription factor, AP-1, composed of *fos* and *jun* polypeptides (51). Examination of TPA-inducible genes in the *Drosophila* system has revealed that both RNA pol III (21) and RNA pol I (7, 67) gene expression is activated by TPA. Under the conditions used, the *Drosophila fos*- and *jun*-related antigen genes, however, were not found to be inducible (21). Therefore, the transcriptional response induced by phorbol ester in *Drosophila* cells may result in the activation of different sets of genes compared with those in the mammalian systems studied.

We are interested in defining the mechanisms governing the regulation of RNA pol III gene expression in *Drosophila* cells. To this end, we have developed an in vitro system that reproduces the TPA-dependent induction of the RNA pol III gene transcription observed in vivo (21). In the present study, we have analyzed the role of the two essential transcription components, TFIIIB and TFIIIC, in this response. We find that only TFIIIB activity is increased in the induced cell fractions. Furthermore, analysis of the TBP revealed that an increase in the amount of this subunit was correlated with the TPA response. Together, these studies demonstrate that the induction of transcription is due to an increase in the activity of TFIIIB which results in an increase in the number of functional transcription complexes.

MATERIALS AND METHODS

Cell cultures. Early-passage Schneider S2 cells (obtained from I. Schneider) were split to 1/40 confluency and grown for 4 days in Schneider medium (GIBCO) supplemented with 10% fetal calf serum. For induction with TPA, adherent cells approaching confluency were serum starved overnight in Schneider medium containing no fetal calf serum. The cells were then split 1:3 in Schneider medium containing no fetal calf serum and allowed to stand for an additional 24 h. Cell cultures were induced with 34 nM TPA for 15 min unless otherwise indicated. We experienced a large variability in serum dependence among S2 cells obtained from various laboratories. In general, the Schneider S2 cells with the largest serum dependence gave the largest TPA induction of RNA pol III transcription. We also found that cells down-regulated in Corning brand flasks reduced RNA pol III transcription only marginally. S2 cells down-regulated in Falcon brand flasks, on the other hand, routinely showed a relatively large reduction in RNA pol III transcription and, consequently, a very large induction by TPA.

Extracts and transcription factor preparation. The combined nuclear and cytoplasmic extracts were prepared by the method of Dignam et al. (13). The *Drosophila* Schneider S2 cell cytoplasmic (S100) extracts used for fractionation were prepared as described previously (15). The TFIIIB and TFIIIC fractions designated as B and C were prepared from cytoplasmic extracts derived from cells grown in Schneider medium containing 10% fetal calf serum. The TFIIIB and TFIIIC fractions designated as control fractions were derived from cells that had been serum deprived, and the fractions designated as induced fractions were prepared from cells that had been serum deprived and then treated with TPA. The TFIIIB and TFIIIC components were prepared from the cytoplasmic extracts by fractionation via phosphocellulose (Whatman P-11) chromatography as previously described (28). The extracts were loaded onto P-11 column at 5 mg/ml of bed volume in buffer A (20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.9], 0.1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) containing 100 mM KCl. The column was washed with this buffer, and proteins were step-eluted with buffer A containing 350 mM KCl (containing TFIIIB and RNA pol III activities) and 700 mM KCl (containing TFIIIC and RNA pol III activities). The fractions were dialyzed against buffer A containing 100 mM KCl and 5 mM $MgCl_2$ and stored at $-70°C$.

Plasmid DNAs. The plasmid DNAs containing genes used as templates for in vitro transcription are pArg, a *Drosophila* $tRNA^{Arg}$ gene, pArg-maxi, a derivative of pArg which contains an additional 12 nucleotides inserted between the internal promoter regions (14); and pDU6-2, a *Drosophila* U6 RNA gene (12).

