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Proximal Sequence Element-Binding Transcription Factor (PTF) Is a Multisubunit Complex Required for Transcription of both RNA Polymerase II- and RNA Polymerase III-Dependent Small Nuclear RNA Genes

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The proximal sequence element (PSE), found in both RNA polymerase II (Pol II)- and RNA Pol III-transcribed small nuclear RNA (snRNA) genes, is specifically bound by the PSE-binding transcription factor (PTF). We have purified PTF to near homogeneity from HeLa cell extracts by using a combination of conventional and affinity chromatographic methods. Purified PTF is composed of four polypeptides with apparent molecular masses of 180, 55, 45, and 44 kDa. A combination of preparative electrophoretic mobility shift and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses has conclusively identified these four polypeptides as subunits of human PTF, while UV cross-linking experiments demonstrate that the largest subunit of PTF is in close contact with the PSE. The purified PTF activates transcription from promoters of both Pol II- and Pol III-transcribed snRNA genes in a PSE-dependent manner. In addition, we have investigated factor requirements in transcription of Pol III-dependent snRNA genes. We show that in extracts that have been depleted of TATA-binding protein (TBP) and associated factors, recombinant TBP restores transcription from U6 and 7SK promoters but not from the VAI promoter, whereas the highly purified TBP–TBP-associated factor complex TFIIIB restores transcription from the VAI but not the U6 or 7SK promoter. Furthermore, by complementation of heat-treated extracts lacking TFIIIC activity, we show that TFIIIC1 is required for transcription of both the 7SK and VAI genes, whereas TFIIIC2 is required only for transcription of the VAI gene. From these observations, we conclude (i) that PTF and TFIIIC2 function as gene-specific factors for PSE- and B-box-containing Pol III genes, respectively, (ii) that the form of TBP used by class III genes with upstream promoter elements differs from the form used by class III genes with internal promoters, and (iii) that TFIIIC1 is required for both internal and external Pol III promoters.

Mammalian small nuclear RNA (snRNA) genes contain related promoter structures, but some (class II) are transcribed by RNA polymerase II (Pol II), whereas others (class III) are transcribed by RNA Pol III (reviewed in references 8, 15, 23, 30, and 37). Class II snRNA genes (e.g., U1 to U5) contain two regulatory elements in the 5′-flanking region: a distal sequence element (DSE) located approximately 220 bp upstream of the transcription start site, and a proximal sequence element (PSE) centered around −55. The DSE contains at least one copy of the octamer motif and functions as an upstream enhancer, whereas the PSE is an essential promoter element which functions in start site selection and may be required for accurate 3′-end formation (17, 33, 35). The promoters of class III snRNA genes (e.g., 7SK and U6) are located solely in the 5′-flanking region and lack intragenic control elements typical of most other class III genes (9, 29). Like their class II snRNA gene counterparts, class III snRNA genes have similar DSE and PSE configurations but additionally contain a TATA-like sequence at position −25. The TATA box of class III snRNA genes is required for efficient transcription and functions as the major determinant of RNA Pol III specificity (25, 28).

The PSE sequence is found almost exclusively in the promoters of snRNA genes and is the only common essential promoter element through which both classes of snRNA genes can be coordinately regulated. PSE swapping experiments between U2 and U6 genes have demonstrated that different PSEs are functionally interchangeable and per se are not responsible for polymerase selection (25, 28, 35). It is likely, therefore, that the transcription factor(s) bound to the PSE elements directly interacts with both class II and class III transcription factors to assist in RNA polymerase selection.

PSE-binding activities have been recently identified in HeLa cells in several laboratories. We have shown that the PSE-binding transcription factor (PTF) binds specifically to the PSE sequences of class II and class III human snRNA genes and that its binding is greatly enhanced by adjacent binding of Oct-1 to the octamer site (31). In addition, PTF and Oct-1 have been shown to activate transcription from the 7SK promoter in an interdependent manner (31). Other laboratories have also shown that a PSE-binding activity is essential for transcription from the mouse U6 (mU6) (41) and human U1 (3, 38) promoters. A PSE-binding activity called SNAPc has been reported to be a TATA-binding protein (TBP)-containing complex distinct from the previously identified complexes SL1, TFIID, and TFIIIB (38). However, since the molecular compositions of PSE-binding activities from different laboratories have not been reported, it is not clear whether these factors are identical or whether there are multiple PSE-binding factors in the cell.

