A Negative Cofactor Containing Dr1/p19 Modulates Transcription with TFIIA in a Promoter-specific Fashion*

(Received for publication, February 1, 1996, and in revised form, May 14, 1996)

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An activity that modulated the relative levels of transcription from the adenovirus major late promoter (MLP), and the immunoglobulin heavy chain μ promoter (μ) was purified as a 90-kDa factor. This factor is suggested to be a heterotetramer of two subunits: a 20-kDa polypeptide identical to the previously described Dr1/p19 and a novel 30-kDa polypeptide. The Dr1/p19 protein has been characterized as a repressor of transcription, and the 30-kDa protein is related to a recently identified yeast gene proposed to encode a repressor of transcription. The 90-kDa factor forms a complex with TATA-binding protein on DNA and at high concentrations of both factors protects over a 150-base pair region around the promoter from DNase I cleavage. The conformation of this complex as assayed by footprinting analysis is altered by the transcription factor TFIIA on the MLP but not on the μ promoter. Similarly, TFIIA reverses the repression of transcription by the 90-kDa factor on the MLP but not on the μ promoter. Thus, the interactions of TATA-binding protein, TFIIA, and the 90-kDa factor are promoter-specific.

Transcription reactions in vitro were developed to analyze the synthesis of pre-mRNA from promoter DNA. Reconstitution of transcription with chromatographic fractions has allowed purification of general transcription factors and isolation of the genes encoding the corresponding polypeptides (for review see Refs. 1–3). Also identified were activities dispensable for the basal levels of transcription that affected the absolute amounts of product RNA. Initially, activities that suppressed transcription in vitro were largely ignored, but as addition of these activities was shown often to increase the relative response to sequence-specific transcriptional activators, interest in their identity and mechanism grew (4–6). These are collectively referred to as negative cofactors of transcription and are distinct, at least operationally, from silencer element-dependent proteins in that they repress a wide variety of promoters.

Several lines of evidence suggest that repression of transcription is a general and important aspect of transcriptional regulation. Both genetic and biochemical experiments suggest that chromatin structure represses transcription (for review see Refs. 7 and 8). Furthermore, studies of SWI/SNF complexes show that controlling the accessibility of chromatin bound promoters to transcription factors is one of the regulatory steps of transcription (9–12). Genetic screening for yeast mutants with elevated basal transcription and for suppressors of mutations to specific upstream activating sequences has led to the identification of several so-called global repressors of polymerase II (pol II) transcription (13–17). Mutations to many of them have pleiotropic effects, often elevating the level of transcription from multiple promoters. The SRB family of proteins, found as suppressors of C-terminal domain truncation mutations of pol II, are implicated as important components of transcription initiation in vivo (for review see Ref. 18). Several of SRB genes have been shown to be essential for survival, and in particular the analysis of SRB4 gene suggested a direct involvement in the transcription of most genes (19, 20). A conditional loss of function mutation in SRB4 gene can be suppressed by a loss of function mutation in other genes, suggesting the presence of a repression system operating in opposition to the SRB proteins.2

Chromatographic fractionation of extracts from mammalian cells identified several activities that repress transcription in vitro (4–6). Among these negative cofactors of transcription is Dr1 (21). It was described as a homotetramer of a 19-kDa phosphoprotein capable of forming protein-DNA complexes with TBP and blocking transcription. The inhibition of transcription was not reversible by the addition of any basal factors including TFIIA. Transfection assays indicated that Dr1/p19 can repress transcription in vivo also and may be a target of regulation by upstream activators (22, 23). Specifically, Dr1/p19 repressed pol II promoters generally when expressed in cells by transfection, and this repression was reversed by co-transfection of activators such as VP16 and E1A135. Additionally, NC2, a negative cofactor with properties similar to Dr1 including its binding to TBP, has been described by Kim et al. to contain Dr1 (24). Immunoprecipitation of labeled extracts with specific antiserum indicated that Dr1/p19 exists inside the cell in association with several proteins (21). The activities of complexes containing these proteins have not been characterized.

