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Resolution of human transcription factor TFIIIC into two functional components

(RNA polymerase III/*in vitro* transcription/DNA binding proteins/internal control region)

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ABSTRACT **tRNA genes and adenovirus viral-associated (VA) genes are transcribed by RNA polymerase III. Transcription of these genes *in vitro* requires two protein fractions containing transcription factors designated TFIIIB and TFIIIC, in addition to RNA polymerase III. We report that the TFIIIC fraction derived from human cells in culture can be separated into two functional components, which we call TFIIIC1 and TFIIIC2. Both TFIIIC1 and TFIIIC2 fractions are required for *in vitro* transcription of the VA1 gene. In DNase I "footprinting" experiments, the TFIIIC2 fraction protects the internal control region termed the B block. Addition of the TFIIIC1 fraction extends the footprint over the internal control region called the A block. TFIIIC1 activity is the limiting transcription factor activity required for VA1 transcription in the crude extract. TFIIIC2 activity sediments as a large component of ≈18 S, while TFIIIC1 activity sediments at ≈9 S. These data indicate that the two activities are unique components and when added together reconstitute TFIIIC activity.**

Genes transcribed by RNA polymerase III (class III genes) encode a variety of small untranslated RNAs such as the 5S RNA, tRNAs, and the adenovirus viral-associated RNAs VA1 and VA2 (for reviews, see refs. 1 and 2). A unique aspect of class III genes is that they contain internal transcriptional control regions. For tRNA and adenovirus VA RNA genes, the internal control region is composed of two segments of DNA, usually separated by ≈35 base pairs (bp), termed the A and B blocks.

Cell-free transcription systems from such diverse sources as yeast, *Drosophila*, and human cells have similar factor requirements for transcription. In addition to RNA polymerase III, transcription of class III genes requires multiple protein factors which form stable transcription complexes with the gene (3–10). Transcription of VA and tRNA genes requires the factors present in the 0.35 M KCl and 0.6 M KCl step eluates from phosphocellulose chromatography of S100 protein extracts (phosphocellulose B and C fractions, respectively; refs. 3, 4, and 6) from human cells. Transcription of 5S RNA requires the same factors plus an additional factor termed TFIIIA (contained in the 0.1 M KCl elution from phosphocellulose).

The limiting component(s) required for VA and tRNA gene transcription in S100 protein extracts is present in the phosphocellulose C (PC-C) fraction (4, 11, 12) from human cell extracts and has been referred to as TFIIIC (3). TFIIIC activity has been defined by workers in several laboratories by its requirement for *in vitro* transcription of VA and tRNA genes when added together with TFIIIB and RNA polymerase III (3–10). In addition, the *in vitro* transcriptional activity of TFIIIC has copurified with an activity that protects the conserved internal control regions (both the A and B blocks) from nuclease digestion (4, 6, 10, 13–17). The protection over these regions is different. TFIIIC from human cell extracts binds strongly to the B-block region of the VA1 gene, but it binds to the A-block region only at high protein concentrations (4, 6). The yeast equivalent to TFIIIC protects the B block of tRNA genes strongly; however, the A-block region demonstrated more variable protection from nuclease depending on the tRNA gene used. The A-block binding of TFIIIC was also more sensitive to assay conditions (10, 15, 16), exonuclease digestion (14), and limited proteolysis (17).

The *in vivo* transcription of transfected VA1 and tRNA genes is greatly stimulated by the expression of adenovirus E1A proteins (12, 18, 19). Extracts prepared from adenovirus-infected HeLa cells also have higher *in vitro* transcriptional activity on these templates than extracts prepared from cells infected with E1A mutants of adenovirus (11, 12). Fractionation of these extracts revealed that the component(s) responsible for the higher transcriptional activity is present in the PC-C fraction.

To pursue the mechanism of class III transactivation by the E1A proteins, we have further characterized the pol III transcription factors in the PC-C fraction. In this report, we demonstrate the separation of a phosphocellulose TFIIIC fraction (PC-C) into two functional components, both required for *in vitro* transcription. One fraction protects the B block of the VA1 gene from DNase I digestion. Addition of the other fraction extends the DNase I "footprint" over a region that includes the A block and the transcription start site.