To characterize the polypeptide(s) associated with the PTF activity, we have purified PTF to apparent homogeneity. We demonstrate that the most purified human PTF protein is a stable complex of four polypeptides with apparent molecular

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masses of 180, 55, 45, and 44 kDa and that it binds to the PSE and activates transcription from both class II and class III snRNA genes in a PSE-dependent manner. We show that the largest subunit of PTF is in close contact with the PSE and is likely to be the major or sole DNA-binding subunit. In addition, we have tested the TBP complex requirements for both the 7SK and U6 promoters. We find that TBP, but not highly purified TFIIIB or TFIID, can support transcription from these promoters in TBP-depleted nuclear extracts. Furthermore, we demonstrate that TFIIIC1 is involved in transcription of the 7SK gene.

MATERIALS AND METHODS

HeLa cells and extracts. HeLa cells were maintained in Joklik minimal essential medium containing 5% bovine calf serum. Nuclear extracts and cytosolic S100 extracts were prepared as described previously (10).

Plasmids. O^+P^+ and O^+P^- 7SK maxigene templates were described previously (31). U1G-, U1G-repPSE, and HTFRG- (a kind gift from M. W. Knuth and R. R. Burgess) were described by Gunderson et al. (14).

EMSA. Probes for electrophoretic mobility shift analysis (EMSA) were prepared by end labeling double-stranded oligonucleotides with the Klenow enzyme and α-^{32}P-labeled deoxynucleoside triphosphates. Twenty femtomoles of probe was used in a 20-μl reaction mixture containing 4% Ficoll type 400, 20 mM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)-KOH (pH 7.9), 70 to 80 mM KCl, 1 mM spermidine, 100 μg of bovine serum albumin (BSA) per ml, 0.03% Nonidet P-40, 1 mM dithiothreitol (DTT), and 2 μg of poly(dI-dC). Reactions were initiated by the addition of protein, and mixtures were incubated for 20 min at 30°C. Samples were loaded directly onto a 4% (37.5:1, acrylamide/bisacrylamide) nondenaturing polyacrylamide gel with 0.5× Tris-borate-EDTA and 0.03% Nonidet P-40 and electrophoresed at 150 V for 2 h at room temperature.

Purification of PTF. HeLa S100 extracts (1.2 liters, 24 g of protein) were adjusted to a salt concentration of 250 mM KCl and subjected to centrifugation at 40,000 rpm for 45 min in a Beckman 45 Ti rotor to facilitate the removal of lipids. The clarified extract was then applied to a 350-ml phosphocellulose column equilibrated with buffer BC (20 mM Tris-HCl [pH 7.9], 20% [vol/vol] glycerol, 0.2 mM EDTA, 0.03% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 5 mM DTT) containing 250 mM KCl (BC250). The column was washed with BC275, and proteins were eluted with BC600. After dialysis to 150 mM KCl, this fraction (830 mg of protein) was applied to a 50-ml Q-Sepharose FF column equilibrated with BC150. The column was washed with BC200, and proteins were eluted with BC300. The column eluates (140 mg of protein) were dialyzed to 120 mM KCl and loaded onto a 20-ml double stranded DNA-cellulose column equilibrated with BC120. The DNA-cellulose flowthrough fraction was directly loaded to a 7-ml Q-Sepharose FF column equilibrated with BC120. Proteins were eluted with BC500. Active fractions (70 mg of protein) were dialyzed to 100 mM KCl and applied to a 1.2-ml octamer-PSE (7SK) DNA affinity column which was saturated with the recombinant Oct-1 POU domain. After the column was washed with loading buffer and then with BC250, PTF was eluted with BC600. Active fractions were dialyzed against BC100 and loaded onto a 0.3-ml PSE (mU6) DNA affinity column equilibrated with the same buffer. The column was washed with BC250, and proteins were eluted with a 1.2-ml linear gradient from 250 to 600 mM KCl. PTF eluted around 350 mM KCl. All procedures were carried out at 4°C. PTF activity was monitored throughout purification by a gel shift assay with an end-labeled *Hin*dIII restriction fragment of O^+P^+ as a probe except that for the DNA affinity steps, an oligonucleotide probe (nucleotides −79 to −39 of the mU6 gene) was used as a probe.