Transcription assays. Standard transcription assay mixtures contained 0.3 μg of DNA template (unless otherwise indicated), 20 mM HEPES (pH 7.9); 5 mM $MgCl_2$; 3 mM dithiothreitol; 100 mM KCl; 10% glycerol; 0.5 mM each ATP, CTP, and UTP; and 0.1 mM [α-^{32}P]GTP (6 Ci/mmol) in a 60-μl final volume. Reactions were incubated for 1 h (unless otherwise indicated) at room temperature and stopped by the addition of 0.1% sodium dodecyl sulfate (SDS) and 400 μg of proteinase K per ml. After 15 min at 37°C, RNAs were purified by phenol extraction and ethanol precipitation and analyzed by electrophoresis on 8 M urea–8% polyacrylamide gels. Transcription products were visualized by autoradiography.

Western blot analysis. Equal amounts of protein from either cytoplasmic extracts or from the B fraction were subjected to SDS-polyacrylamide gel electrophoresis. Immunoblotting was carried out as described by Sambrook et al. (54). TBP was detected with affinity-purified antibodies raised against *Drosophila* TBP and were provided by Curtis Tyree and James Kadonaga, University of California, San Diego. Polyclonal antibodies against *Drosophila* eIF-2α were a gift from Douglas Cavener, Vanderbilt University.

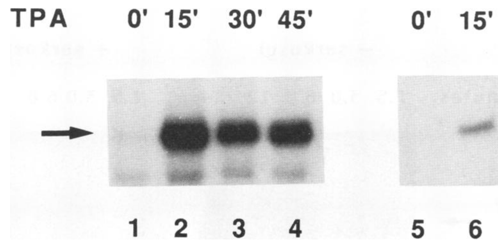

FIG. 1. Enhanced transcription of tRNA and U6 RNA genes in extracts derived from TPA-treated *Drosophila* Schneider cells. In vitro transcription assays were performed as described in Materials and Methods. The assay mixtures contained 0.6 μg of pArg as template (lanes 1 to 4) or 3.0 μg of pDU6-2 (lanes 5 and 6). Nuclear extracts (125 μg of protein) and cytoplasmic extracts (250 μg of protein) isolated from cells treated with TPA for the times indicated above each lane were combined and added to each reaction.

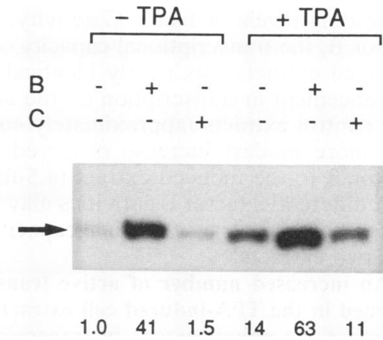

FIG. 2. TFIIIB is the limiting component in noninduced and induced cell extracts. Noninduced (−TPA) or induced (+TPA) S100 extracts (60 μg of protein) were used for transcription assays in vitro as described in Materials and Methods with the pArg template. The presence (+) or absence (−) of additional TFIIIB (60 μg) or TFIIIC (30 μg) in the transcription reaction is indicated above each lane. TFIIIB and TFIIIC fractions were prepared from S100 extracts as described in Materials and Methods.

Alkaline phosphatase-linked anti-rabbit antibodies (Vectastain) and enhanced chemiluminescence reagents (Amersham) were used to detect bound antibodies.