MATERIALS AND METHODS

Plasmids and Probes. pVA was used previously (11). pVA1 contains the VA1 gene only (nucleotides 10,584–10,812) in pUC18. ^{32}P-labeled probes were generated by digestion with *Sal* I (−26), 3′-end filling, and secondary cleavage with *Hin*dIII (pVA, 317-bp fragment) or *Eco*RI (pVA1, 247-bp fragment).

Cells and Extract Fractionation. 293 cells were cultured as described except 5% newborn calf serum was used (11). S100 extracts were prepared as described by Wu (20), except that 0.5 mM phenylmethylsulfonyl fluoride and 100 Kallikrein inhibitor units (KIU) of aprotinin (Trasylol; FBA Pharmaceuticals, New York) were added after cell lysis. The S100 was adjusted with solid ammonium sulfate to 25% saturation while stirring on ice, incubated for 30 min, and centrifuged at 15,000 × *g* for 20 min. The supernatant was brought to 50% saturated ammonium sulfate, centrifuged, and the pellet was dissolved in and dialyzed against 100 mM KCl in buffer A [20 mM Hepes, pH 7.9/0.2 mM Na_2EDTA/0.5 mM dithiothreitol/20% (vol/vol) glycerol], and chromatographed on phos-

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Abbreviation: phosphocellulose C, PC-C.

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phocellulose (Whatman P-11) as described (11). The 0.6 M KCl eluate (designated the PC-C fraction) was dialyzed for 2 hr against 50 mM KCl in buffer B [50 mM Tris·HCl, pH 7.5/0.5 mM Na_2EDTA/0.5 mM dithiothreitol/20% (vol/vol) glycerol], and 10–16 mg of protein was loaded onto a Mono Q HR 5/5 column (Pharmacia) previously equilibrated with 50 mM KCl in buffer B. Protein was eluted with a 30-ml 100–600 mM KCl linear gradient in buffer B using a flow rate of 0.5 ml/min at room temperature. Acetylated bovine serum albumin (21) was added to the fractions to 0.1 mg/ml, and pooled fractions were dialyzed in 50 mM KCl in buffer A. Extracts and pooled fractions were stored and protein concentration was determined as described (11).

The TFIIIB/pol III fraction was prepared from the PC-B fraction from 293 cell extracts by dialysis in 100 mM KCl in buffer A, applied onto a phosphocellulose column (bed volume, 4 mg of protein per ml), and eluted with a linear 0.1–0.4 M KCl gradient in buffer A. The TFIIIB/pol III activity was identified by *in vitro* transcription with the PC-C fraction (B assay; ref. 11). Added purified RNA polymerase III (22) did not stimulate transcription in reactions using the TFIIIB/pol III fraction, indicating that pol III was in excess to TFIIIB.

***In Vitro* Transcription.** Salt concentrations were adjusted to 75 mM KCl, and 0.35 μg of pVA1 and 0.15 μg of pUC18 plasmids were used in reactions as described (11).

DNase I Footprint Analysis (23). End-labeled DNA probes (2000 Cerenkov cpm), corresponding to 1–3 fmol of gene, were preincubated with protein samples and carrier DNA (10 μg of pUC18 DNA per ml) in 75 mM KCl/5 mM $MgCl_2$/50 mM Tris·HCl, pH 7.9/1 mM EDTA/0.5 mM dithiothreitol/15% (vol/vol) glycerol for 30 min at 20°C. After adjustment of $MgCl_2$ to 7.5 mM, DNase I (Cooper Biomedical, Malvern, PA) was added to a final concentration of 4 μg/ml. After 1 min, reactions were stopped with RNA extraction buffer (11) and 5 μg of tRNA, then the mixtures were phenol/chloroform-extracted and ethanol-precipitated. Labeled DNA was resolved on 12% (see Fig. 1*A*) or 8% polyacrylamide (see Fig. 4) gels with 8 M urea. Gels were dried and exposed to autoradiographic film with a Dupont Cronex intensifying screen.

