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Identification of a 150-Kilodalton Polypeptide That Copurifies with Yeast TFIIIC and Binds Specifically to tRNA Genes

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The transcription in vitro of eucaryotic tRNA genes by RNA polymerase III requires two transcription factors, designated TFIIIB and TFIIIC. One of the critical functions of TFIIIC in the transcription of tRNA genes is that it interacts directly and specifically with the two internal promoter elements of these genes. We have partially purified *Saccharomyces cerevisiae* TFIIIC by chromatography on Bio-Rex 70, DEAE-cellulose, and phosphocellulose resins. A 150-kilodalton (kDa) DNA-binding polypeptide copurified with TFIIIC activity. This 150-kDa protein coeluted with the DNA-binding activity of TFIIIC after rechromatography of TFIIIC on phosphocellulose and its elution with a linear salt gradient. The stable and high-affinity interaction of this protein with tRNA genes was demonstrated by the maintenance of a protein-DNA complex under conditions of high ionic strength. Finally, we showed by two criteria that the interaction of this protein with tRNA genes was specific. First, the protein-DNA complex was competed with only by DNA-containing tRNA genes; second, the protein preferentially bound to DNA fragments containing a tRNA gene. These results strongly suggest that the DNA-binding domain of the yeast TFIIIC is contained within this 150-kDa polypeptide.

In eucaryotic cells, RNA polymerase III is involved in the expression of a variety of small untranslated RNAs. These include tRNAs, 5S RNAs, virus-associated RNAs (e.g., VA1 and VA2 of adenovirus), and other small nuclear RNAs (e.g., U6 RNA) (22). In addition, there is increasing evidence that RNA polymerase III plays a role in the regulation of expression of certain RNA polymerase II genes (5, 6). Unique to the class III genes is their ability to direct transcription via internal control regions (ICRs): tRNA genes contain two ICRs, the 5′ ICR, designated the A box, and the 3′ ICR, called the B box (22). In addition, 5′-flanking sequences of these genes appear to modulate the efficiency of transcription (11, 17, 19). A variety of cell-free transcription systems from mammalian, insect, and yeast sources have been used to examine DNA sequence requirements for gene expression. Fractionation of these cell extracts to identify the protein factors involved in the transcription of these genes has revealed that two fractions, designated TFIIIB and TFIIIC, are required to reconstitute specific and efficient transcription with RNA polymerase III in vitro (3, 9, 21). These factors are used by all class III genes. In addition to TFIIIB and TFIIIC, another component, TFIIIA, is required for the transcription of 5S RNA genes. Analysis of the function of the TFIIIB and TFIIIC components in the transcription of tRNA genes has shown that both are involved in the formation of stable transcription complexes with the DNA template. These complexes are stable for many rounds of transcription initiation (20). The interaction between TFIIIC and TFIIIB is important for the formation of productive transcription complexes (3, 12).

By template competition and nuclease-protection analysis, TFIIIC has been shown to bind specifically to tRNA genes in the absence of TFIIIB (22). Analysis of the DNA-binding property of yeast TFIIIC showed that its primary recognition site is the 3′ ICR of tRNA genes, and to a lesser extent (depending on the template), 5′ ICR binding also occurs (4, 10). The TFIIIC fraction from both human cells (28) and *Bombyx mori* (16) has been further fractionated into two functional components. Whether the putative DNA-binding and protein-binding domains of TFIIIC are contained within one polypeptide or are contained in different polypeptides of a multisubunit protein is not yet known. Detailed biochemical analysis of the ordered interactions that specify the transcription process necessitates the identification of the various polypeptides that compose the subunit structures of both TFIIIB and TFIIIC. Accordingly, we have used a protein-blotting method to determine the molecular mass of a DNA-binding polypeptide of *Saccharomyces cerevisiae* TFIIIC.