Bacterial expression of Oct-1 and the POU domain of Oct-1. Recombinant Oct-1 and the POU domain of Oct-1 were produced in bacteria as described previously (31).

In vitro transcription. Transcription reactions for adenovirus VAI and human 7SK genes were performed for 1 h at 30°C in a 25-μl reaction volume with 8 μl of HeLa cell nuclear extract (8 mg/ml) and 200 to 400 ng of 7SK and 4 to 25 ng of VAI supercoiled DNA templates. The standard reaction mixtures contained 10 mM HEPES-KOH (pH 7.9), 70 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 500 μM each ATP, CTP, and UTP, 25 μM GTP, 2.5 μCi of [α-^{32}P]GTP (3,000 Ci/mmol), 5 mM creatine phosphate, and 2 μg of α-amanitin per ml. Following incubation, 200 μl of stop mix (10 mM Tris-HCl [pH 7.8], 1 mM EDTA, 1.1 M ammonium acetate, 100 μg of yeast tRNA per ml) was added, and the mixture was extracted with phenol-chloroform and ethanol precipitated. The precipitates were resuspended in 90% formamide–10 mM EDTA–0.1% bromophenol blue–0.1% xylene cyanol, heat denatured, and analyzed on 6% polyacrylamide gels with 8 M urea.

Transcription reactions from the U1G- and HTFG- templates were performed in a final volume of 25 μl containing 11 μl of HeLa nuclear extract, 20 mM HEPES-KOH (pH 7.9), 5.0 mM DTT, 1.0 mM 3′-*O*-methyl GTP, 500 μM ATP, 500 μM CTP, 0.26 μM [α-^{32}P]UTP (20 μCi), 2.0 U of RNase T_1, and 1.0 μg of a supercoiled template. Transcription proceeded for 90 min at 30°C, and RNA products were extracted and analyzed as described above.

UV cross-linking. DNA fragments containing either a wild-type or mutated 7SK PSE sequence linked to an octamer sequence were cloned into M13 mp18. Single-stranded DNA was prepared, and the forward sequencing primer for M13 was used to prime synthesis of radiolabeled, 5′-bromodeoxyuridine (BrdU)-substituted double-stranded DNA as described previously (1), using 5 mM BrdU–5 mM TTP for the extension reaction. Cleavage by the appropriate restriction endonucleases released the desired DNA fragments, which were then purified by polyacrylamide gel electrophoresis (PAGE). The probes obtained corresponded to the O^+P^+ and O^+P^- probes described by Murphy et al. (31). For cross-linking studies, approximately 20 fmol of each probe was used in each 20-μl DNA binding reaction mixture prepared as described for EMSA except that no Ficoll was added. After incubation at 30°C for 1 h, the open tubes were irradiated for 30 min, using an upturned UVP TM-36 transilluminator (320 nm) on the high setting, at a distance of 5 cm, in a cold room. Five microliters of the reaction mixture was then loaded onto a polyacrylamide gel for EMSA. The remainder of the reaction mixture was processed as described previously (1) and loaded on a sodium dodecyl sulfate (SDS)–7.5% polyacrylamide gel. The gel was dried after electrophoresis and autoradiographed. Ten femtomoles of Oct-1 purified from HeLa cells as described by Pierani et al. (36) was added to the appropriate reaction mixtures. Five to 10 fmol of PTF (Q-Sepharose FF column fraction) was added to the appropriate reaction mixtures.