Sedimentation Velocity Gradient Analysis. Mono Q TFIIIC1 and TFIIIC2 fractions were concentrated 4-fold with Centricon 30 microconcentrators (Amicon) and dialyzed against buffer C [20 mM Hepes, pH 7.8/0.15 M KCl/0.5 mM Na_2EDTA/0.5 mM dithiothreitol/0.1% Nonidet P-40/10% (vol/vol) glycerol] with 5% sucrose. Two hundred microliters of the concentrated samples were analyzed in 5 ml of neutral sucrose gradients containing 5–27% sucrose in buffer C and 0.125 mM phenylmethylsulfonyl fluoride and 50 KIU of Trasylol per ml, centrifuged for 21 hr at 45,000 rpm and 4°C in a Beckman SW50.1 rotor. Molecular size markers (29–700 kDa; Sigma) were sedimented in a parallel gradient. Two-hundred-microliter fractions were collected dropwise from the bottom.

RESULTS

Enriched Source of TFIIIC from 293 Cell Extracts. Previously, we and others have shown that the *in vivo* expression of adenovirus E1A proteins stimulates the *in vitro* activity of pol III transcription factors, which fractionate in the PC-C fraction (11, 12). From those results, we reasoned that 293 cells (adenovirus-transformed human embryonic kidney cells that constitutively express E1A) would provide us with the most enriched source of TFIIIC. We found that S100 extracts from 293 cells yielded ≈10 times greater *in vitro* transcriptional activity per microgram of protein than HeLa cell S100 extracts (11). Phosphocellulose TFIIIC fractions (PC-C) prepared from 293 cells were very active in both *in vitro* transcription (see Fig. 3) and DNase I footprint (see Fig. 1*A*) assays.

Separation of Transcription Factors in the PC-C Fraction by FPLC-Mono Q Chromatography. The proteins in the PC-C fraction were resolved by chromatography on a Pharmacia Mono Q column and eluted with a linear salt gradient from 100 to 600 mM KCl. DNase I footprint analysis was conducted to identify specific DNA binding activities in the PC-C and Mono Q fractions (Fig. 1*A*). The PC-C fraction demonstrated strong protection in the region of the B block and the 3′ terminus of the VA1 gene (lanes 35–37). The Mono Q column separated the specific DNA binding activities (lanes 1–34). A majority of the 3′ terminus binding activity flowed through the column while some bound and eluted with the beginning of the linear gradient (125 mM KCl). Weak B-block protection was observed with fractions that eluted between 200 and 250 mM KCl, and strong B-block binding activity was reproducibly eluted from the Mono Q column between 350 and 400 mM KCl.

Transcription factor activity of the Mono Q column fractions was assayed in *in vitro* transcription reactions containing the TFIIIB/pol III fraction plus individual Mono Q column fractions (Fig. 1*B*). This reconstituted transcription reaction assays for TFIIIC activity. A barely detectable amount of specific VA1 transcription was observed in fractions that generally corresponded to the strong B-block binding activity. Coeluting with this weak activity was an activity that produced high molecular weight RNAs. S1 nuclease analysis indicated the large RNAs were not initiated at the proper VA1 transcriptional start sites (data not shown).

To determine whether the loss of activity was due to the separation of transcription factors in the PC-C fraction into two or more components, we performed *in vitro* transcription complementation analysis. We reasoned that if the TFIIIC activity in the PC-C fraction separated into two components, one component might be identified by its specific B-block binding activity. Addition of the B-block binding component into a transcription assay with TFIIIB and pol III would then assay for a component that would complement the binding factor for *in vitro* transcription. Therefore, we reassayed the Mono Q column fractions in an *in vitro* transcription assay that contained 1 μg of protein of the pooled B-block binding activity and 5 μg of protein of the TFIIIB/pol III fraction (Fig. 1*C*). Specific VA1 transcription was readily observed with fractions eluting between 250 and 300 mM KCl from the column. The peak of this transcriptional complementing activity (270 mM KCl; lane 15) did not coincide with the weak B-block protection activity, which eluted around 230 mM KCl. In the footprint analysis, the equivalent fractions (270 mM KCl, corresponding to Fig. 1*A*, lanes 18 and 19) did not show B-block protection. Weaker complementing activity eluted over a wide range lower in the gradient (130–230 mM KCl). Reconstitution of *in vitro* transcriptional activity with the pooled B-block binding activity and the Mono Q eluate between 250 and 300 mM KCl demonstrates that the TFIIIC fraction is composed of at least two separable factors. Fractions containing the strong B-block binding activity were pooled and designated TFIIIC2 and the fractions that complemented TFIIIC2 for *in vitro* transcription were termed TFIIIC1.