MATERIALS AND METHODS

Preparation of TFIIIC. Cell extracts were prepared from *S. cerevisiae* 20B-12 (αtrp1 pep 4-3) according to the method of Klekamp and Weil (9). The resultant ammonium sulfate precipitate pellet (prepared from 75 to 95 g of cells) was suspended and dialyzed in buffer A (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.9], 10% [vol/vol] glycerol, 0.2 mM EDTA, 0.5 mM dithiothreitol) containing 0.1 mM phenylmethylsulfonyl fluoride to a concentration of 50 to 60 mg of protein per ml. This fraction was loaded onto a 150-ml column of Bio-Rex 70 (Bio-Rad Laboratories) equilibrated in the same buffer. The column was washed with 2 column volumes of buffer A containing 0.28 M KCl, and TFIIIC was then eluted with buffer A containing 0.5 M KCl. The 0.5 M KCl-eluted fractions were dialyzed against buffer A containing 0.05 M ammonium sulfate and loaded onto a DEAE-cellulose column (10 mg of protein per ml of bed volume). The column was washed with the same buffer and eluted with buffer containing 0.3 M ammonium sulfate. The terminal wash step containing TFIIIC activity was diluted with buffer A to a final ammonium sulfate concentration of 0.1 M. This material was loaded onto a phosphocellulose column (P-11; Whatman, Inc.) (4 mg of protein per ml of bed volume) equilibrated with buffer A containing 0.1 M ammonium sulfate. TFIIIC activity was then eluted with buffer A

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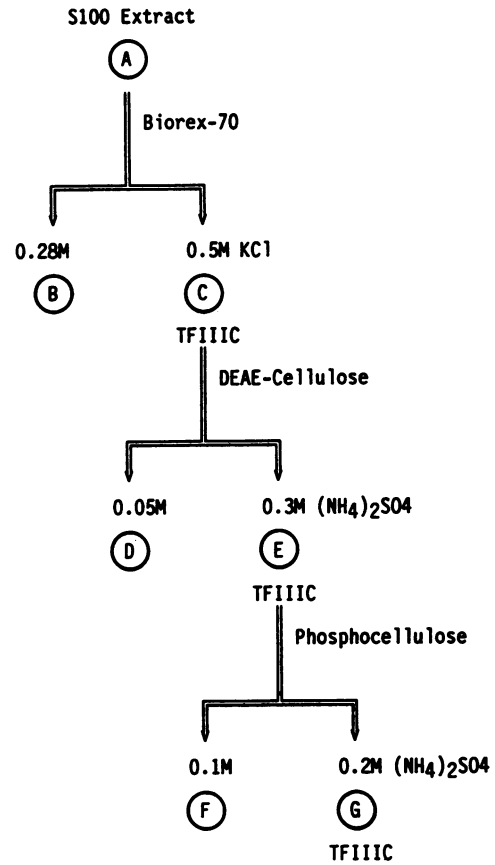

FIG. 1. Scheme for the partial purification of *S. cerevisiae* TFIIIC.

containing 0.2 M ammonium sulfate. This fraction was either directly dialyzed against buffer A without phenylmethylsulfonyl fluoride but containing 4 mM $MgCl_2$, 0.1 M NaCl, and 20% glycerol or concentrated first by precipitation with ammonium sulfate (0.4 g/ml) such that the protein concentration was 6 to 10 mg/ml. The resultant phosphocellulose-derived TFIIIC fraction was devoid of TFIIIB and RNA polymerase III activities and was stored at −90°C.

Column fractions were assayed by their ability to support tRNA gene transcription when reconstituted with partially purified RNA polymerase III and TFIIIB. The partially purified RNA polymerase III and TFIIIB were kindly supplied by Jacqueline Segall and prepared according to the method of Taylor and Segall (24). Transcription assays were performed according to the method of Willis et al. (27) by using a *Drosophila melanogaster* $tRNA^{Arg}$ gene (pArg) as a template (20). The concentration of proteins was monitored by the method of Lowry et al. (13) and by measuring the absorbance at 280 nm. Chromatography of the final TFIIIC-derived fraction on phosphocellulose was carried out by using 5 mg of the protein fraction G (Fig. 1), which was loaded onto a 1-ml phosphocellulose column equilibrated in buffer A containing 0.05 M $(NH_4)_2SO_4$. The column was washed with 2 column volumes of buffer, and bound protein was eluted with a 10-ml gradient of 0.05 to 0.4 M $(NH_4)_2SO_4$. Fractions of approximately 0.4 ml were collected.