Depletion of PTF and TBP from HeLa nuclear extracts. To deplete PTF, nuclear extract was incubated twice with mU6 PSE affinity resin prewashed with BC100 for 1 h at 4°C with constant mixing. After depletion, the resin was removed by centrifugation and the supernatant was used in transcription reactions.

To deplete TBP, 300 μl of anti-TBP antiserum was incubated with 100 μl of protein A-Sepharose (Oncogene Science) for 2 h at 4°C with constant mixing. After three washes with BC100 and three with BC400, the resin was incubated for 2 h with 300 μl of HeLa nuclear extract adjusted to 400 mM KCl. After centrifugation, the supernatant was dialyzed against BC100, and aliquots were quickly frozen in liquid nitrogen.

Sedimentation velocity gradient analysis. mU6 PSE affinity peak fractions were dialyzed against buffer BC300 with 10% glycerol. One-hundred-microliter aliquots of the samples were analyzed in 4 ml of glycerol gradients containing 15 to 40% glycerol in buffer BC300 by centrifugation for 20 h at 45,000 rpm in a Beckman SW60 rotor at 4°C. Molecular weight markers were sedimented in a parallel gradient. Fractions were collected dropwise from the bottom and assayed for PTF activity by EMSA.

Heat treatment of HeLa nuclear extract. Heat-treated nuclear extract was prepared by incubating 250-μl aliquots of extract at 47°C for 15 min as described previously (32). Aliquots were quickly frozen in liquid nitrogen.

Preparation of DNA affinity resins. To prepare an octamer-PSE (7SK) affinity column, the following 5′ phosphorylated oligonucleotides were synthesized: OCTA-PSE (7SK)-1, 5′-CAGCACCTTATTTGCATTTATTTGCATAAGC-3′; OCTA-PSE (7SK)-2, 5′-GAACAACTTGACCTAAGTGTAAAGTTGAGA-3′; OCTA-PSE (7SK)-3, 5′-GGTGCTGTCTCAACTTTACACTTAGGTCAA-3′; and OCTA-PSE (7SK)-4, 5′-TTGTTCGCTTATGCAAATAAATGCAAATAA-3′. The single-stranded oligonucleotides were gel purified, annealed, ligated, and coupled to Sepharose CL-2B (Pharmacia) as described by Kadonaga and Tjian (19). mU6 PSE affinity resin was prepared with the following oligonucleotides: PSE (mU6)-1, 5-TGTGAAGGAAACTCACCCTAACTGTAAAGTAAT-3′; and PSE (mU6)-2, 5′-CACAATTACTTTACAGTTAGGGTGAGTTTCCTT-3′. The oligonucleotides were processed as described above.

RESULTS

Purification of PTF. We have previously shown by gel shift assay that PTF can form an Oct-1 · PTF · DNA complex with a DNA fragment containing closely juxtaposed octamer and 7SK PSE sequences and that partially purified PTF can complement a HeLa nuclear extract depleted of PTF in an in vitro transcription assay (31). In the early stages of purification, we used the gel shift assay to monitor the PTF activity, but as shown below, purified PTF was also active in an in vitro transcription assay. A number of PTF preparations were analyzed during the course of this study, and the purification scheme for a typical preparation is summarized in Fig. 1. Details of a representative purification are given in Materials and Methods. The essential features of the purification included the use of two ion-exchange chromatography columns, phosphocellulose (P11) and Q-Sepharose FF, a nonspecific DNA affinity column (DNA-cellulose), and two specific DNA affinity columns. Although the recovery of PTF activity from the DNA-cellulose column was low (50 to 60%), it proved to be a key