We have purified a 90-kDa transcriptional cofactor consisting of Dr1/p19 and a novel protein with a molecular mass of 30 kDa. This report describes the initial characterization of the cofactor complex. The results suggest that it is primarily a negative cofactor with differential effects depending on the identity of the core promoter element. The mechanism of the repression involves formation of complexes with TBP at the...
promoter, and variations between core promoters were manifest in the presence of TFIIA.

EXPERIMENTAL PROCEDURES

Purification of the 90-kDa Factor—The 90-kDa factor was purified by following its stimulatory activity on MLP transcription from the Hela nuclear extract-derived 0.3 M KCl DEAE fraction (C1) prepared as described previously (25). The C1 fraction was adjusted to buffer A (20 mM Hepes-NaOH (pH 7.9), 20% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonfyl fluoride) plus 500 mM KCl and applied to a Superdex 200 26/60 column (FPLC, Pharmacia Biotech Inc.). Fractions corresponding to the molecular mass of 90 kDa were pooled, equilibrated to buffer A plus 100 mM KCl, and loaded onto a Mono-S HR 5/5 column (FPLC, Pharmacia). The bound material was eluted with a 15-mL linear gradient of 100 to 400 mM KCl in buffer A. The active fractions, found between 200 and 300 mM KCl, were pooled, equilibrated to buffer A plus 100 mM KCl, and then applied to a Mono Q HR 5/5 column (FPLC, Pharmacia). The loaded material was eluted with a 12-mL linear gradient from 100 to 500 mM KCl in buffer A. The activity was eluted near 400 mM KCl. The active fractions were dialyzed to buffer A plus 500 mM KCl and loaded onto a Superdex 200 16/60 column (FPLC, Pharmacia). The active fractions of the 90-kDa factor are shown in Fig. 1A.

Binding to Immobilized DNA—The DNA fragment containing the MLP region was the HindIII and XhoI fragment of the MLP transcription template plasmid (26, 27). Coupling of the biotin-labeled DNA to magnetic beads has been described (28). The protein preparations were incubated with the immobilized DNA in the transcription buffer plus 0.1% Nonidet P-40 in a total volume of 30 µl. 120 ng of yeast TBP (29) and a 400-fold dilution of the 90-kDa factor preparation were used. The protein-DNA complexes were subsequently isolated and washed three times with 30 µl of the incubation buffer. Subsequently, a semi-stringent wash with 30 µl of the transcription buffer with 150 mM KCl and 0.1% Nonidet P-40 was done. The washed beads were resuspended in SDS-polyacrylamide gel electrophoresis buffer, boiled, and loaded onto the SDS gel directly.

Amino Acid Sequencing of p30—An estimated amount of 2.5 µg of p30 was acetone-precipitated from the final gel filtration chromatography fractions and resolved on 10% SDS gel. After being transferred to nitrocellulose membrane, proteins were visualized by Ponseau S staining. Subsequently, the band representing p30 was excised, destained, and digested by endoproteinase Lys-C. The resulting peptides were separated on a C4 column using an Applied Bioscience (Biosystems) HPLC. Amino acid sequencing was done by Richard Cook (MIT Biopolymers Laboratory) with an Applied Biosystems model 477A protein sequencer with on-line model 120 PTH-derivative analyzer.

Cloning of p30—An adult human frontal cortex cDNA library (Clontech) was screened with a hybridization probe derived from the rat cDNA (30) and a partial peptide sequencing (data not shown). The activity enhanced transcription from the MLP of adenovirus and inhibited transcription from the µ promoter of the immunoglobulin heavy chain gene. In addition, the 90-kDa activity formed protein-DNA complexes in association with TBP on the MLP. This activity was purified to near-homogeneity by assaying for its stimulatory effect on the MLP. The final preparation contained three polypeptides of 20 (p20), 30 (p30), and 34 kDa (Fig. 1A). In the Mono S chromatographic step prior to the gel filtration chromatographic step, the 34-kDa protein did not copurify with the other two proteins or with the MLP-stimulating activity (data not shown) and thus is most likely not a component of the activity. Coomassie Brilliant Blue and Ponseau S stainings of the proteins in the preparation indicated one-to-one stoichiometry of p20 and p30 (data not shown). The purified activity chromatographed as a protein factor of 90 kDa as reported for the initial preparation (25).