DNA-binding gel retardation assays. Each of the DNA-binding assays was performed in a solution containing 20 μl of 20 mM HEPES (pH 7.9), 1 mM dithiothreitol, 4 mM $MgCl_2$, 150 mM KCl, 0.5 mg of bovine serum albumin per ml, 0.05% Nonidet P-40, 5% glycerol, 0.5 to 1 μg of double-stranded poly(dI · dC), 0.5 to 2 ng of labeled VA1 RNA gene fragment, and 0.5 to 2 μg of protein sample. The amount of poly(dI · dC) and protein used was determined by titration experiments in which both parameters were varied to obtain clearly resolved retarded DNA bands. Under these conditions the amount of complexed DNA formed was proportional to the amount of protein added. Incubations were carried out for 30 min at 20°C, and the reaction mixture was loaded onto a 4% polyacrylamide gel (acrylamide:bis ratio, 1:29) which contained 20 mM Tris hydrochloride (pH 7.9), 2 mM EDTA, and 5% glycerol. The electrode buffer was the same, except that glycerol was omitted. The samples were loaded onto the prerun gel at room temperature, and after the samples entered the gel the electrophoresis was carried out further at 4°C and the buffer was continuously recirculated.

To quantitate the amount of complexed DNA, the DNA was visualized by autoradiography, the retarded bands were excised from the gel, and Cerenkov radiation was measured.

Protein blotting and detection of DNA-binding proteins. The yeast-derived fractions containing TFIIIC were subjected to sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis (50 μg of protein per lane), and the proteins were transferred to nitrocellulose electrophoretically for 12 to 15 h at 4°C. The nitrocellulose was cut into strips and blocked with buffer (10 mM HEPES [pH 7.9], 5% nonfat dry milk) as described previously (15). The filters were then incubated for 1 h at 20°C in binding buffer (10 mM HEPES [pH 8.0], 50 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 0.25% nonfat dry milk) containing 10^5 cpm of ^{32}P-labeled DNA probe per ml. The filters were washed in the same buffer for 1 h without the DNA probe with 100 mM NaCl unless otherwise indicated. The filters were dried and autoradiographed for 1 to 3 days with an intensifier screen.

DNA probes. The *Drosophila* $tRNA^{Arg}$ gene (pArg) fragment used was a 508-base-pair *Hin*dIII fragment which has been previously described (20). The VA1 fragment was a 247-base-pair *Sal*I-*Eco*RI fragment which encodes the VA1 RNA gene from the adenovirus type 2 genome (2). The sup3-e fragment used was a 346-base-pair *Hin*dIII-*Ava*II fragment from *Schizosaccharomyces pombe* containing a suppressor $tRNA^{Ser}$ gene (27). The DNA fragments were end labeled by using [^{32}P]dATP and T4 DNA polymerase with a specific activity of 10^7 cpm/μg. The amount of DNA fragments used for detection of the DNA-binding proteins was quantitated by using the diaminobenzoic acid procedure (25).

RESULTS

TFIIIC was partially purified from *S. cerevisiae* S100 extracts by means of the fractionation scheme shown in Fig. 1. TFIIIC was detected by mixing the various fractions with partially purified TFIIIB and RNA polymerase III and examining tRNA gene transcription. The transcription activities of the three fractions containing TFIIIC are shown in Fig. 2A. Equal amounts of protein from each fraction were reconstituted with nonlimiting amounts of RNA polymerase III and TFIIIB. These fractions were also analyzed for specific binding of TFIIIC to a labeled DNA fragment containing the adenovirus VA1 RNA gene by using a DNA-binding gel retardation assay. This technique allows the separation of DNA-protein complexes from free DNA fragments because of their retarded mobility on polyacrylamide gels (7). We used the VA1 RNA gene fragment as a probe for

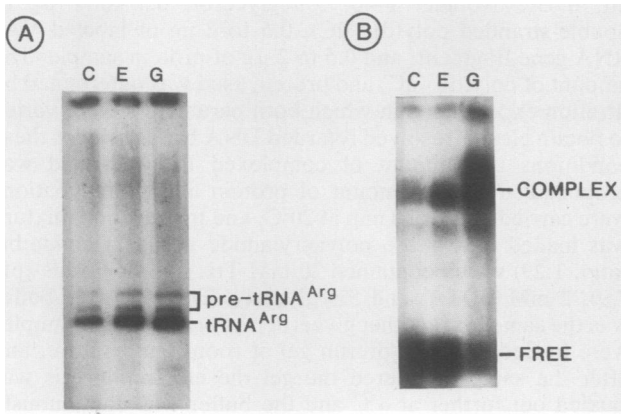

FIG. 2. Analysis of TFIIIC transcription and DNA-binding activities in purified yeast fractions. (A) Results of the transcription reconstitution assay. Ten micrograms of each fraction (C, E, and G, derived from the fractionation scheme shown in Fig. 1) was reconstituted with partially purified preparations of yeast RNA polymerase III and TFIIIB and the pArg gene template. The resultant RNA transcripts were analyzed on polyacrylamide gels. (B) Results of the gel retardation assay. Two micrograms of each fraction was incubated with the VA1 gene fragment in each DNA-binding reaction, and the DNA-protein complexes were resolved from free DNA on polyacrylamide gels as described in Materials and Methods.