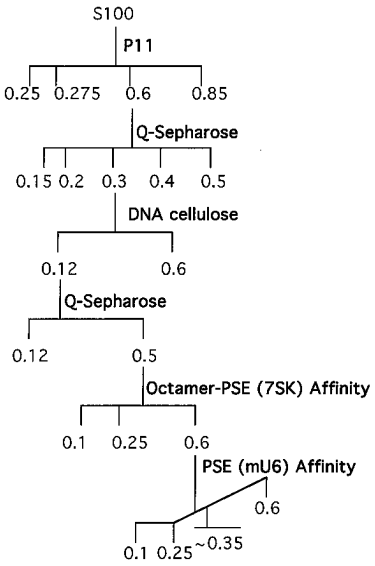

FIG. 1. Scheme for purification of PTF. See text for details.

purification step since most of the PTF activity was in the flowthrough fraction whereas nonspecific DNA-binding proteins were retained on the column. A preparation obtained by using a scheme without the DNA-cellulose step contained a number of contaminants, as judged by SDS-PAGE analysis (data not shown).

In designing the first DNA affinity column, we took advantage of the fact that the POU domain of Oct-1 (or Oct-2) bound to an octamer site potentiates the binding of PTF to the 7SK PSE, which normally is a weak binding site for PTF alone. Therefore, a DNA affinity column containing an octamer and the 7SK PSE was prepared (31) and saturated with recombinant Oct-1 POU domain before the PTF activity from the DNA-cellulose column was loaded. Active PTF fractions from the first DNA affinity column were further purified on a second DNA affinity column containing the mU6 PSE site, which itself binds PTF efficiently without adjacently bound Oct-1 (31, 41). PTF bound to the second affinity column was eluted with a linear KCl gradient. Figure 2A shows a silver-stained SDS-polyacrylamide gel of the collected fractions. The PSE-binding activity of each fraction was measured by gel shift assay using an oligonucleotide probe containing the mU6 PSE rather than a probe with an octamer and the 7SK PSE, since the input contained a high concentration of recombinant POU domain which could complicate the Oct-1 · PTF supershift assay. In fact, excess recombinant POU domain in the input bound nonspecifically to the mU6 PSE probe (Fig. 2B, lane 1). Gel shift analysis demonstrated that the PSE-binding activity was present maximally in fractions 7 and 8 (Fig. 2B).

To assay the transcriptional activity of PTF in the eluates, PTF was selectively depleted from HeLa nuclear extracts with DNA affinity resin carrying the mU6 PSE; the depleted extracts were then complemented with the affinity column fractions (Fig. 2C). A mutation in the PSE of the 7SK gene lowered the transcription to the basal level in a mock-depleted extract (Fig. 2C, lanes 1 and 2) as well as in untreated extracts (data not shown). Both wild-type PSE- and mutant PSE-containing templates were transcribed at the same basal level in PTF-depleted extracts (Fig. 2C, lanes 3 and 4), indicating that PTF was efficiently depleted from the extracts. Complementa-

