Separation of TFIIC into two functional components by sequence specific DNA affinity chromatography

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ABSTRACT

Recently, it has been shown that mammalian transcription factor IIC (TFIIC) activity can be separated by anion exchange FPLC chromatography into two functional components (1), both of which are required for transcription of tRNA and the adenovirus VA RNA genes. Here we show that these two functional components, designated TFIIC1 and TFIIC2, can also be separated by sequence specific DNA affinity chromatography. These results confirm the observation that TFIIC can be fractionated into two components, which are both required for transcription of VA I and tRNA genes *in vitro*. Thus in the mammalian reconstituted system, a minimum of three proteins, in addition to RNA polymerase III, are required for the transcription of the VA and tRNA genes *in vitro*. The DNA binding component, TFIIC2, binds specifically to the 3' segment of the internal promoter (the B block), demonstrated by its ability to protect this region from digestion by DNase I. TFIIC2 is the limiting, titratable component in the phosphocellulose C fraction required for the formation of a stable preinitiation complex on the VAI RNA gene *in vitro*, as demonstrated with a template competition and rescue assay (2).

INTRODUCTION

RNA polymerase III - directed transcription of eukaryotic genes has been intensively studied over the last decade, with respect to both the cis and trans elements that are involved in promoter recognition and template commitment (for review, see ref. 3). Mutational analysis of a variety of class III genes, including those which encode the 5S RNA (4), tRNAs (5) and Adenovirus VAI RNA (6,7,8), has provided a detailed description of sequences which constitute the promoter elements of these genes. While sequences flanking the coding regions have been shown to play some role in modulating transcription efficiency (9), these genes all share the common feature of an internal split promoter. For the VA and tRNA genes, these highly conserved intragenic elements consist of two non- contiguous sequences of eleven base pairs each, termed the A and B blocks. These are centered at approximately +13 and +57, respectively in tRNA genes (10), and at +19 and +64 in the Ad2 VAI RNA gene (8).

Recognition of these genes by RNA Polymerase III requires accessory transcription factors, which interact with these sequence elements to form stable preinitiation complexes (11). These class III transcription factors have been analysed by chromatographic fractionation of cell extracts. In addition to RNA Polymerase III (Pol III), at least two chromatographically separable proteins are required for reconstituted transcription in systems from human (12), yeast (13),

Drosophila (14), and *Xenopus* (15). These have been designated TFIIB and TFIIC, and are required for the transcription of all class III genes. An additional factor, TFIIIA, is a gene-specific transcription factor required for 5S RNA synthesis only (12). Based on both DNA binding and template competition assays (2, 11,16), the interaction between TFIIC and the intragenic control regions of both the VA and tRNA genes has been shown to be the primary event required for template commitment in vitro and occurs prior to the association of TFIIB with the preinitiation complex. The inability to detect any DNA binding activity for TFIIB has led to the inference that its association with the complex is through protein:protein interactions. In contrast to the mammalian homologues, much progress has been made in the purification and characterization of these two factors in the yeast system. TFIIB prepared from yeast appears to be a single monomeric protein of 60 Kd (17), while TFIIC is a multimeric protein of about 300 Kd (18).

Our purpose for pursuing purification of TFIIC stems from our interest in the transactivating function of the adenovirus EIA protein. The EIA protein stimulates the transcription of class III genes (19,20,21), and appears to do so by modulating the activity of TFIIC (21,22). The purification of this factor is required to understand the molecular details of its activation by EIA .

Recently, it was observed that FPLC MonoQ chromatography of partially purified TFIIC activity results in the separation of TFIIC into two functional components, designated TFIIC1 and TFIIC2. In addition to TFIIB and Pol III, both of these components are required to reconstitute transcription of the VA and tRNA genes in vitro (1). TFIIC2 binds specifically to the B block region of the internal promoter, while TFIIC1 exhibits a transcription complementing activity. Though no DNA binding activity has yet been unequivocally demonstrated for TFIIC1 alone, when it is added to TFIIC2 , binding of both the B and A blocks is observed.