analyzing TFIIIC DNA-binding activity because previous studies with HeLa cell TFIIIC have indicated that human TFIIIC has a higher affinity for this gene than for tRNA genes (2, 12). Therefore, use of the VA1 gene as a probe should increase the sensitivity of this assay for detecting the DNA-binding activity of TFIIIC. In the gel retardation assay, DNA-protein complexes were formed and resolved on polyacrylamide gels. A major retarded band was observed in all fractions that exhibited TFIIIC activity in the transcription reconstitution assay (Fig. 2B). By competition analysis we determined this band to be a specific TFIIIC-DNA complex (Fig. 3). The addition of a 10-fold molar excess of a plasmid containing the VA1 RNA gene (pVA) to the assay reduced the amount of complex obtained, whereas the addition of plasmid alone did not affect the amount of complex formed. By the same criteria, the faster mobility complexes sometimes observed with the VA1 RNA gene

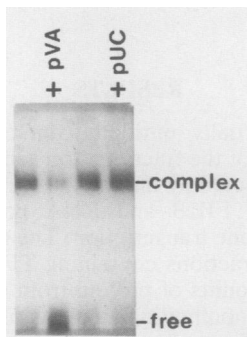

FIG. 3. Competition analysis of specific TFIIIC-gene binding by the gel retardation assay. Each reaction mixture contained 1 μg of fraction G and 0.5 ng of the labeled VA1 gene fragment. In the two lanes indicated, a 10-fold molar excess of competitor DNA (either plasmid containing the VA1 gene [pVA] or plasmid alone [pUC]) was added. The resultant DNA-protein complexes were resolved by polyacrylamide gel electrophoresis.

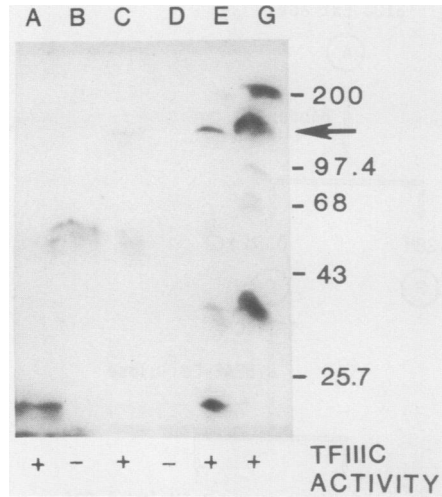

FIG. 4. Detection of DNA-binding proteins in purified yeast fractions by a protein blot assay. *S. cerevisiae* extracts were fractionated according to the scheme shown in Fig. 1. Each fraction (50 μg of protein) was electrophoresed on sodium dodecyl sulfate-10% polyacrylamide gel, the separated proteins were transferred to nitrocellulose, and the bound proteins were incubated with a labeled *Schizosaccharomyces pombe* $tRNA^{Ser}$ gene fragment, sup3-e. The bound DNA was detected as described in Materials and Methods. All fractions from the purification scheme are shown except F, which did not contain any detectable DNA-binding activity. The relative migrations of molecular mass standards are indicated and include myosin (200 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), and α-chymotrypsin (25.7 kDa).

probe do not appear to result from specific TFIIIC-DNA complexes.

To identify the polypeptide responsible for the specific DNA-binding function of TFIIIC, fractions derived from the purification procedure were analyzed by a protein-blotting procedure involving a tRNA gene probe. Equal amounts of protein derived from each chromatographic step were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were then transferred to nitrocellulose. The nitrocellulose was probed with a ^{32}P-labeled DNA fragment containing a $tRNA^{Arg}$ gene (Fig. 4). In the fractions containing TFIIIC activity, several DNA-binding proteins were detected. Polypeptides with approximate sizes of 200, 150, and 50 kilodaltons (kDa) were enriched in each chromatographic step. We found that the relative amounts of the 200- and 50-kDa DNA-binding proteins varied in different TFIIIC-containing fraction G preparations but that the 150-kDa protein signal was reproducibly observed.