Several lines of circumstantial evidence suggested that the p20 was the previously cloned transcriptional cofactor, Dr1/p19 (21). These included the similarity of chromographic behavior, the ability to form protein-DNA complexes with TBP, and the modulatory effect on transcription. Their identity was confirmed by a Western assay using an anti-Dr1 antisera (gift of D. Reinberg; Fig. 1B) and partial peptide sequencing (data not shown).

Dr1 was originally described as a homotetramer of the p19 phosphoprotein (21). Thus, it was of interest to determine whether p30 was associated with Dr1/p19 (p20) or was an incidentally copurifying species. The association of the two polypeptides was tested by a DNA binding assay, taking advantage of the proposed interaction between TBP and the 90-
and sequenced leading to a composite peptide sequence containing all of the microsequenced peptides and a potential N-terminal methionine (Met\(^{12}\); Fig. 3A). The polypeptide predicted from the open reading frame consists of 216 amino acids with a calculated mass of 23.7 kDa. This molecular mass is smaller than that indicated by SDS gel electrophoresis (30 kDa) and suggests that the cDNA may be incomplete. Interestingly, the predicted p30 peptide sequence shows a strong homology to HAP5 (32% identity; score of 142 using BLOSUM62 matrix; p value versus Non-Redundant Data Base as of April 18, 1996 of 3.5e-11), a subunit of yeast CCAAT binding heteromeric protein complex also containing HAP2, HAP3, and HAP4 (33). Considering that Dr1/p19 (p20) has homology to HAP3, another subunit of the CCAAT binding complex, the homology between p30 and HAP5 further suggests that Dr1/p19 (p20) and p30 form a complex. p30 also shows a strong homology to a Saccharomyces cerevisiae protein (gene YER159c; GenBank\(^{\text{TM}}\) accession number, U18917). In fact, the sequence relationship of p30 to the yeast gene is greater than that to HAP5 (45% identity; score of 192; p value of 5.1e-20). Interestingly, this protein has recently been identified as a suppressor of mutations in the SRB4 gene, which encodes a component of the pol II holoenzyme in yeast (see “Discussion”).

The 90-kDa Factor in Transcription—Dr1 has been described as a negative cofactor of basal transcription (21), yet the 90-kDa factor has been purified based on its stimulatory effect on the MLP (25). Systematic transcription assays with a defined set of transcription factors revealed that the 90-kDa factor stimulated transcription only in the presence of TFIIA and saturating levels of TBP (data not shown). Further analysis indicated that the stimulatory activity of the 90-kDa factor was due to a derepression from an inhibitory effect of TFIIA. Specifically, in the presence of saturating levels of TBP, TFIIA repressed transcription from the MLP by 50% (Fig. 4A, compare lanes 1, 6, and 11), and the addition of increasing levels of the 90-kDa factor relieved this inhibition (Fig. 4A, lanes 6-10 and lanes 11-15). This was despite the fact that identical levels of the 90-kDa factor by itself inhibited transcription 6-fold (Fig. 4A, lanes 1-5).