Here we present evidence that mammalian TFIIC can also be separated into two functional components by sequence-specific DNA affinity chromatography, as predicted by the initial characterization of the DNA binding activities of TFIIC1 and TFIIC2 (1). This result confirms the previous observation that mammalian TFIIC consists of at least two activities. B block-specific DNA affinity chromatography results in a bound fraction representing a 30% yield of activity with an approximately 500X increase in specific activity compared to the initial nuclear extract. This fraction is highly enriched for TFIIC2, the component of TFIIC which binds with high affinity to the B block region of the internal promoter. In addition to being required for transcription of both the VAI and tRNA genes, it is the limiting titratable component in the phosphocellulose C fraction required for the formation of a stable pre-initiation complex on these genes.

MATERIALS AND METHODS

Preparation of cell-free extracts and phosphocellulose chromatography

Nuclear extracts (23) were prepared from 293 cells, a human embryonic kidney cell line

transformed by adenovirus 5, which constitutively express the EIA and EIB proteins (24). These were grown in suspension culture in SMEM (GIBCO) with 5% newborn calf serum (GIBCO), and harvested at 8×10^5 cells/ml.

The nuclear extract was subsequently chromatographed through phosphocellulose (12) for the preparation of the B fraction (the 0.35 M KCl eluate containing TFIIB and Pol III activity) and the C fraction (the 0.6 M KCl eluate containing TFIIC activity). The B fraction was subsequently rechromatographed through phosphocellulose equilibrated at 0.1 M KCl and eluted with a linear gradient, from 0.1- 0.4 M KCl in buffer A [20 mM HEPES-KOH (pH7.9); 1 mM EDTA; 1 mM DTT; 20% glycerol]. Fractions eluting at 0.35 M KCl contained both TFIIB and Pol III activity and were free of any detectable TFIIC1 or TFIIC2 activity, as assayed by in vitro transcription in reactions reconstituted with TFIIC1 or TFIIC2 prepared by FPLC chromatography.

TFIIC1 was prepared by FPLC chromatography of the phosphocellulose C fraction on Mono Q Sephabeads (Pharmacia), as described (1).

Preparation of oligonucleotide affinity resin

An oligonucleotide, of the sequence 5' GGATCCGGGGTTCGAACCCC 3', containing the B Block sequence of the Ad2 VA1 gene, was synthesized (Applied Biosystems, Inc.) and purified by electrophoresis on 20% acrylamide; 7 M urea polyacrylamide gels. The 20mer was visualized by UV shadowing, excised and eluted overnight, at 37 °C in elution buffer [10 mM magnesium acetate; 0.5 M ammonium acetate; 0.1% SDS ; 0.1 mM EDTA], ethanol precipitated and resuspended in TE buffer [10 mM Tris, pH 8.0, 1.0 mM EDTA].

DNA was phosphorylated in a reaction containing 250 U/ml polynucleotide kinase (Pharmacia); 7 μ M oligonucleotide; 66 μ M ATP; 70mM Tris (pH 7.6); 10 mM MgCl_2 ; 5 mM DTT for 60 min at 37 °C. A trace amount of DNA (1 μ g / 500 μ g) was kinased in the presence of $\gamma^{32}\text{P}$ - ATP (ICN; 7000 Ci/mmol) and added to the unlabeled phosphorylated DNA. This reaction mix was heated to 100 °C for one min, and cooled slowly (over eight hours) to room temperature to allow strand hybridization. The concentration of ATP was then raised to 600 μ M, and 500 U/ml of T4 DNA ligase (BRL) and 100 U/ml of polynucleotide kinase were added. The ligation reaction was carried out at 12 °C for 12-16 hours. The DNA was phenol extracted, ethanol precipitated, and resuspended in H_2O (1 μ g DNA/ μ l). Unincorporated ATP was removed from the DNA by chromatography through G-25 Sephadex equilibrated in H_2O . Fractions devoid of ATP were pooled, ethanol precipitated, and resuspended in H_2O at 1 μ g / μ l to be used for coupling to Sepharose CL-2B (Pharmacia) (25). After coupling, the DNA affinity resin was found to contain approximately 31 μ g DNA/ml resin, or 2.4 μ M in binding sites.