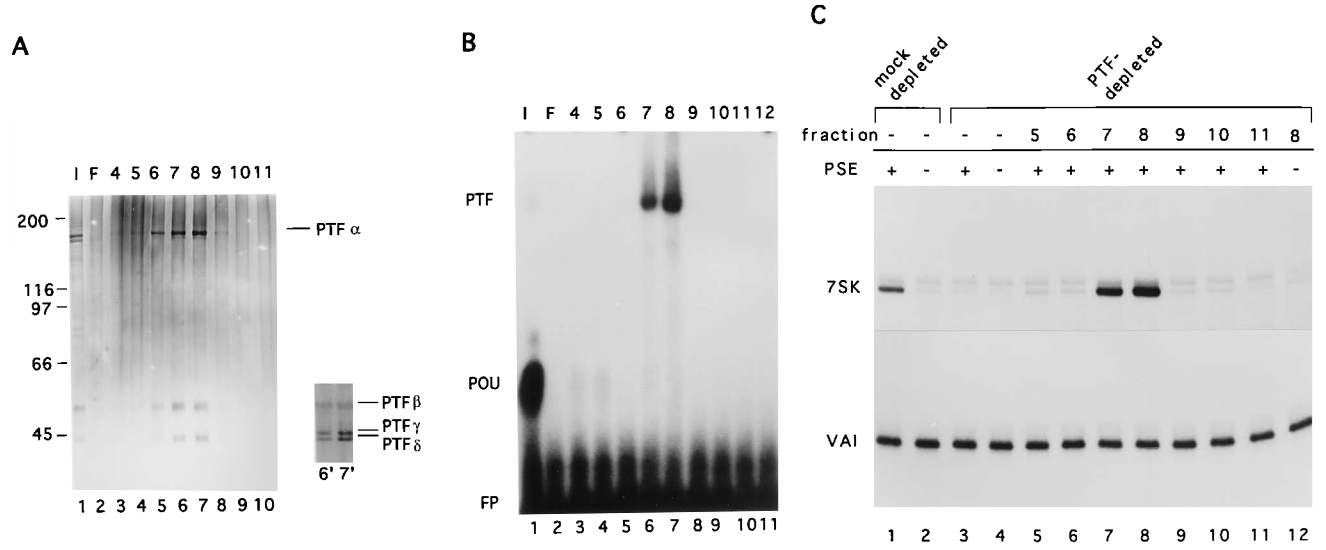

FIG. 2. Coelution of four polypeptides with DNA-binding and transcription activities during mU6 PSE affinity chromatography. (A) SDS-PAGE analysis of mU6 PSE affinity fractions. Polypeptides in various fractions (5 μl of each) were separated by SDS-PAGE and visualized by silver staining. Lane I, input to the affinity column; lane F, flowthrough from the affinity column. The remaining lanes are fractions that eluted from the column between 250 and 600 mM KCl. Numbers at the left indicate the positions (in kilodaltons) of molecular weight markers. In lanes 6′ and 7′, the protein samples in fractions 7 and 8 were analyzed on an SDS–10% polyacrylamide gel instead of a 7.5% gel to show separation of PTFγ and PTFδ. PTF subunits are indicated at the right. (B) EMSAs using affinity column fractions. EMSAs were performed with 1 μl of each of the fractions represented in panel A. The positions of the PTF · PSE complex (PTF) and the nonspecific complex containing Oct-1 POU (POU) are indicated. FP indicates the position of the free probe. The DNA probe contains mU6 sequences from −79 to −39. (C) In vitro transcription assay of the 7SK gene with affinity column fractions. One microliter of each of the same column fractions was assayed for the ability to direct 7SK gene transcription in PTF-depleted HeLa nuclear extract as described in Materials and Methods. The adenovirus VAI gene was included as an internal control for general transcription factor activity. Positions of the 7SK and VAI transcripts are indicated at the left. The 7SK templates used are indicated above the lanes. PSE + and PSE − represent O^+P^+ and O^+P^-, respectively. The upper portion of the gel containing 7SK transcripts was exposed for 2 h, and the lower portion containing VAI transcripts was exposed for 5 min to prevent overexposure.