TFIIA has been associated with a stimulation of transcription, and both the repression by TFIIA and the derepression by a second repressor were paradoxical. An insight was obtained by observing another transcript of 90 nucleotides. This internally initiated transcript originating within the G-less cassette is probably generated by a TATA-like sequence, 5'–TATATT-3' (designated as the CTE for the cryptic TATA element), located 30 bases upstream of its 5' end (data not shown). TFIIA stimulated transcription from the CTE under conditions in which it repressed transcription from the MLP (Fig. 4A, compare lanes 1, 6, and 11) as if it was modulating a competition between the MLP and the CTE. Consequently, the ratio of transcription from the MLP and CTE was changed from 6.7-fold (Fig. 4A, lane 1) to 2.2-fold (Fig. 4A, lane 11). Undoubtedly, TBP binds to a number of other TATA like sequences along the length of the plasmid templates, but initiations at only a limited subset such as the CTE would be detected in the GTP minus conditions used in these assays. The high concentrations of TBP used in these assays would generate competition between various TBP-promoter complexes for other limiting basal factors. Under such conditions, TFIIA could alter in a promoter-specific fashion the affinity of the TBP-promoter complexes for the other basal factors and thus alter the relative levels of transcription. Consistent with this model, TFIIA still stimulated the CTE but did not repress transcription from the MLP when TBP was added at subsaturating levels and the other basal transcription factors were abundant relative to TBP (Fig.
The 90-kDa factor likely prevents the progression of the initiation complex assembly beyond TBP binding for both the MLP and the CTE in the absence of TFIIA. The presence of TFIIA blocked therepressionbythe90-kDa factor on the MLP but not on the CTE. Under TBP saturating conditions, this block increased binding of limiting basal factors to the MLP, thus accounting for the stimulation of the MLP (Fig. 4A, lanes 6–10 and lanes 11–15), and under TBP limiting conditions, it resulted in the maintenance of the level of transcription from the MLP (Fig. 4B, lanes 6–10 and lanes 11–15). The inability of TFIIA to function equivalently at the CTE resulted in the repression of transcription from the CTE by the 90-kDa factor at all levels of TBP and TFIIA. Such promoter-specific activities of TFIIA and the 90-kDa factor resulted in a wide range of the ratio of transcription from the MLP and CTE from as low as 2.2-fold (Fig. 4A, lane 11) to as high as 9.6-fold (Fig. 4A, lane 10).

It is of interest that TFIIA has a variable effect on different core promoters. On the CTE, it had a net stimulatory effect but did not block repression by the 90-kDa factor. On the MLP, TFIIA did not have a net stimulatory activity but restored (Fig. 4A) or maintained (Fig. 4B) the capability of TBP to associate with the other basal factors in the presence of the 90-kDa factor. Again, consistent with this interpretation, under conditions of limiting TBP relative to the other basal factors, addition of the 90-kDa factor did not stimulate the MLP at any level of TFIIA (Fig. 4B).

The above results indicated that both TFIIA and the 90-kDa factor differentially affect core promoters. To study this possibility, the effect of the 90-kDa factor in combination with TFIIA was tested on a series of core promoters. In the absence of TFIIA, the 90-kDa factor repressed transcription from all of the tested promoters (Fig. 5, lanes 1–5 for each promoter). It should be noted that the repression showed highly variable efficiencies. The adenovirus E4 promoter (E4) was completely repressed in the presence of 2 ng of the 90-kDa factor while at the same level, the interleukin-2 promoter showed substantial residual activity. In the presence of TFIIA (lanes 6–10 for each promoter), the E4 and the interleukin-2 promoters were not repressed by the 90-kDa factor. However, repression by the 90-kDa factor was not blocked by TFIIA for either the immunoglobulin heavy chain \( \mu (\mu) \) or the immunoglobulin light chain \( \kappa (\kappa) \) promoter. Thus, TFIIA and the 90-kDa factor show promoter-specific effects individually and together result in a large change in the ratio of expression levels of various promoters.

Protein-DNA Complexes on Promoters Formed by the 90-kDa

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Factor, TBP, and TFIIA—Interactions of TBP, TFIIA, and the 90-kDa factor with the core promoters were examined by DNase I footprinting assay. TBP protected the TATA region (−18 to −35) of the MLP as expected (Fig. 6A, compare lanes 1 and 2). As the 90-kDa factor was titrated, a series of DNase I hypersensitive sites and protected regions emerged (Fig. 6A, lanes 2–5). Specifically, at the highest level of the 90-kDa factor (Fig. 6A, lane 5), several hypersensitive sites (−140, −115, −100, −85, −75, −60, −15, and +10) either became more sensitive to or maintained the level of cleavage by DNase I, but in between these sites the bases were protected by DNase I digestion. Within the TATA region the cleavage pattern also changed; bases at −31, −29, −27, −22, and −20 showed slightly increased sensitivity (Fig. 6A, compare lanes 2 and 5). As anticipated, the addition of TFIIA in combination with TBP induced a slight extension of the protection from −35 to −38 followed by hypersensitive sites at −42 and −43 (Fig. 6A, compare lanes 2 and 14, for example). Adding TFIIA and the 90-kDa factor together showed that the pattern induced by the 90-kDa factor was modulated by TFIIA. At low and intermediate concentrations of TFIIA, high concentrations of the 90-kDa factor generated its typical pattern (Fig. 6A, lanes 9 and 13). However, at the highest concentration of TFIIA, the 90-kDa factor induced protection between the hypersensitive sites diminished, and the cleavage pattern within the TATA region was restored to the TBP-TFIIA induced pattern (Fig. 6A, lanes 14–17). It should be noted however that even at the highest concentration of TFIIA and the lowest concentration of the 90-kDa factor, there were detectable alterations of the digestion pattern outside TATA element (Fig. 6A, compare lanes 14 and 15). This raised the possibility that on a given molecule of DNA, both the 90-kDa factor and TFIIA can coexist. It should also be added that the 90-kDa factor and TFIIA in the absence of TBP show no alterations on the footprint pattern either individually or together at the level assayed (data not shown).