We compared the enrichment of the activity of TFIIIC and the DNA-binding activity of the 150-kDa protein in these steps. In a representative purification procedure, enrichment of the DNA-binding activity of TFIIIC from the Bio-Rex 70 step to the DEAE-cellulose step was approximately 3.5-fold; from the DEAE-cellulose step to the phosphocellulose step, enrichment was 5-fold. The DNA-binding activity of the 150-kDa protein was enriched approximately 3-fold from the Biorex-70 step to the DEAE-cellulose step and 7-fold from the DEAE-cellulose step to the phosphocellulose step. Thus, the enrichment observed for the DNA-binding activity of TFIIIC correlated well with that observed for the 150-kDa protein. The transcription activity of TFIIIC was less stable and variable compared with its DNA-binding activity but

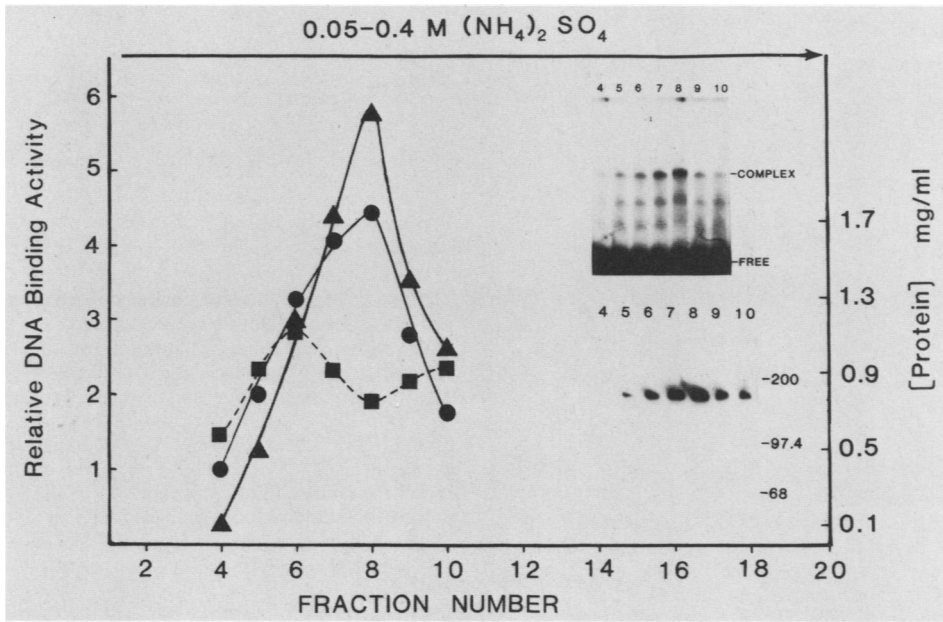

FIG. 5. Analysis of the elution profile of the DNA-binding activities from a phosphocellulose gradient. Fraction G, derived from the purification scheme shown in Fig. 1, was rechromatographed on phosphocellulose. Fractions eluted in the linear gradient from 0.05 to 0.4 M $(NH_4)_2SO_4$ were analyzed by a gel retardation assay (●) and by a protein blot assay (▲) as described in Materials and Methods. Fractions 4 to 10 contained the peaks of the DNA-binding activities. The protein concentrations of these fractions are also shown (■). For the gel retardation assay, the labeled VA1 gene fragment was used with 3 μl of each fraction. For the protein blot assay, 70 μl of each fraction was loaded onto the gel, and the labeled pArg gene fragment was used as a probe.

was also enriched approximately 2.5-fold from the Bio-Rex 70 step to the DEAE-cellulose step and 3.5-fold from the DEAE-cellulose step to the phosphocellulose step.

The copurification of the 150-kDa DNA-binding protein with TFIIIC activity suggested a possible association of this polypeptide with TFIIIC. To further test this possibility, the phosphocellulose-derived TFIIIC fraction G was rechromatographed on phosphocellulose. A linear salt gradient of 0.05 to 0.4 M $(NH_4)_2SO_4$ was used to elute the bound proteins. Fractions eluted from the column were assayed for DNA-binding activity by using the protein blot and gel retardation assays. We found that the 150-kDa DNA-binding activity coeluted from the phosphocellulose column with the specific DNA-binding activity of TFIIIC (Fig. 5).