RESULTS

TFIIIB is the limiting component in both noninduced and induced cell extracts. Using nuclear run-on assays, we have previously shown that RNA pol III gene expression can be specifically induced in cell cultures treated with the phorbol ester TPA (21). The preparation of extracts from these cells and analysis of their transcriptional capacities revealed that a substantial increase in RNA pol III transcription occurs with extracts derived from induced cells compared with extracts from noninduced cells. Since our initial studies were carried out with cytoplasmic extracts, it was still conceivable that differences in the extractability of the transcription components could account for the observed differences in the transcriptional capacities of the noninduced and induced cell extracts. We therefore examined whether combined nuclear and cytoplasmic extracts still exhibit the same relative difference in transcription activities as the cytoplasmic extracts alone. Cytoplasmic S100 and nuclear extracts derived from *Drosophila* Schneider cells were prepared as described in Materials and Methods after the cells had been incubated for various periods with TPA (Fig. 1). When we combined the cytoplasmic and nuclear extracts and used the mixture to transcribe a $tRNA^{Arg}$ gene template (pArg) in vitro, we observed the maximum increase in transcription 15 min after TPA treatment of the cells (Fig. 1, lane 2) when compared to nontreated cells (lane 1). Thus, comparisons of these combined extract preparations from induced and noninduced cells revealed substantial increases in the transcriptional capacities of the induced extracts. By using the nuclear extracts alone, the same relative levels of transcription were observed between the noninduced and induced extracts; however, the level of transcription in these extracts was significantly lower than that in the cytoplasmic extracts (data not shown). Although there was some difference in the levels of transcription stimulation observed when the combined extracts were used and when only cytoplasmic extracts were used (Fig. 2), the difference was comparable to the variation observed between two sets of cytoplasmic extracts. Therefore, it does not appear that the substantial increase in transcription that we observed in vitro resulted from either differential partitioning of the transcription components between the nuclear and cytoplasmic compartments or differential extractability of these components. Since the transcription of both tRNA and 5S RNA class RNA pol III genes was previously shown to be TPA inducible (21), we also examined whether a different class of RNA pol III genes could also be similarly induced. Using a *Drosophila* U6 RNA gene as template (12), we observed a significant increase in transcription in the reaction containing the TPA-induced cell extracts over that in the noninduced extracts (Fig. 1, lanes 5 and 6). Thus, all three classes of *Drosophila* RNA pol III genes appear to be inducible by the phorbol ester TPA.

To examine the molecular mechanisms and components that mediate this regulatory event, we first determined whether there was a difference in RNA pol III in the induced and noninduced cell extracts. We previously showed that RNA pol III is not limiting in these *Drosophila* extracts (28). Examination of its catalytic activity revealed that it remained constant in the induced cell extracts (21). Furthermore, mixing experiments showed that the increased transcriptional activity of the induced cell extracts was due to an increase in the activity of a positive-acting factor. These results suggested that the increased transcriptional capacity of the induced cell extract could be a result of increased TFIIIB or TFIIIC activity or both.

To further determine the components responsible for the enhanced activity levels of the induced cell extracts, we first examined which of the transcription components were limiting within the cytoplasmic extracts. Transcription reactions were performed with extracts derived from noninduced and induced cells. Partially purified fractions containing the transcription factor TFIIIB (fraction B) or TFIIIC (fraction C) were added to these reactions. As shown in Fig. 2, the addition of fraction B to either the control or induced cell extracts produced an increase in transcription, whereas the addition of fraction C did not change the transcription activity of either extract. Further increasing the amount of fraction B or C did not change the transcriptional activities of these extracts (data not shown). Although the level of stimulation with the addition of B factor varied for each independently prepared extract, we always observed a pronounced increase in transcription with the addition of fraction B compared with no increase with the addition of factor C. Thus, the *Drosophila* Schneider cell cytoplasmic extracts contain limiting amounts of factor B activity and relatively

saturating levels of factor C activity. With the addition of factor B, the transcriptional capacity of the noninduced and induced extracts was nearly identical. The relatively large enhancement in transcription on the addition of factor B to the control extracts (approximately 40-fold) compared with the more modest increase observed with the addition of factor B to the induced extract (4.5-fold) further suggested that differential factor B activities may be responsible for the difference in the transcriptional capacities observed between the two extracts.