The extension of footprints outside TATA box required a higher level of TBP than necessary to protect the TATA box. Specifically, although 10 ng of TBP was sufficient to protect the TATA region completely, adding a high level of the 90-kDa factor resulted in no extended footprint alterations beyond the TATA region (data not shown). Under such conditions, the 90-kDa factor still induced its typical pattern within the TATA region that was reversible by TFIIA. These data indicate that the 90-kDa factor probably forms oligomeric structures by association with multiple TBP molecules. Further confirmation was obtained from EMSA (see below). Also of interest, the addition of nonspecific competitor DNA such as poly(dGdC) deprotected the TATA site from TBP in the presence but not in the absence of the 90-kDa factor (data not shown). It is likely that the 90-kDa factor weakly binds to DNA as well as to TBP and under these conditions sequesters TBP away from the promoter onto the heteropolymer DNA.

DNase I footprinting was repeated with the μ promoter using identical levels of the proteins. As in the case of the MLP, TBP protected the TATA region from −18 to −35 (Fig. 6B, compare lanes 1 and 2). Additional partial protection of the AT-rich sequences 3′ to the TATA box occurred as a consequence of the high level of TBP used in the absence of poly(dGdC). Titration of the 90-kDa factor induced alterations that were qualitatively similar to those with the MLP. At the highest level of the 90-kDa factor (Fig. 6B, lane 5), hypersensitive sites (−125, −118, −110, −95, −65, −21, −11, and +13) were generated, and the regions in between were protected from the cleavage (Fig. 6B, lanes 2–5). The addition of TFIIA to intermediate levels of the 90-kDa factor partially restored the footprint pattern around the TATA region to the TBP-TFIIA induced pattern in that sensitivity of the base −39 increased and that of the bases −35 and −21 decreased (Fig. 6B, compare lanes 4, 8, 12, and 13).
However, unlike in the case of the MLP, the extended footprint as well as alterations around the TATA region induced by the highest level of the 90-kDa factor were resistant to TFIIA (Fig. 6B, lanes 5, 9, 13, and 17), indicating a significant difference between the MLP and the m promoter in their interaction with the 90-kDa factor. As in the case with the MLP, low levels of TBP and high levels of the 90-kDa factor induced a TATA region-contained alteration of the footprint. However, unlike in the case of the MLP, TFIIA could not restore the footprint to the TBP-TFIIA induced state (data not shown).

The interactions of the factors with the MLP and the m promoter were also analyzed by EMSA. Titration of increasing levels of TBP in the presence of the 90-kDa factor resulted in a series of protein-DNA complexes with increasingly lower mobilities (Fig. 7A). At the highest concentration of TBP assayed, a single low mobility complex was formed with both the MLP and the m promoter. This was not a nonspecific complex that could not enter the gel matrix because prolonged electrophoresis resulted in a further migration (Fig. 7B). Titration of the 90-kDa factor with the high levels of TBP showed that formation of this low mobility complex also requires high levels of the 90-kDa factor (data not shown). Under the EMSA conditions used, TBP alone did not form a stable protein-DNA complex. Therefore, it is likely that this complex represents an oligomeric structure including multiple TBPs and the 90-kDa factors as suggested by the footprinting assay. As in the case of the footprinting assays, the EMSA reactions did not contain poly(dGdC). The 90-kDa factor alone produced a smearing of the probe DNA consistent with the proposed interactions with DNA (Fig. 7A, lanes 1 and 7).