Since the chromatographic procedures we used indicated an association between the 150-kDa polypeptide and TFIIIC, we examined the DNA-binding properties of this polypeptide in more detail. To assess the stability of these observed protein-DNA interactions, we examined the effect of ionic strength on the DNA-binding activities present in the phosphocellulose-derived TFIIIC fraction (fraction G) (Fig. 6). Using the protein blot assay, we examined the polypeptides in this fraction that would interact with a ^{32}P-labeled DNA fragment containing a $tRNA^{Arg}$ gene under conditions of different salt concentrations. When the filters were washed in a low-ionic-strength buffer, the DNA probe bound to many proteins. Increasing the salt concentration in the wash buffer to more than 200 mM eliminated most of the protein-DNA interactions, except for a major signal at 150 kDa. The DNA-binding activity of this protein was still detectable at 1 M KCl. This is consistent with the results of an analysis of preformed transcription factor-tRNA gene complexes in which TFIIIC was not dissociated from the template in the presence of 1 M KCl (8).

We next analyzed the specificity of the DNA-protein interactions (Fig. 7). A protein blot assay was done in which increasing amounts of unlabeled competitor DNA were added to each nitrocellulose filter in addition to the labeled tRNA gene fragment. We found that this preparation of TFIIIC contained two major DNA-binding proteins of 150 and 50 kDa. The 150-kDa DNA-binding signal was effectively eliminated with increasing amounts of competitor $tRNA^{Arg}$ gene contained in pBR322, whereas no competition

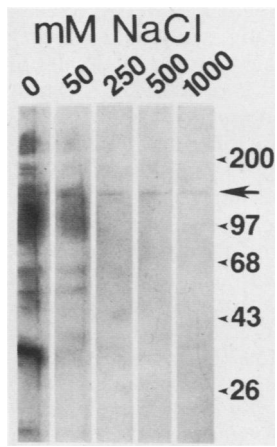

FIG. 6. Effect of ionic strength on the binding of proteins to a tRNA gene fragment. A protein blot analysis was carried out as described in Materials and Methods with the TFIIIC phosphocellulose-derived fraction. After incubation of the protein-bound nitrocellulose filters with DNA probe, the filters were then washed in the same buffer containing the salt concentrations indicated. The filters were exposed to film for 3 days with an intensifier screen. The indicated molecular mass standards are as described in the legend to Fig. 4.

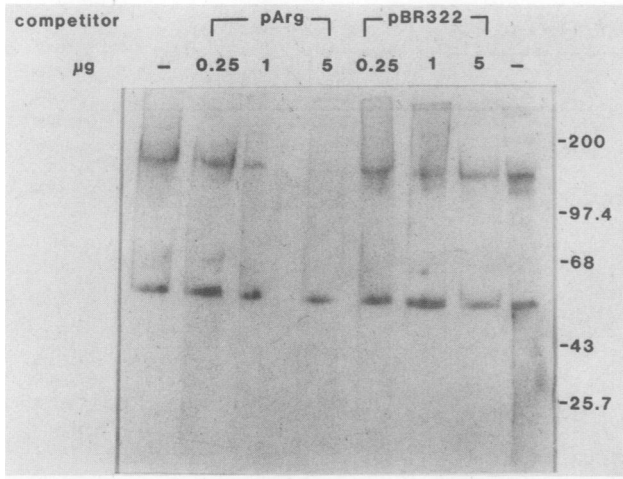

FIG. 7. Competition analysis of the DNA-binding activities. Equal amounts of TFIIIC containing fraction G were used in each lane. The protein blot assay was carried out by using the $tRNA^{Arg}$ gene fragment as a probe. In addition, the filters were simultaneously incubated with various amounts of unlabeled (uncut) plasmid DNAs (shown at the top). pBR322 containing the $tRNA^{Arg}$ gene (left) or PBR322 alone (right) were used. The indicated molecular mass standards are as described in the legend to Fig. 4.