Fig. 2 shows the results of *in vitro* transcription complementation assays using the pooled TFIIIC1 and TFIIIC2 fractions. Addition of only TFIIIC1 or TFIIIC2 to the TFIIIB/pol III fraction did not reconstitute transcription (lanes 4 and 5). However, when TFIIIC1 and TFIIIC2 were both added to the TFIIIB/pol III fraction (lane 6), transcription was reconstituted to levels dramatically greater than the activity observed using the individual fractions. To assay TFIIIC2 activity, conditions were determined in which

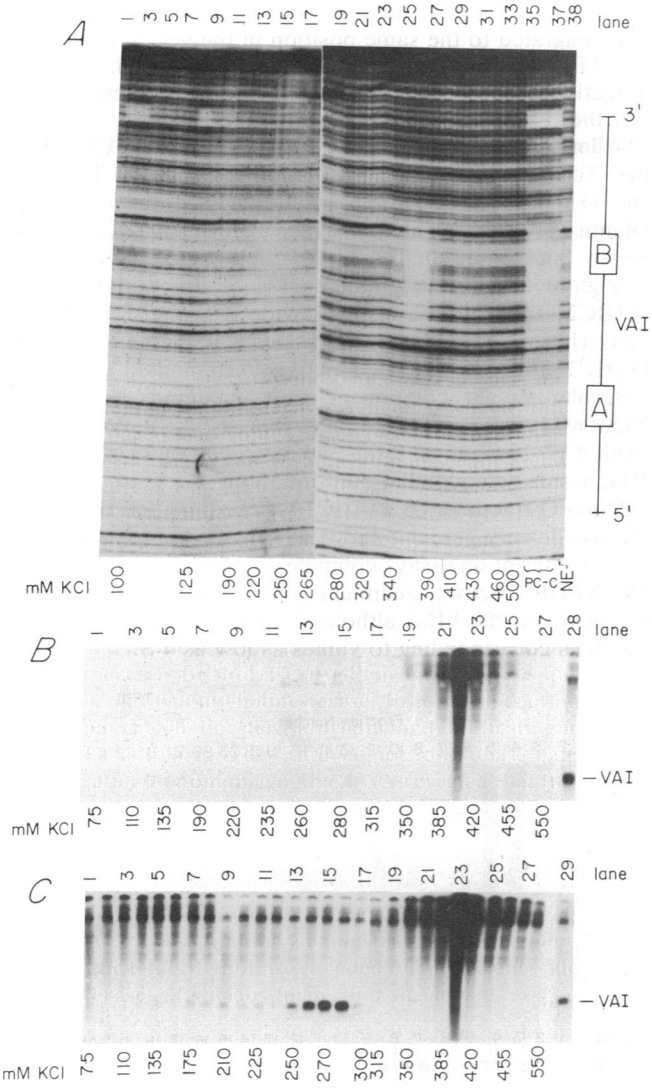

FIG. 1. Mono Q chromatography of the 293 cell PC-C fraction. (*A*) DNase I footprint analysis of the VA1 gene with the PC-C fraction and Mono Q gradient fractions. A schematic representation of the VA1 gene aligned with the footprint pattern is shown on the right. The orientation of the gene and the A and B blocks are indicated. PC-C fractions were assayed in lanes 35–37 using 4, 8, and 16 μg of protein, respectively. Mono Q eluate fractions (4 μl) were assayed in lanes 1–34. Footprint analysis without added extract (NE) is in lane 38. The reaction volume was 25 μl, and 1–2 ng of pVA probe (317 bp, 3′ end-labeled) was used. (*B*) *In vitro* transcription analysis of Mono Q column fractions (lanes 1–27). Reactions assayed fractions from the Mono Q column supplemented with 5 μg of the TFIIIB/pol III fraction. Decreasing amounts of eluate fractions (20–3 μl) were added to reaction mixtures to assay the maximum sample, while considering the salt concentration of each fraction, to maintain the transcription reactions at 75 mM KCl. A positive control for VA1 transcription appears in lane 28. An autoradiograph is shown of the labeled RNA products from the reactions described in *Materials and Methods*. (*C*) *In vitro* transcription analysis of Mono Q column fractions for complementing activity (lanes 1–28). *In vitro* transcription analysis was conducted identically to the experiment described in *B*, except that 1 μg of protein of the pooled strong B-block binding activity (350–400 mM KCl) was added to each reaction mixture. A positive control for VA1 transcription appears in lane 29.