Surprisingly, the mobilities and number of protein-DNA complexes produced by TBP and the 90-kDa factor were unaffected by TFIIA under these conditions. This was true with both the MLP and the m promoter. Because the addition of TFIIA under similar binding conditions altered the DNase I footprint patterns of the MLP, this result was unexpected. One possibility was that TFIIA was part of the protein-DNA complexes but had no effect on the mobilities of the complexes. This possibility was examined on the low mobility complexes by the addition of anti-TFIIA antibody (gift of H. Handsa) to the EMSA reaction. The presence of the antibody caused a smearing of the protein-DNA complexes only if TFIIA was added to the reaction (Fig. 7B, lanes 1-8). This likely reflects the binding of the anti-TFIIA antibodies to TFIIA in the protein-DNA complex. Consistent with the footprinting analyses, there was a difference in the response between the MLP and the m promoter. The smearing effect was readily visible at the lowest level of TFIIA in the reactions containing the MLP (Fig. 7B, lane 6), but not until the highest level of TFIIA was there a hint of a smear in the reactions containing the m promoter (Fig. 7B, lane 16). These data suggest that the alterations observed with the addition of TFIIA to the MLP footprinting assays were largely conformational and that TFIIA and the 90-kDa factor coexist on a single DNA molecule under these conditions (see "Discussion").
Reinberg and co-workers have reported two separate forms of Dr1 (21). The first form was described as a homotetramer of phosphorylated Dr1/p19 protein with a molecular mass of 90 kDa that elutes in the 0.5 M KCl fraction from a phosphocellulose column. The second form is unphosphorylated and eluted in the 1.0 M KCl fraction from the same column. The 90-kDa factor eluted from a phosphocellulose column in the 0.6 M KCl fraction, and comparison of the mobility with the Dr1 found in the 1.0 M KCl fraction by Western blotting indicated that Dr1/p19 (p20) subunit of the 90-kDa factor was likely phosphorylated (data not shown). In addition, NC2, one of the negative cofactors reported by Roeder and co-workers, has been shown to contain a phosphorylated form of Dr1/p19, although the subunit composition of the purified NC2 and its molecular size have not been described (24). We suggest that all three factors, the 90-kDa factor, the native Dr1 complex, and NC2 are the same activity and are a heterotetramer of p20 and p30 polypeptides. This proposal is consistent with the repression of transcription and interaction with TBP reported for all three factors.

The 90-kDa factor is a repressor of basal transcription. Although it will probably repress transcription from all promoters, the efficiency of repression varies depending on the identity of the core promoter. The mechanism of repression appears to be formation of transcriptionally inert complex with TBP on promoters. An intriguing aspect of transcriptional regulation by the 90-kDa factor is the promoter-specific modulation by TFIIA. In the presence of high levels of TFIIA, transcription from the MLP is refractory to inhibition by the 90-kDa factor. In contrast, under the same condition, transcription from the immunoglobulin \( \mu \) promoter is strongly inhibited. Correlatable differences between these two promoters were observed in the footprint assays. High levels of TFIIA restored the footprint within the TATA region of the MLP to the TFIIA-induced pattern from the 90-kDa factor-TBP-induced pattern. In contrast, on the \( \mu \) promoter, the addition of high levels of TFIIA did not alter the footprint induced by the 90-kDa factor as effectively. Undoubtedly, TFIIA is part of the protein-DNA complex on the MLP in the presence of the 90-kDa factor because it is detectable by supershifting of the complex by anti-TFIIA antibody.

Interestingly, the 90-kDa factor and TBP can form a series of extended protein-DNA complexes. Dr1/p19 has been shown to bind to TBP directly. Because the 90-kDa factor is probably a heterotetramer of two Dr1/p19 (p20) subunits and two p30 subunits, each complex is likely bivalent for binding to TBP. Furthermore, the purified Dr1/p19 (p20) subunit oligomerizes into a tetramer when prepared separately from p30 (21), indicating the potential to form higher order structures of the 90-kDa factor under appropriate conditions. Also of interest, both Dr1 and p30 is distantly related to histone proteins through the so-called histone fold motif, a feature found among proteins that make protein-protein and protein-DNA contacts (36). Therefore, the oligomerization of the 90-kDa factor is likely a reflection of its innate structural features and important for its function in vivo. The formation of the more extended protein-DNA complex is nucleated by TBP binding to TATA element and subsequent binding of the 90-kDa factor. The addition of higher amounts of TBP results in the formation of a complex that migrates slowly during the gel electrophoresis and has an extended footprint spanning over 150 base pairs of the probe. Thus, most likely, this complex is composed of multiple 90-kDa factors and TBP, and the promoter is inaccessible to the other basal factors necessary to mediate transcription. It should be noted, however, that formation of the more extended structure is probably not required for the inhibition of tran-