An increased number of active transcription complexes is formed in the TPA-induced cell extracts. We next examined whether the enhancement of transcription observed in the TPA-induced cell extract resulted from an increase in the number of active transcription complexes or whether it reflected an increase in the rate of reinitiation events from the same number of active transcription complexes. We used the anionic detergent Sarkosyl to analyze the number of active transcription complexes formed in both the noninduced and induced cell extracts. Previously, Sarkosyl has been used in transcription assays with HeLa cell extracts to limit reinitiation events from RNA pol III transcription complexes (36). To determine whether Sarkosyl could be similarly used in our in vitro reactions to limit initiation by RNA pol III to a single round per active gene complex, we performed transcription assays in the absence and presence of Sarkosyl (Fig. 3A). In these assays, stable preinitiation complexes were first allowed to form by preincubating the tRNA gene template with the induced cell extract before the addition of the nucleoside triphosphates (NTPs) and Sarkosyl. The reactions were then allowed to proceed for the times indicated. As shown, in the absence of Sarkosyl, multiple rounds of transcription initiation produced an increase in RNA synthesis with corresponding increases in reaction times. In the presence of Sarkosyl, a single round of transcription occurred within 1.5 min. Further increasing the reaction times, however, did not increase the amount of RNA synthesized, indicating that subsequent rounds of initiation were blocked. Kinetic analysis of the transcription reaction has shown that one full-length transcript is produced within 30 s (36; data not shown). We therefore observed an approximately threefold difference in the amount of tRNA transcript produced in 1.5 min in the absence compared with the presence of Sarkosyl (Fig. 3A). Thus, these conditions allowed us to examine the relative numbers of active transcription complexes that formed within the noninduced and induced cell extracts. With the inclusion of Sarkosyl in the transcription reactions, we observed a substantial increase in the amount of RNA synthesized in the induced cell extracts compared with that produced in the noninduced cell extracts (Fig. 3B). It is possible, however, that the transcription complexes formed in the induced and noninduced cell extracts are differentially sensitive to Sarkosyl, which could preferentially disrupt complexes formed in the noninduced extracts. We therefore compared the level of transcription from these two extracts in reactions carried out for 1 min in the absence of Sarkosyl (Fig. 3B). This period should allow the synthesis of approximately two full-length transcripts per gene complex. As shown, we again observed a substantial difference in the level of RNAs produced from each extract. Taken together, these results indicate that the TPA-induced cell extracts form an increased number of functional transcription complexes that is proportional to their overall increase in transcription.

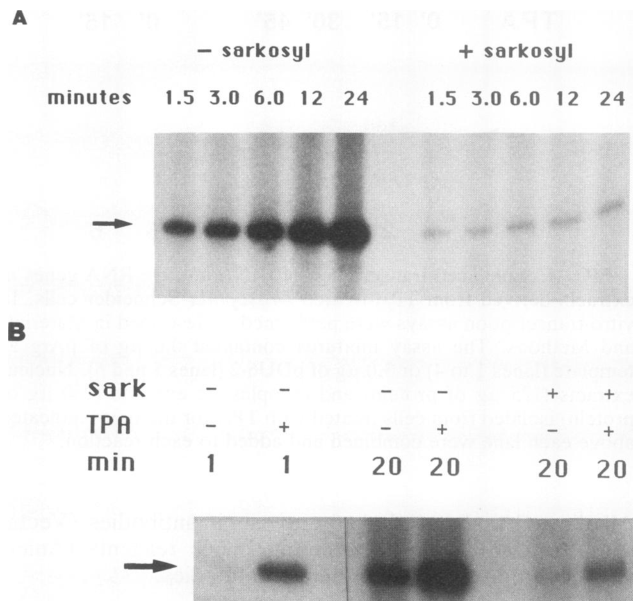

FIG. 3. The number of functional transcription complexes is increased in the TPA-induced cell extracts. (A) The addition of Sarkosyl to stable preinitiation complexes produces a single round of transcription. Transcription reactions were carried out with cytoplasmic extracts (200 μg of protein) for the times indicated. Stable complexes were allowed to form by preincubation of the extract with the pArg template for 30 min. The reactions were allowed to proceed by the addition of NTPs; where indicated, Sarkosyl was added to a final concentration of 0.08%. (B) Limiting reinitiation from stable transcription complexes, induced cell extracts exhibit an increase in the amount of transcripts produced. Extracts from TPA-induced (+) or noninduced (−) cells were used in the transcription reactions. Stable complexes were allowed to form with the pArg template prior to the addition of NTPs and, where indicated, 0.08% Sarkosyl (sark). The reactions were carried out for the times indicated.