TFIIIB, pol III, and TFIIIC1 were present and the transcription reaction was responsive in a linear fashion to added TFIIIC2 fraction (lanes 7–11). Conversely, TFIIIC1 levels were assayed under conditions in which TFIIIC2 and the TFIIIB/pol III fraction were in excess and increasing

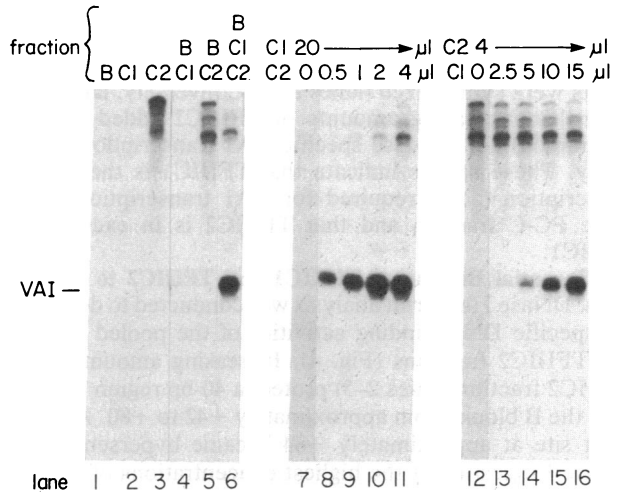

FIG. 2. *In vitro* transcription analysis for complementing activity using pooled TFIIIC1 and TFIIIC2 fractions. An autoradiograph of labeled RNA products from the reactions is shown. The pooled TFIIIC1 (C1) and TFIIIC2 (C2) fractions were combined in reaction mixtures as indicated at the top of the figure. Five micrograms of TFIIIB/pol III fraction (B) was added to all reaction mixtures except those represented in lanes 2 and 3. Unless indicated, 20 μl of TFIIIC1 and 4 μl of TFIIIC2 were used in the reactions. Lane 7 (TFIIIC1 and TFIIIB/pol III) and lane 12 (TFIIIC2 and TFIIIB/pol III) were overexposed to detect background activity. Protein concentration of the pooled TFIIIC1 fraction was 0.8 mg/ml and the pooled TFIIIC2 fraction was 1.2 mg/ml.

amounts of TFIIIC1 were added (lanes 12–16). The results demonstrate assay conditions that sensitively monitor TFIIIC1 and TFIIIC2 activities. Using these assays we measured the TFIIIC1 and TFIIIC2 activities present in the crude PC-C fraction (data not shown). Transcription activity quantitation indicated that ≈10% of the TFIIIC1 activity and 15% of the TFIIIC2 activity in the applied PC-C fraction were recovered from the Mono Q column.

Determination of the Limiting Component in the PC-C Fraction. To determine which component was limiting in the PC-C fraction, we supplemented the crude fraction with the TFIIIC1 or TFIIIC2 fractions in reconstituted transcription reactions (Fig. 3). Lanes 1 and 6 show the labeled RNA products generated by the PC-C fraction with the TFIIIB/pol