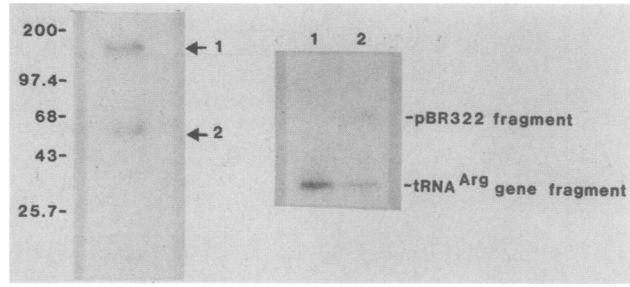

FIG. 8. Examination of sequence preference of the DNA-binding activities. Protein blots were performed, and the filters were incubated with equal molar amounts of two labeled DNA probes, the *Hin*dIII-$tRNA^{Arg}$ gene fragment and a *Hin*dIII-pBR322 fragment. The indicated molecular mass standards are as described in the legend to Fig. 4. After autoradiography and detection of the DNAs bound (left panel), the labeled DNAs bound to the 150- and 50-kDa proteins (1 and 2, respectively) were eluted from the two regions of the nitrocellulose filter by incubation of each radioactive band in 1 ml of 0.1% sodium dodecyl sulfate for 1 h. The filter was removed, 100 μg of carrier RNA and 50 μl of 5 M NaCl were added, and the DNA fragments were precipitated overnight with 3 volumes of ethanol at −20°C. The precipitated DNAs were suspended and fractionated on 1% agarose gel. The gel was dried and autoradiographed for 5 days (right panel), and the amounts of separated fragments originally bound to the 150- and 50-kDa proteins (lanes 1 and 2, respectively) were determined.

was observed with pBR322 as the competitor DNA. Neither DNA competed with the DNA-binding activity of the 50-kDa protein at these concentrations of competitor DNA. At fivefold-higher concentrations of competitor DNAs, both DNAs effectively eliminated the 50-kDa DNA-binding signal (data not shown). These results show that the 150-kDa protein interacts specifically with tRNA genes, whereas the 50-kDa protein binds nonspecifically to DNA.

To further test the DNA-binding specificity of the 150- and 50-kDa proteins, we asked whether either protein would bind preferentially to a DNA fragment containing a tRNA gene or to a fragment of vector DNA. If the protein bound in a sequence-specific manner, we would expect a greater amount of the tRNA gene fragment to be bound relative to the vector DNA fragment. On the other hand, if the protein bound nonspecifically, we would expect equal amounts of the tRNA gene fragment and vector fragment to be bound. When these two ^{32}P-labeled DNA probes were incubated with the electrophoretically separated polypeptides bound to nitrocellulose, both 150- and 50-kDa DNA-binding proteins were detected (Fig. 8). The bound DNAs were eluted from each region of the nitrocellulose, and the fragments were separated on agarose gels. Analysis of the bound fragments revealed that approximately 90% of the total DNA bound to the 150-kDa protein was the tRNA gene-containing fragment. The 50-kDa protein showed a 60:40 ratio of bound tRNA gene to bound vector DNA. These results further establish that the 150-kDa protein is highly specific for tRNA genes. The high degree of specificity exhibited by the DNA-binding activity in the 150-kDa region also suggests that it is the predominant (if not the only) active DNA-binding protein that migrates in this region of the gel. The 50-kDa protein, on the other hand, bound approximately equal amounts of the DNA fragments, demonstrating that its binding is nonspecific.

DISCUSSION

TFIIIC was originally described as a protein fraction required to reconstitute specific RNA polymerase III gene transcription together with another fraction, TFIIIB, and RNA polymerase III (21). The subsequent demonstration that TFIIIC binds specifically to DNA has allowed its activity to be measured by other assays which detect protein-DNA complexes (1, 28). The advantage of these assays is that they depend simply on the ability of TFIIIC to bind to DNA, whereas reconstitution of transcription depends on additional protein-protein and protein-DNA interactions. In the present study we used a gel retardation assay to follow the activity of TFIIIC through several steps of chromatography on ion-exchange columns. We find this assay to be a reliable measure of TFIIIC activity. We have subsequently used this assay for determining apparent equilibrium constants for the binding of TFIIIC to tRNA genes and to tRNA genes with mutations within the specific DNA-binding sites (unpublished results).