Neither the DNA-binding activity of TFIIIC nor its ability to reconstitute transcription is altered in the induced cell extracts. Since there was a significant difference in the number of active transcription complexes formed within the two extracts, we next determined whether this difference could be accounted for by alterations in the ability of the C component to bind to the DNA template. Using template commitment assays, we examined the relative DNA-binding activities of factor C derived from the noninduced and induced cell extracts (Fig. 4). In each reaction, saturating levels of the factor B fraction were reconstituted with a factor C fraction isolated from either cell extract. These fractions were preincubated with increasing amounts of a competitor tRNA gene template, pArg, to allow stable complexes to form. A constant amount of the reference template, pArg-maxi, was then added with the NTPs, and the reaction was allowed to proceed. As shown, with factor C fraction from either extract, increasing amounts of competitor DNA reduced the level of transcription from the reference template to the same extent. These results indicate that the relative DNA-binding activities of factor C from the noninduced and induced cell extracts are essentially unchanged. Therefore, although there is a dramatic difference in the number of active gene complexes between the extracts, the two factor C preparations still maintain a similar capacity to form these complexes. This suggests that the

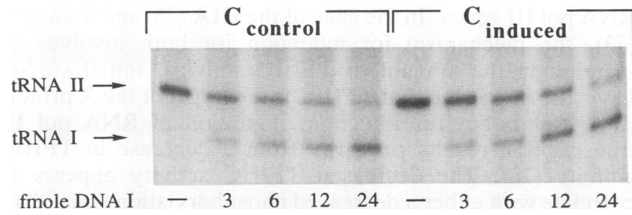

FIG. 4. The DNA-binding activity of TFIIIC is unchanged in the TPA-induced cell extracts. In vitro transcription assays were carried out with 60 μg of the TFIIIB fraction in each reaction. Control and induced TFIIIC fractions (5 μg) were preincubated for 30 min with increasing amounts of the pArg template (DNA I) and the TFIIIB fraction before the addition of 24 fmol of the pArg-maxi template (DNA II) and the NTPs. Where necessary, pUC19 was added to maintain a total of 48 fmol of DNA. Transcription reactions were allowed to proceed as described in Materials and Methods. Labeled RNAs transcribed from DNA I and DNA II are designated tRNA I and tRNA II, respectively.

total number of stable C-DNA complexes is unaltered in this regulatory event.

In addition to the specific interaction of factor C with the DNA template, the formation of active tRNA gene transcription complexes involves the subsequent association of factor B with the C-DNA complex (30, 38). Since the association of factor B involves direct contacts with factor C, it is conceivable that alterations in the factor B interaction site on the C component could render it incapable of forming active transcription complexes. We therefore measured the relative abilities of the factor C fractions derived from these extracts to reconstitute tRNA gene transcription (Fig. 5A). Transcription reactions were reconstituted with a saturating level of the B factor fraction and increasing amounts of the factor C fraction derived from either noninduced or induced cell extracts. Approximately the same amount of the factor C fraction in each case was required to generate the same level of transcription. Generally, depending on the factor C preparation, the relative difference between noninduced and induced factor C activities varied between 0.8- and 1.7-fold. These results indicate that the ability of factor C to reconstitute transcription remains constant in the induction process.

TFIIIB activity is enhanced in the induced cell extracts. We next determined the relative levels of the factor B activities in the noninduced and induced cell extracts by reconstituting increasing amounts of each of the B factor preparations with a constant and saturating amount of the factor C fraction (Fig. 5B). These results show that the relative level of transcription was substantially increased in the reactions containing the factor B fraction from induced cell extracts. Furthermore, this large difference in the factor B activities approximates the difference in the transcriptional activities observed in the unfractionated extracts.

The increased factor B activity in the induced cell extracts could be a result of either an increase in the levels of protein or modification(s) in the preexisting protein. To begin to address this, we used Western immunoblot analysis to examine whether we could detect either quantitative or qualitative changes in the TBP subunit of TFIIIB in the noninduced and induced cell extracts (Fig. 6). Equal amounts of protein derived from each of the control and induced cell extracts (lanes 2 and 3) and factor B fractions (lanes 7 and 8) were compared for the amount of cross-reactivity with affinity-purified *Drosophila* TBP antibodies.