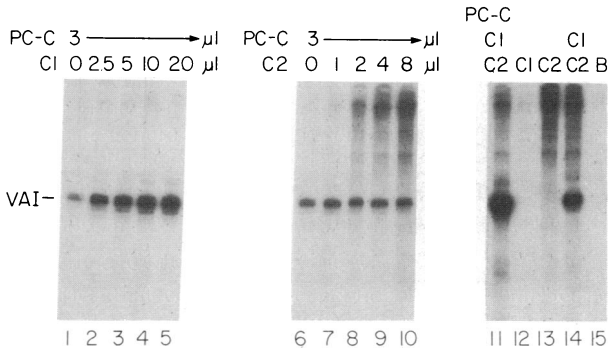

FIG. 3. Analysis of the limiting component in the PC-C fraction. Three microliters of PC-C fraction (1 mg of protein per ml) was supplemented with increasing amounts of TFIIIC1 (C1) fraction (lanes 2–5, and 11) or TFIIIC2 (C2) fraction (lanes 7–11). Five micrograms of the TFIIIB/pol III (B) fraction was used in all reactions. For lanes 11–14, 10 μl of TFIIIC1 and 4 μl of TFIIIC2 were added to the reaction mixture. Protein concentrations of fractions used in this experiment and all subsequent experiments are indicated in the legend to Fig. 2. An autoradiograph of the labeled RNA products is shown.

III fraction. When an increasing amount of the TFIIIC1 factor was added to reactions containing the PC-C and TFIIIB/pol III fractions, increased amounts of VA1 transcripts were synthesized (lanes 2–5). Conversely, lanes 7–10 show that increasing amounts of TFIIIC2 added into the reaction did not increase specific VA1 transcription significantly. These results indicate that TFIIIC1 is the limiting transcription factor required for VA1 transcription in the crude PC-C fraction and that TFIIIC2 is in excess over TFIIIC1.

Differential Binding of TFIIIC1 and TFIIIC2 to the VA1 Gene. DNase I footprint analysis was conducted to determine the specific DNA binding activities of the pooled TFIIIC1 and TFIIIC2 fractions (Fig. 4). Increasing amounts of the TFIIIC2 fraction (lanes 2–5) protect a 40-bp region centered over the B block, from approximately +42 to +80. A DNase I cut site at approximately +84 became hypersensitive to DNase I digestion at the highest concentrations of TFIIIC2 added (lanes 4 and 5). When increasing amounts of the TFIIIC1 fraction were added to a constant amount (20 μl) of TFIIIC2, the highest amounts of added TFIIIC1 (lanes 9 and 10) altered the TFIIIC2 footprint to include the region from the start site of transcription into the A block (approximately +1 to +18). The footprint 3′ to the B block was also modified. The hypersensitive site at approximately +84 produced by TFIIIC2 alone was suppressed and there was partial protection extending to the 3′ end of the gene. An apparent hypersensitive site at approximately +80 was evident, but the significance of this is unclear, since renatured full-length probe migrated to the same position in the gel (not shown). The TFIIIC1 fraction alone demonstrated much weaker protection over the 5′ region and no detectable protection over the B block (lanes 12 and 13).