Oligonucleotide Affinity chromatography

The 0.6 M phosphocellulose eluate (C fraction) was dialysed against 0.1M KCl in Buffer D [20 mM Hepes (pH 7.9); 5mM MgCl_2 ; 1.0 mM EDTA; 0.5mM DTT; 10% glycerol; 0.1%

NP-40]. Prior to DNA affinity chromatography, the C fraction was incubated with 30 μ g of sonicated salmon sperm DNA/ mg of protein for 10 min on ice. All further steps were at 4 °C.

The protein-DNA mixture (2-4 mg protein/ml resin), was added to affinity resin equilibrated in 0.1M KCl in buffer D, mixed gently using siliconized glass rods, and left for 45 min, with occasional mixing, to allow binding. The unbound protein fraction was collected, and the column was washed with 6 column volumes of 0.1M KCl in buffer D followed by 4 column volumes of 0.25 M KCl in buffer D, collecting one column volume fractions. 1 column volume of 2.0 M NaCl, 0.1mg/ml BSA in buffer D was applied, mixed gently, and left for 10 min. The high salt eluate was collected and the column washed with 2 additional volumes of 2.0 M NaCl in buffer D. High salt eluates containing transcription and DNA binding activities were dialysed 2 hours against 0.1 M KCl in buffer D. Aliquots were frozen in dry ice and stored at -70 °C. The activity was stable for at least one month at -70°C, but was not stable to multiple rounds of freeze/thaw.

Plasmids and Preparation of Labeled DNA

pArg (26) contains a 508 base pair Hind III fragment containing a *Drosophila melanogaster* tRNA^{Arg} gene inserted into the Hind III site of pBR322.

pVAI contains the pVA (22) Sal I/ Bal I fragment, which includes nucleotides 10,589 to 10,810 of the Ad2 DNA sequence, including the VAI RNA gene, inserted in the Sal I/ Sma I site of pUC18. When used for DNase I footprinting assays, pVAI was digested with Sal I, dephosphorylated with calf intestinal phosphatase (Boehringer Mannheim) and 5' end labeled with T4 polynucleotide kinase in the presence of γ -³²P-ATP (ICN; 7000 Ci/mmol). Secondary digestion with Eco RI resulted in a 245 bp fragment, labeled at -26 (relative to the start site of transcription).

pAsn8 (27) contains a 1.72 Kb Hind III fragment containing a *Drosophila melanogaster* tRNA^{Asn} gene inserted into the Hind III site of pUC8. For use in DNase I footprinting assays, pAsn8 was digested with Hind III, 5' end labeled (as described above) and secondarily digested with Sal I, resulting in a 248 bp fragment, labeled at -48, relative to the start site of transcription. Labeled DNA fragments were electrophoresed on 5% polyacrylamide gels, excised, eluted overnight in elution buffer, ethanol precipitated and resuspended in TE buffer.

In vitro transcription and DNase I Footprinting Assays

The standard reaction for the reconstituted transcription of the VAI or tRNA genes was carried out in a volume of 40 μ l containing 0.12-0.4 μ g of the DNA affinity resin high salt eluate (TFIIIC2), 1-2 μ g of TFIIIC1, 4 μ g of phosphocellulose B fraction. The final conditions (including contributions of added fractions) consisted of 75 mM KCl; 5mM MgCl₂; 20mM Hepes-KOH (pH 7.9); 10% glycerol; 0.5 mM EDTA; 0.5 mM DTT; 1.0 μ g/ml α -amanitin; 4.0 nM DNA template. NTP's were added to 500 μ M of ATP, UTP,CTP and 25 μ M GTP. α -³²P GTP was added at 2 μ Ci / reaction (Amersham; 3000Ci/mmol). Reactions were initiated by the addition of protein, carried out for 60 min at 30° C, and transcription products

were processed for electrophoresis on 8% polyacrylamide gels in TBE plus 7 M urea.