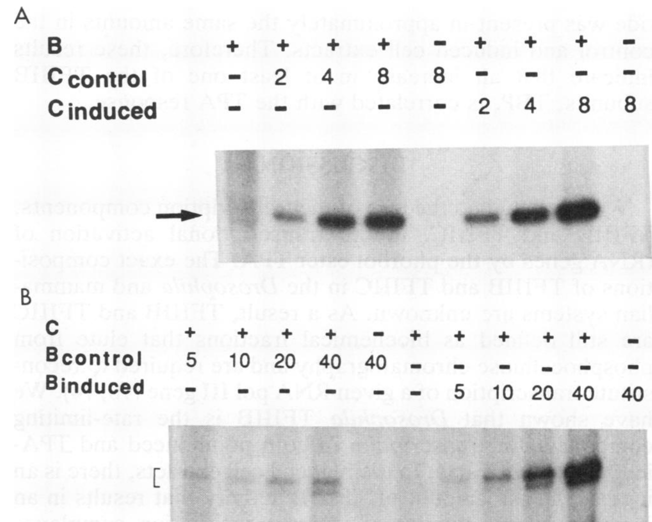

FIG. 5. Analysis of TFIIIB and TFIIIC activities from TPA-induced and noninduced cell extracts. (A) The ability of TFIIIC to reconstitute transcription is the same in the noninduced and induced cell extracts. Transcription assays were carried out with pArg as template as described in Materials and Methods. The transcription factor fractions used were prepared as described in Materials and Methods. As indicated, reactions contained 60 μg of TFIIIB and 2 to 8 μg of TFIIIC derived from either TPA-induced or noninduced cell extracts. (B) The activity of TFIIIB is increased in the induced cell extracts. Transcription assays were performed with pArg as template. As indicated, reactions contained 11 μg of TFIIIC and 5 to 40 μg of TFIIIB prepared from either TPA-induced or noninduced cell extracts.

These results revealed that there was an approximate 4- to 10-fold increase in the amount of TBP present in both the extract and factor B fractions derived from induced cells relative those derived from noninduced cells. To further ascertain whether TBP was specifically increased relative to other proteins in these extracts, we also examined the relative amounts of the translational component, eIF-2α (lanes 4 and 5). In contrast to the TBP, this 38-kDa polypep-

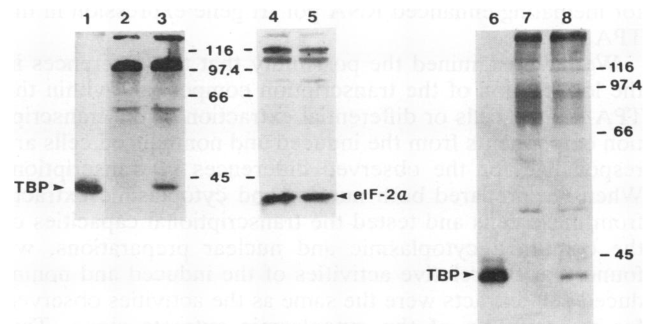

FIG. 6. An increase in the amount of the TBP subunit in induced cell fractions is observed by Western blot analysis. Western blots were prepared as described in Materials and Methods. Lanes 1 to 3 and 6 to 8 were probed with anti-*Drosophila* TBP antibodies. Lanes 4 and 5 were probed with anti-*Drosophila* eIF-2α antibodies. Lanes: 1, 2 ng of recombinant TBP; 2 and 4, 25 μg of cytoplasmic extract from control cells (−TPA); 3 and 5, 25 μg of cytoplasmic extract from induced cells (+TPA); 6, 2 ng of TBP; 7, 12 μg of control TFIIIB fraction (−TPA); 8, 12 μg of induced TFIIIB fraction (+TPA).

tide was present in approximately the same amounts in the control and induced cell extracts. Therefore, these results indicate that an increase in at least one of the TFIIIB subunits, TBP, is correlated with the TPA response.