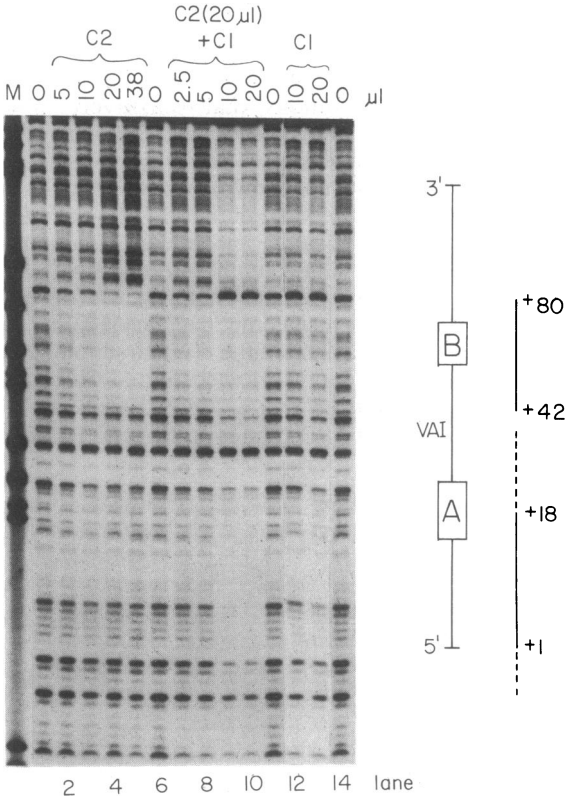

FIG. 4. DNase I footprint analysis of the VA1 gene with the TFIIIC1 and TFIIIC2 fractions. Amounts of TFIIIC1, TFIIIC2, and PC-C used in each assay are indicated at the top of the figure. Increasing amounts of TFIIIC2 were added to reaction mixtures shown in lanes 2–5. Constant amounts of TFIIIC2 in addition to increasing amounts of TFIIIC1 were assayed in lanes 7–10. TFIIIC1 alone (lanes 12 and 13) was also assayed for binding activity. A schematic representation of the VA1 gene with the protected regions designated is on the right. End-labeled *Hae* III digest of pBR322 was used as marker (lane M). DNase I footprint reaction volume was 50 μl, and 1–2 ng of pVA1 probe (247 bp) was used.

Sedimentation Analysis of the TFIIIC1 and TFIIIC2 Activities. To determine the sedimentation value of the TFIIIC1 and TFIIIC2 activities, the fractions from the Mono Q column were concentrated, dialyzed against sucrose gradient buffer, and individually sedimented by centrifugation through sucrose density gradients (Fig. 5). Fractions from the TFIIIC2 sedimentation analysis were analyzed for *in vitro* transcription activity in an assay complemented with TFIIIC1 (Fig. 5*A*). TFIIIC2 activity sedimented as a high molecular weight component with maximum activity in fractions 4 and 5, corresponding to a sedimentation coefficient of 17–18 S and an estimated molecular mass of 400–500 kDa, assuming a globular conformation.

Mono Q fractionated TFIIIC1 was sedimented through a sucrose density gradient and fractions were analyzed by *in vitro* transcription assays complemented with TFIIIC2 (Fig. 5*B*). TFIIIC1 activity sedimented primarily as a component of ≈9 S (or 200 kDa) although some activity trailed into fractions corresponding to values as low as 4 S.