DNase I footprinting reactions contained 1-3 fmoles end labeled DNA restriction fragments, with salt and buffer conditions exactly as those described for *in vitro* transcription reactions. Protein was added as indicated in the figure legend. The reaction was initiated by the addition of protein, binding was at room temperature for 45 min, followed by digestion with DNase I at 4 ng/ μ l for 1 min at room temperature. The DNA was then phenol/chloroform extracted, ethanol precipitated, resuspended in loading buffer and subjected to electrophoresis on 8% polyacrylamide/7M urea sequencing gels.

RESULTS

The approach used for TFIIC2 purification was based on the sequence specific interaction between TFIIC2 and its cognate DNA, the B block region of the VA I RNA gene. This gene contains a B block that correlates precisely with the consensus sequence 5'-GGTTCGANN-3' (10,29), in an 18 base pair region of perfect dyad symmetry centered on the B block. TFIIC2 binds with extremely high affinity to this sequence, with a measured affinity constant of $2 \times 10^{11} \text{ M}^{-1}$ (30). An oligonucleotide containing this sequence was synthesized for use in sequence specific DNA affinity chromatography by the method of Kadonaga and Tjian (25).

The hybridization and ligation of this sequence resulted in a polymer containing an average of 15-25 binding sites per molecule. Initial experiments (data not shown) demonstrated that the polymer, but not the monomer, could stably sequester TFIIC activity from a phosphocellulose C fraction with an efficiency comparable to the intact VA RNA gene, per binding site. This polymer was covalently coupled to Sepharose CL-2B and used for affinity chromatography.

The use of nuclear extracts prepared from 293 cells as starting material was based on findings that the E1A proteins of adenovirus (which are constitutively expressed in this cell line along with the E1B genes) transactivate class III genes, and appear to do so by increasing the activity of TFIIC (21,22). The 5-10 fold increased transcriptional activity contained in the phosphocellulose C fraction prepared from 293 cells, as compared to HeLa cells, thus serves as an initial enrichment for TFIIC activity.

As described for the DNA affinity purification of other sequence specific DNA binding proteins (31), prior to affinity chromatography, the phosphocellulose C fraction was first incubated with nonspecific DNA to enable the binding, and subsequent partitioning of non specific DNA binding proteins present in the fraction. The optimum concentration of sonicated salmon sperm DNA was found to be 30 μ g/mg of protein. This mixture was then applied to the affinity resin. A 45 min incubation of this mix with the affinity resin, with occasional stirring, rather than slow chromatography, resulted in more efficient binding. Optimal monovalent and divalent cation conditions for sequence specific binding during affinity chromatography were found to be 0.1M KCl and 5mM MgCl_2 . Inclusion of the nonionic detergent NP-40, at 0.1% decreased the amount of activity in the flow through. After extensive washing with 0.1 M KCl,

FIGURE 1. DNase I footprint analysis of TFIIIC2 interaction with the VAI and tRNA^{Asn} genes.

5' end labeled restriction fragments containing the Adenovirus VAI gene (lanes 1-7) or the *Drosophila* tRNA^{Asn} gene (lanes 8-14) were incubated with the high salt DNA affinity resin eluate in amounts indicated beneath each lane and subjected to DNase I digestion, as described in Materials and Methods. Lanes 7, 8, and 14 demonstrate control reactions of DNase I digestion without added protein. Marker lanes (M) contain labeled pBR322 Hpa II restriction fragments, with numbers indicating size, in b.p. A representation of the location of the A and B block regions in each gene is shown to the right of each figure. Numbers indicate nucleotide position relative to the start site of transcription.