DISCUSSION

We have studied the role of the transcription components, TFIIIB and TFIIIC, in the transcriptional activation of tRNA genes by the phorbol ester TPA. The exact compositions of TFIIIB and TFIIIC in the *Drosophila* and mammalian systems are unknown. As a result, TFIIIB and TFIIIC are still defined as biochemical fractions that elute from phosphocelluose chromatography and are required to reconstitute transcription of a given RNA pol III gene (70, 74). We have shown that *Drosophila* TFIIIB is the rate-limiting component for transcription in both noninduced and TPA-induced cell extracts. In the induced cell extracts, there is an increase in the amount of TFIIIB activity that results in an increase in the amount of active transcription complexes formed. In addition to inducing tRNA and 5S RNA gene expression (21), we have shown in the present study that TPA enhances the transcription of a U6 RNA gene. Although it has not yet been examined in *Drosophila* cells, TFIIIB but not TFIIIC is required for the transcription of U6 RNA genes in yeast and mammalian systems (44, 52, 69). It has been recently shown, however, that in the yeast system TFIIIC may stimulate U6 RNA gene transcription and relieve repression of U6 RNA gene transcription by chromatin (6, 17). If TFIIIC is not required for U6 RNA gene transcription in the *Drosophila* system, the mechanism to induce transcription of the U6 RNA gene could only conceivably involve alterations in TFIIIB. Since our previous studies showed that the catalytic activity of RNA polymerase was unaltered in this response (21) and our present studies have revealed that both the DNA-binding activity of TFIIIC and its ability to reconstitute transcription are unchanged in the induced cell extracts, the increase in TFIIIB activity alone appears to account for the difference in the transcriptional capacities between the noninduced and TPA-induced cell extracts. We have also demonstrated that the amount of at least one of the TFIIIB subunits, TBP, is increased in this regulatory event. Together, these results indicate that it is the TFIIIB component that is responsible for mediating enhanced RNA pol III gene expression in the TPA response.

We have examined the possibility that the differences in the localization of the transcription components within the TPA-treated cells or differential extraction of the transcription components from the induced and noninduced cells are responsible for the observed differences in transcription. When we prepared both nuclear and cytoplasmic extracts from these cells and tested the transcriptional capacities of the combined cytoplasmic and nuclear preparations, we found that the relative activities of the induced and noninduced cell extracts were the same as the activities observed for transcription of the cytoplasmic extracts alone. This indicates that the increased activity of the induced cell extracts does not simply result from an increased partitioning of TFIIIB into the cytoplasmic compartment.

Examination of the regulation of RNA pol III gene expression has focused largely on mammalian cells and the effects of specific viral transactivator proteins. The adenovirus E1A protein (4, 22, 27), the hepatitis B virus X protein (2), and the simian virus 40 large T antigen (42, 73) have all been shown to enhance transcription of transfected cellular and viral RNA pol III genes. In the case of the E1A (26) and T antigen (73), the mechanism for induction for both involves an increase in the amount of TFIIIC activity. Initial studies have also suggested that TFIIIC is the target of the X protein (2). After poliovirus infection, inhibition of RNA pol III transcription results primarily from a decrease in TFIIIC activity (18). The decreased TFIIIC activity appears to correlate with either a decreased phosphorylation state (8) or proteolytic cleavage of the protein by the poliovirus proteinase 3C (9). Thus, the regulation of RNA pol III gene expression in mammalian systems by viral proteins appears to involve changes in TFIIIC to either increase or decrease its activity.

The regulation of *Xenopus* 5S RNA genes has been extensively studied (for reviews, see references 23 and 47). The developmental switch from the oocyte to somatic genes is attributed to changes in the levels of TFIIIA as well as to a differential ability of TFIIIC to stabilize the binding of TFIIIA to these genes (75). In another developmental event, the differentiation of F9 embryonic carcinoma stem cells into endoderm results in a down-regulation of RNA pol III transcription (72). In this case, a decrease in TFIIIB activity is thought to account for the decreased rate in transcription. The down-regulation of RNA pol III genes in growth-restricted cells was similarly found to be a result of decreased TFIIIB activity (66). Taken together, the various regulatory mechanisms governing RNA pol III gene regulation that have so far been examined appear to involve changes in the activity of at least one of the required transcription components. In most cases, the up-regulation of these genes appears to be correlated with increased TFIIIC activity, whereas events that result in down-regulation of these genes can occur via either decreased TFIIIC or TFIIIB activities. Our studies represent the first example of a non-virus-mediated event which induces RNA pol III transcription through an increase in TFIIIB activity. Whether other events that serve to modulate the activity of these genes in *Drosophila* cells are similarly mediated by TFIIIB remains to be determined.