We have also used another DNA-binding assay to gain more information about the nature of the TFIIIC protein. Protein-blotting procedures have been useful both for examining the DNA-binding functions of proteins of known subunit structures (26) and for identifying polypeptides responsible for sequence-specific interactions (15). In the study reported here we used this approach to determine the molecular mass of a DNA-binding polypeptide that is probably a subunit of yeast TFIIIC. This polypeptide is approximately 150 kDa in size and copurifies with TFIIIC activity. That it coelutes on a phosphocellulose column gradient with the DNA-binding activity of TFIIIC corroborates the view that it is associated with TFIIIC. This polypeptide interacts specifically with tRNA genes and does so with high affinity. These results provide the first evidence for the size of the DNA-binding subunit of yeast TFIIIC.

Despite the efforts of a number of investigators, TFIIIC has not yet been purified to homogeneity by any system. The human TFIIIC fraction appears to be composed of two or more nonidentical subunits, since it can be separated into two fractions (TFIIIC1 and TFIIIC2) that can be reconstituted in transcription assays (28). TFIIIC2 was shown to

contain a DNA-binding activity which interacts with the 3′ ICR of adenovirus VA1 DNA by DNase 1 protection analysis. The addition of the TFIIIC1 fraction to the reaction extends the footprint over the 5′ ICR. Whether TFIIIC1 interacts directly with this region or indirectly facilitates TFIIIC2 binding to the 5′ ICR is not yet known. Sedimentation analysis revealed that the molecular masses of human TFIIIC2 and TFIIIC1 are 400 to 500 and approximately 200 kDa, respectively (2). Further studies involving UV-cross-linking of a 5-bromo-2′-deoxyuridine-containing VA1 DNA probe with TFIIIC2 indicated that the polypeptide interacting with this gene is approximately 250 kDa in mass.

In *S. cerevisiae*, TFIIIC has been shown to have a molecular mass of 300 kDa (18, 23). This large protein complex bound specifically to DNA has been visualized by electron microscopy (23). Partial purification of the protein by Ruet et al. (18) revealed that three polypeptides with molecular masses of approximately 146, 144, and 100 kDa copurified with TFIIIC activity. The approximately 150-kDa DNA-binding protein we detected in *S. cerevisiae* is, therefore, consistent with those results. Other preparations of yeast TFIIIC that have been partially purified by different procedures (D. Riggs and P. Geiduschek, unpublished results) also contain a 150-kDa DNA-binding activity. Taken together, these results suggest that the DNA-binding function of yeast TFIIIC is contained in a protein complex composed of at least two identical or nonidentical subunits.

Limited proteolysis of *S. cerevisiae* TFIIIC-tRNA gene complexes and subsequent analysis by a gel retardation assay revealed that a smaller, specific complex is maintained (14). Analysis of the larger unproteolyzed complex by DNase I protection showed that TFIIIC bound to both the 5′ and 3′ ICRs. The smaller proteolyzed complex did not bind to the 5′ ICR of the gene, but binding to the 3′ ICR was maintained. These results show that the "core" 3′-ICR-binding function can be physically separated from a distinct 5′-ICR-binding domain. It is conceivable that the proteolysis releases a protein fragment analogous to the human TFIIIC1 and that the remaining core protein is the equivalent of TFIIIC2. Whether these two DNA-binding domains are located within a single polypeptide remains to be established. The inability so far to resolve yeast TFIIIC into more than one fraction by column chromatography (as can be done with its human counterpart) suggests that if there are two distinct polypeptides, they are tightly associated. In any case, yeast TFIIIC appears to be a considerably smaller protein than human TFIIIC.

The direct interaction of TFIIIB with TFIIIC is important in the formation of productive transcription complexes (3). Given this function of TFIIIC in the transcription process and its DNA-binding properties, TFIIIC appears to contain a minimum of three functional domains responsible for TFIIIB interactions, 5′-ICR binding, and 3′-ICR binding. Our studies strongly suggest that in *S. cerevisiae* at least one of the DNA-binding domains is contained within a 150-kDa protein and that this subunit alone is capable of a highly specific DNA interaction. Further work will be necessary to determine whether any of the other domains necessary for TFIIIC function are contained within this polypeptide.

Detailed characterization of the TFIIIC protein is critical for our understanding of its role in controlling the expression of a wide variety of cellular genes. Determination of the molecular mass of a TFIIIC polypeptide subunit will now greatly facilitate its further purification and the generation of specific probes for the isolation of the gene encoding this important protein.

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