FIGURE 2. *In vitro* transcriptional activity of DNA affinity chromatography fractions. Transcription of the Ad2 VAI gene was carried out as described in Materials and Methods. Reactions contained 4 μ g of B fraction (containing TFIIB and Pol III), to which was added 4 μ g of onput C fraction (lane 1); 0.2 μ g of high salt eluate (bound protein) (lane 2); 0.2 μ g bound protein and 1 μ g TFIIC1 (lane 3); Lanes 4 and 5 are controls with 1 μ g of TFIIC1 + B fraction, and B fraction alone demonstrating that transcriptional activity is dependent upon all components.

and then 0.25 M KCl, the bound protein was step eluted with 2.0 M NaCl in buffer D. Inclusion of BSA (0.1mg/ml) in the elution buffer was found to be critical for the recovery and stability of activity. Fractions were assayed for both transcriptional and sequence specific DNA binding activity.

DNA binding was assayed by the method of DNase I footprinting (32), monitoring protection of the B block in both the VAI and tRNA^{Asn} genes. As shown in Fig.1, sequence specific DNA binding activity eluted in the 2.0 M salt fraction evidenced by protection of the B block region of both the VAI (lanes 1-7) and tRNA^{Asn} gene (lane 8-14) from digestion by DNase I. In contrast to the binding specificity exhibited by the C fraction, which spans both the A block and B block region of these genes (1), binding of protein bound to the DNA affinity resin is restricted to only the B block of both the VAI and tRNA^{Asn} genes.

Earlier results (1) demonstrated that the A and B-block binding activities can be separated by

FIGURE 3. In vitro transcriptional complementation by bound and flow-through fractions.

Reconstituted transcription of the VAI gene was carried out in reactions containing 4 μ g of B fraction and: 1 μ g output (lane 1); 1 μ g flow through (lane 2); 0.2 μ g bound fraction (lane 3); 1 μ g flow-through fraction + 0.2 μ g bound protein (lane 4).

anion exchange chromatography. The results of Fig. 1 confirm these findings. Specific DNA sequence affinity chromatography using the B-block sequence alone yielded a protein fraction which produced no significant DNase I protection over the region of the A block, even at five times higher protein concentration than required for clear protection of the B-block region (Fig. 1; compare lanes 3 and 6, or lanes 10 and 13). B-block binding activity of the bound fraction was not observed when an otherwise identical column containing reiterated copies of an SP1 binding site (25) was substituted for the B-block column (data not shown). This control demonstrated that sequence specific affinity chromatography is achieved with the B-block column.

Transcriptional activity was assayed first using TFIIB plus Pol III fraction and the bound fraction from affinity chromatography. As shown in Fig. 2, lane 2, no VAI RNA synthesis was observed in a reaction containing TFIIB, Pol III, and the high salt eluate. When TFIIC1, prepared by FPLC Mono Q chromatography of a phosphocellulose C fraction was added to the reconstituted reaction containing TFIIB, pol III and the high salt eluate containing protein bound to the DNA affinity resin, specific VAI RNA transcription was observed (Fig. 2, lane 3). This

result demonstrated that proteins bound to the affinity resin were separated from a functional component required for transcription, TFIIC1.

Evidence for the separability of two TFIIC components by DNA affinity chromatography was further demonstrated by the ability of the flow-through fraction to complement the bound fraction for transcription. Neither the flow-through fraction nor the bound fraction alone efficiently reconstituted transcription of the VAI gene when added to TFIIB and Pol III (Fig. 3, lanes 2, 3). However when both the flow-through and the bound fraction were included in the reaction, much more VAI RNA synthesis occurred (Fig. 3, lane 4).