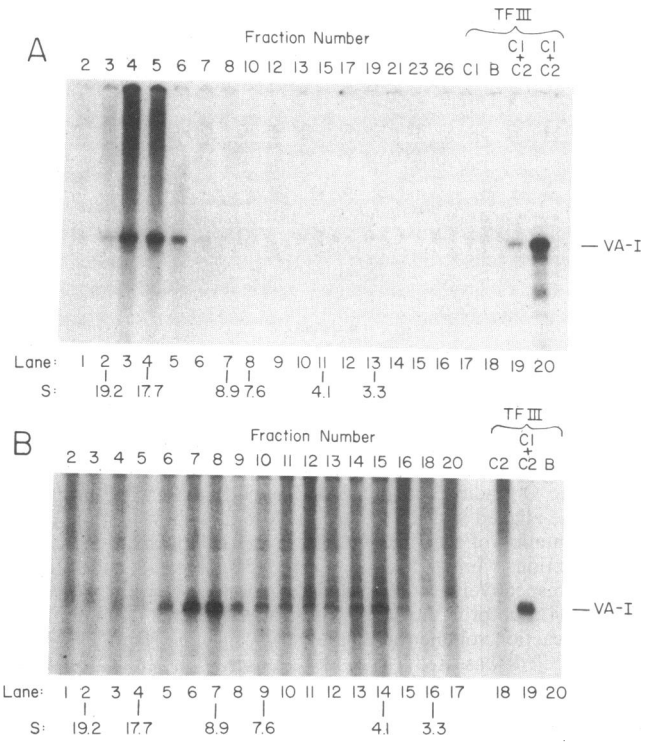

FIG. 5. Sedimentation velocity gradient analysis of the Mono Q TFIIIC2 and TFIIIC1 activities. Autoradiographs of the labeled RNA products are shown. (*A*) *In vitro* transcription assay for TFIIIC2 activity in the sucrose gradient fractions from Mono Q TFIIIC2 fraction sedimentation. Reaction mixtures containing 5 μg of TFIIIB/pol III fraction (B), 5 μl of Mono Q TFIIIC1 fraction, and 12 μl of the sucrose gradient fractions from TFIIIC2 sedimentation (lanes 1–16). Five micrograms of protein TFIIIB/pol III fraction was added either alone (lane 18), or with 5 μl of TFIIIC1 fraction and 0, 3, or 6 μl of TFIIIC2 fraction (lanes 17, 19, and 20, respectively). (*B*) *In vitro* transcription assay for TFIIIC1 activity in the sucrose gradient fractions from Mono Q TFIIIC1 fraction sedimentation. Reactions contained 5 μg of protein of the TFIIIB/pol III fraction (B), 2.5 μl of Mono Q TFIIIC2 fraction, and 12.5 μl of the sucrose gradient fractions from TFIIIC1 sedimentation (lanes 1–17). Five micrograms of protein TFIIIB/pol III fraction was added alone (lane 20), or with 2.5 μl of TFIIIC2 (lane 18), or with 2.5 μl of TFIIIC2 and 5 μl of TFIIIC1 (lane 19) in the transcription reaction mixtures.

DISCUSSION

These results demonstrate that TFIIIC activity can be separated into at least two components, which we designate TFIIIC1 and TFIIIC2. The TFIIIC1 fraction is needed to complement TFIIIC2 for the efficient initiation of VA1 transcription *in vitro*. The transcription of high molecular weight RNAs in reactions containing TFIIIC2 raised the possibility that TFIIIC1 might be a termination or processing factor, generating the correct 3′ end of transcripts initiated at the VA1 start sites. However, S1 nuclease analysis demonstrated that the high molecular weight RNAs did not have 5′ ends at the correct start sites, and their transcription did not require a pol III promoter (data not shown).

Transcription assays using a PC-C fraction supplemented with the TFIIIC1 and TFIIIC2 fractions indicate that TFIIIC1 is the limiting transcriptional component(s) in the PC-C fraction. Since the expression of E1A proteins appears to increase the activity of the limiting transcription factor present in the PC-C fraction (11, 12), E1A proteins may modulate transcription by regulating the amount or specific activity of the TFIIIC1 component(s).

The DNase I footprint results (Fig. 4) demonstrate the differential binding activities of the TFIIIC1 and TFIIIC2 fractions to the VA1 gene. The TFIIIC2 fraction binds strongly to the B-block region in the absence of other fractions. The TFIIIC1 fraction alone at best weakly protects the 5′ region of the gene (including the A block). However, the TFIIIC1 fraction causes clear protection of the 5′ region when TFIIIC2 is present and partial protection 3′ of the B block. It is possible that TFIIIC2 stabilizes the binding of TFIIIC1 to the 5′ region. Alternatively, TFIIIC1 may not be a DNA-binding protein itself, but it may alter the DNA binding specificity of TFIIIC2. Further work is needed to clarify the DNA–protein as well as the potential cooperative protein–protein interactions that may be responsible for the increased protection elicited by TFIIIC1. At present, it is not clear whether all these changes in the DNase I footprint produced by the addition of the TFIIIC1 fraction to the TFIIIC2 fraction are due to the same component(s) responsible for the transcriptional complementing activity. The DNase I protection properties of the two TFIIIC fractions could explain the differential protection of the A- and B-block regions of VA1 and tRNA genes by various TFIIIC preparations using different assay conditions (4, 6, 10, 13–17).

TFIIIC2 behaves as a large molecular mass component of 400–500 kDa, while TFIIIC1 activity sediments as a smaller component of ≈200 kDa. Further purification is required to determine whether these components are composed of a single polypeptide chain, or of several equivalent or non-equivalent subunits.

The separation of TFIIIC1 and TFIIIC2 raises many questions regarding their individual activities. Are one or both factors needed for (*i*) stable complex formation? (*ii*) TFIIIB and RNA polymerase III interaction in complex formation? (*iii*) 5S RNA gene transcription? Also, it will be important to determine whether TFIIIC1, TFIIIC2, or both factors are increased in activity or amount due to E1A expression. Since E1A proteins are central to the process of oncogenic transformation by adenovirus (24), and expression of E1A stimulates TFIIIC activity (11, 12), the biochemical characterization of TFIIIC1 and TFIIIC2 may help elucidate the molecular mechanisms of viral oncogenesis.

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