The increase in TFIIIB activity observed after treatment of the cells with TPA could result from either an increase in the level of the TFIIIB protein, or a modification(s) of the preexisting protein which enhances its activity. Recent studies have revealed that the TFIIIB component contains the TBP and TBP-associated factors (TAFs) (for a review, see reference 74). The regulation of RNA pol III genes through modulation of TFIIIB activity therefore presents the interesting possibility that TBP participates in this regulatory event. We have therefore examined the TBP in our *Drosophila* fractions by Western blot analysis with antibodies directed against *Drosophila* TBP. Using both the noninduced and induced cell extracts and TFIIIB fractions, we find that the amount of this polypeptide present in these fractions is increased in the induced cell fractions. These results further substantiate the conclusion that the TPA-responsive factor is TFIIIB and not just another component that coelutes with the TFIIIB component. Although the other polypeptides that compose *Drosophila* TFIIIB have not yet been identified, it is conceivable that additional quantitative and/or qualitative changes in these polypeptides could be produced by this regulatory event.

The TBP directs transcription by all three eucaryotic RNA polymerases (for reviews, see references 24, 25, and 58). In extracts from mammalian and *Drosophila* cells, TBP is found in distinct high-molecular-weight complexes (16, 49, 50, 63). The TBP complex contained in the phosphocellulose

TFIIIB fraction from HeLa extracts was found to be part of the TFIIIB activity and essential for transcription of TATA-less RNA pol III genes such as VAI RNA and 5S RNA genes (41). At least two unique TAFs have been identified from this phosphocellulose fraction as part of the HeLa TFIIIB complex (62, 64). TBP does not form a stable complex with TATA-less RNA pol III genes alone, but it associates specifically with a component within the HeLa TFIIIB phosphocellulose fraction (70). Thus, it appears that although TBP is used for the transcription of all cellular genes, recruitment of TBP to a particular gene is dictated by the specific TBP-TAF complex and its ability to interact with other factors assembled on the DNA template.

Our finding that the amount of TBP is increased in both the induced extract and the TFIIIB fraction in response to TPA suggests that there could be an increase not only in the levels of the TFIIIB complex but possibly also in the levels of other TBP-TAF complexes. For certain TAFs that are present in excess amounts in *Drosophila* cells relative to TBP, increasing the amount of cellular TBP could produce increases in the levels of these TBP-TAF complexes. In HeLa extracts, all TBP is found to be present in complexed form (65). It is unlikely, however, that TBP is limiting relative to all cellular TAFs. If this were the case, we would have expected to observe induction of all cellular genes by the phorbol ester TPA. However, in the *Drosophila* system, the TPA response did not produce a detectable increase in the expression of several RNA pol II genes examined (21). The TPA response has also been shown to induce the transcription of RNA pol I genes (7, 67). Whether the activity of the TBP-containing RNA pol I factor, SL1, is enhanced in this regulatory event remains to be determined. It is also conceivable that the TPA response could induce increases in other TAF components in addition to TBP, increasing the levels of active TFIIIB complexes. Alternatively, modifications of certain TAFs could alter the abilities of these TAFs to associate with specific TBP-TAF complexes, again increasing the relative amounts of active TFIIIB complexes. The specific roles of the TBP and other TFIIIB subunits in this TPA-mediated regulatory event await further investigation. Since the TBP is a central factor in the transcription of all cellular genes, this system may provide a unique opportunity to study the regulation of specific TBP-containing complexes.

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