The recovery of TFIIC2 activity from the phosphocellulose C fraction was approximately 30 %, while the specific activity (as measured by pmoles GMP incorporated into VAI RNA/ hour/ μ g protein for transcription, and fmoles of DNA protected from DNase I cleavage/ μ g protein for DNA binding) was increased about 50 - 200 fold. This represents an approximately 500- 2000 fold increase in specific activity from the nuclear extract. The protein profile, observed by SDS- polyacrylamide gel electrophoresis followed by silver staining, indicated at least 10 discernible polypeptides, ranging from 45 -250 KD in molecular weight (data not shown).

TFIIC prepared from HeLa cells has been defined not only on the basis of its site specific DNA binding activity and requirement for transcription, but has also been shown to be the limiting, titratable component in HeLa cell S100 extracts that is required for the formation of a stable preinitiation complex (2). This was demonstrated using template competition assays in which the first template, in a preincubation with extract, was titrated to determine the concentration which excluded the transcription of a second template subsequently added to a transcription reaction. Under these conditions, which indicate that a limiting component had been sequestered by the first template, different chromatographic fractions were added to the second template to determine which could "rescue" its transcription. Only addition of fractions containing the equivalent of TFIIC, TfrX, could restore transcription of the second template (2). We have used this second template rescue assay to determine whether one or both components of TFIIC is the limiting titratable factor required for VAI transcription in a phosphocellulose C fraction. Figure 4, lanes 1-4 show a titration of the amount of VAI DNA present during the first preincubation with fraction B and fraction C. After a 25 min incubation, a constant amount of tDNA^{Arg} was added, along with nucleotide triphosphates. Fig. 4, lane 4 indicates that addition of 60 fmoles of the first template was sufficient to sequester the limiting component required for complex formation, as indicated by exclusion of tRNA synthesis. Under these conditions, either TFIIC1 or TFIIC2 or both were added along with the second template after the first preincubation. As shown in Fig. 4, the addition of TFIIC2 (lane 6) or TFIIC2 and TFIIC1 (lane 7) rescued transcription of the second template, while addition of TFIIC1 alone (lane 5) could not restore tRNA synthesis. These results indicate that TFIIC2 is the limiting component in the C fraction that is required for complex formation on the VAI gene.

FIGURE 4. TFIIC2 is the limiting, titratable component in C fraction required for stable complex formation.

Panel A depicts the experimental protocol, where DNA I is pVAI, and DNA II is pArg. Each reaction contains 4 μg of C and B fractions. In lanes 1-4, these were preincubated with increasing amounts of pVAI, as indicated beneath each lane, after which a constant amount of pArg was added (60 fmoles), along with nucleotide triphosphates and no added fraction. In lanes 5, 6, and 7, 60 fmoles of pVAI was preincubated with fractions C and B (as above), after which 60 fmoles of pArg was added along with: 1 μg TFIIC1 (lane 5); 0.24 μg of TFIIC2 (lane 6); or 1 μg TFIIC1 and 0.24 μg TFIIC2 (lane 7). I and II indicate position of ^{32}P -labeled VAI RNA and tRNA^{Arg}, respectively.

DISCUSSION

Sequence specific DNA affinity chromatography using the B block of the Ad2 VAI gene separated TFIIC activity into two activities required for *in vitro* transcription. These results confirm the observation (1) that mammalian TFIIC activity can be fractionated into at least two components. The results are consistent with the model that separate proteins, TFIIC1 and TFIIC2, interact with the A and B block promoter elements, respectively. The bound component, TFIIC2, bound specifically to the B block of both the VAI and tRNA^{Asn} genes, as

shown by DNase I footprinting assays, without protecting the A block portion of the internal promoter. TFIIC2 was also found to be the limiting, titratable component required for stable complex formation on the VA I gene in C fraction prepared from 293 cells.