### DISCUSSION

We have identified and purified a cofactor of transcription that consists of Dr1/p19 (p20) and a novel protein with a molecular mass of 30 kDa (p30). The heteromeric composition of the complex has been suggested by co-association with TBP on the MLP as well as copurification over several chromatographic steps. The heteromeric complex has a molecular mass of 90 kDa and is probably composed of two subunits of Dr1/p19 (p20) and two subunits of p30. The formation of heteromeric structure by Dr1/p19 (p20) and p30 is also supported by their homology to HAP3 and HAP5 proteins, respectively. These two yeast proteins associate into a CAATT binding regulatory complex, and the peptide sequences necessary for their interaction is conserved in the Dr1/p19 (p20) and p30 polypeptides (33, 35).³

³ D. McNabb and L. Guarente, personal communication.
scription by the 90-kDa factor or for the promoter-specific derepression by TFIIA, because both phenomena occur at low levels of TBP as well.

Promoter specificity for the activities of TFIIA factor has not been previously described. This specificity was only apparent under conditions of excess TBP where complexes of this protein bound to DNA competed for other basal factors necessary for initiation. This suggests that different combinations of TBP, TFIIA, and promoter DNA have varying affinities for other factors such as TFIIIB, TFIIIF, and pol II. As discussed above, the combined activities of the 90-kDa factor and TFIIA were more dramatically promoter-specific than either factor alone. This specificity could be observed at both low and high TBP concentrations. It is interesting to speculate that DNA bound TBP complexes might compete for basal factors such as the holopolymerase II in vivo. If this were the case, then the promoter specificity detected in this study might be more relevant to conditions in vivo than the typical reaction using limiting concentrations of TBP.

It is now well accepted that the regulation of pol II transcription involves negative modulation of basal and activated transcription. The 90-kDa factor is probably one of these negative components and suppresses transcription in vivo as well as in vitro. Cotransfection assays indicate that an overexpression of Dr1/p19 subunit represses transcription in vivo, and a subset of activation domains including VP16 and E1A-CR3 can derepress transcription (22, 23). Comparison of sequences of the p30 subunit of the 90-kDa factor allowed identification of a related protein from S. cerevisiae encoded by the YER159c gene. A mutation in YER159c can suppress a conditional loss of function mutation in SRB4, a component of the yeast pol II holoenzyme complex (20).2 Shifting the SRB4 mutant strain to restrictive temperature results in rapid cessation of mRNA synthesis from many pol II promoters, indicating that SRB4 is an important component of transcriptional activation (20). That a mutation in the p30 homologue suppresses the loss of function mutation of SRB4 is consistent with the proposal that the p30 homologue is a component of a general repressor activity such as the 90-kDa factor. Furthermore, the results presented here indicate that transcriptional regulation by the repressor 90-kDa factor may have a measure of promoter specificity dictated by the content of the core promoter and the flanking sequences.

Acknowledgments—We thank B. Blencowe, K. Cepok, D. Chasman, J. Pomerantz, and Q. Zhou for critical reading of this manuscript. We thank D. Reinberg for anti-Dr1/p19 antiserum, H. Handa for anti-TFIIA antibody, and D. Chasman for yeast recombinant TBP. We also thank Richard Cook of the MIT Biopolymers Laboratory for expert help in HPLC and amino acid sequencing. We are indebted to D. Munroe for providing the human cDNA library and technical assistance in isolating p30 cDNA. We are particularly grateful to E. Gadbois, R. Young, D. McNabb, and L. Guarente for sharing unpublished results.

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