The separation of B block and A block binding activities in extracts from mammalian cells was in contrast to the properties of the yeast homologue of TFIIC, the τ factor. Highly purified τ factor binds to both the A and B block regions (33). Interestingly, in limited protease digestion studies of τ (34), a protease resistant form of τ could be isolated and shown to interact with the B block region only. However this form of τ was not active in *in vitro* transcription. From these studies, it was suggested that this protein contains two DNA binding domains, termed τ_A and τ_B and that these may be linked via a flexible protease sensitive hinge (34). Thus far no functional τ_A domain has been isolated. This model raises the possibility that TFIIC1 and TFIIC2 are in fact proteolytic fragments of mammalian TFIIC. However, several observations argue against this. First, separation of these components has been demonstrated by two different methods, FPLC and oligonucleotide affinity chromatography. Secondly, inclusion of protease inhibitors does not affect the separation of TFIIC1 and TFIIC2 on either FPLC or DNA affinity chromatography. Both of these methods allow very rapid chromatography, and thereby minimize the time in which proteolysis might occur.

Recently, a comparative analysis of TFIIC activity prepared from either S100 or nuclear HeLa cell extracts was undertaken (35). While both TFIIC preparations had similar levels of transcriptional activity, sequence specific binding over the VAI B block region could only be detected with TFIIC prepared from nuclear extracts. This led the authors to hypothesize that two forms of B block-binding TFIIC exist, a predominant transcriptionally inactive form enriched in the nuclear extract, and a minor functionally active form. The inability to detect DNA binding activity with the S100 preparation was proposed to reflect the less sensitive nature of the DNase I footprinting assay, whose limit of detection was insufficient to detect the minor, though functionally active form. Based on our observation that TFIIC can be separated into two functional components, we suggest that the differential behavior of TFIIC prepared from either S100 or nuclear extracts may be a function of the ratio of TFIIC2 and TFIIC1 in these preparations. TFIIC2 may be enriched in nuclear extracts, while TFIIC1 may be at similar concentrations in both S100 and nuclear extracts. The requirement of both of these components in reconstituted *in vitro* transcription would argue against the proposition that TFIIC1 and TFIIC2 represent active and inactive forms of TFIIC.

The result demonstrating that TFIIC2 is the limiting component required for stable transcription complex formation was not unexpected, based upon previous biochemical data demonstrating the strong correlation between protein binding over the B block region and template commitment (2), as well as mutational studies indicating that the 3' promoter element is most critical for stable complex formation (7,36,37).

With regards to the question of EIA transactivation this result was of particular interest to us. An early indication providing evidence that the increased transcriptional activity in extracts

prepared from cells expressing the EIA proteins was due to an increase in the activity of TFIIC was based on results using a template competition assay (22). By comparing the amount of the first template required to sequester the limiting component required for stable complex formation, and thereby exclude transcription of a second template, we observed that there was an increase in the concentration of this component in extracts prepared from cells expressing the EIA proteins (22). Furthermore, in extracts prepared from either uninfected or adenovirus infected Hela cells this limiting component is in the C fraction, as addition of this fraction could rescue transcription of the second template (2, 22). These previous results, in conjunction with the data presented here showing that TFIIC2 is the limiting component in the C fraction required for complex formation, indicate that TFIIC2 is a target of EIA-mediated transactivation. These results do not, however, rule out the possibility that EIA proteins may also affect the activity of TFIIC1.

The separation of TFIIC1 and TFIIC2 enables a more rigorous analysis of these two components with regards to their function in transcription and their role in the assembly process of the stable preinitiation complex. As sequence specific DNA binding activity has not been demonstrated for any of the other components involved in the transcription of the VA and tRNA genes, and the B block is the critical sequence required for formation of a stable complex on these genes, it seems likely that the binding of TFIIC2 to the B block precedes further protein:protein, or TFIIC2 induced protein:DNA interactions. Experiments are currently underway to define the order and nature of these interactions.

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