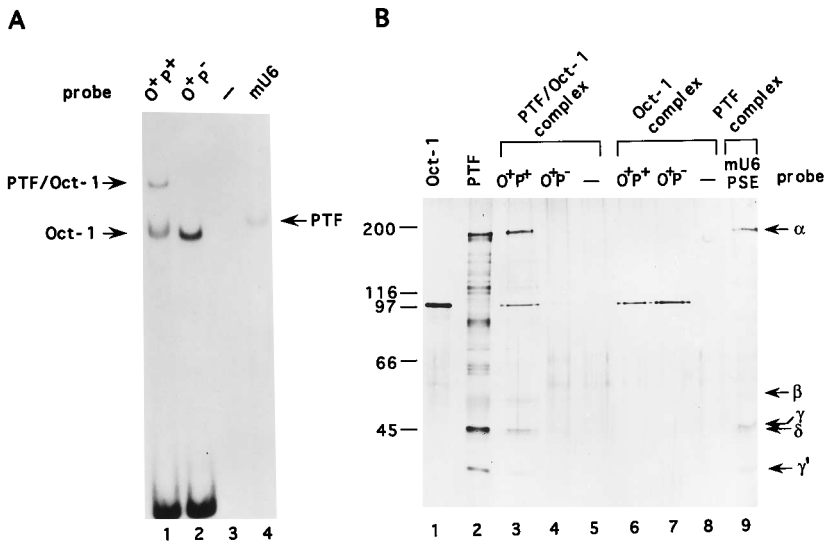

FIG. 3. PTF is composed of four polypeptide subunits. (A) EMSA using various DNA probes. Recombinant Oct-1 (panel B, lane 1) and partially purified PTF (panel B, lane 2) were analyzed with the DNA probe indicated above each lane. The nondenaturing EMSA gel was exposed for 4 min without drying. The positions of specific protein-DNA complexes containing either PTF · Oct-1, Oct-1, or PTF are indicated. (B) SDS-PAGE analysis of PTF and PTF · Oct-1 DNA complexes. EMSA gel pieces corresponding to PTF · Oct-1 complex (lanes 3 to 5), Oct-1 complex (lanes 6 to 8), or PTF complex (lane 9) were loaded on an SDS–10% polyacrylamide gel after brief treatment with a twofold-concentrated SDS gel sample buffer. In lanes 1 and 2, recombinant Oct-1 and partially purified PTF used for EMSA in panel A, respectively, were analyzed. The DNA probes used are indicated above lanes 3 to 9. The positions (in kilodaltons) of molecular size markers are indicated at the left. PTF subunits are indicated at the right. PTFγ′ (36-kDa polypeptide) is likely a degradation product of PTFγ because of its variable appearance in different preparations of PTF and because its peptide sequence is related to that of PTFγ.

tion with column fractions showed that the peak of transcriptional activity was present in fractions 7 and 8 (Fig. 2C). Transcriptional activation by PTF was dependent on the PSE site, since the mutant template was inactive (Fig. 2C, lanes 4 and 12). Importantly, transcription of an adenovirus VAI gene included as an internal control was not affected by either depletion or addition of PTF. Comparison of the protein profiles across the gradient with the DNA-binding and transcription activities revealed that four polypeptides coeluted with both activities. These polypeptides migrated with apparent molecular masses of 180, 55, 45, and 44 kDa on a 7.5% polyacrylamide gel. Although the 45- and 44-kDa polypeptide doublet appeared as a single band on a lower-percentage (7.5%) gel, they were all separated on higher-percentage (10% and up) gels (Fig. 2A, lanes 6′ and 7′). The 55-kDa polypeptide band often stained negatively with silver (see also Fig. 3).

PTF is a complex of four polypeptide subunits. Extensive efforts to renature PTF activity from polypeptides in SDS-polyacrylamide gel slices failed. We therefore examined whether these four polypeptides were present in the specific PTF · DNA complexes formed with recombinant Oct-1 and partially purified PTF, using either the O^+P^+ or O^+P^- DNA probe. Protein · DNA complexes were first separated by electrophoresis in a 4% nondenaturing polyacrylamide gel (Fig. 3A). After locating the complexes by brief autoradiography, we subjected proteins in the gel slices corresponding to the upper and lower complexes to SDS-PAGE analysis (Fig. 3B). The lower complexes formed on both O^+P^+ and O^+P^- probes contained only Oct-1 (lanes 6 and 7). Oct-1 without DNA present did not migrate to the same position in the gel (lane 8). The supershifted complex formed on the O^+P^+ probe contained, in addition to Oct-1, all four polypeptides present in the most purified PTF preparation and one additional polypeptide of 36 kDa (lane 3). The polypeptides present in the supershift complex were specific to the PSE site and did not fortuitously migrate to that particular position of the nondenaturing gel, since these proteins were not detected with the O^+P^- probe or with no DNA probe (Fig. 3B, lanes 4 and 5). Since only some of the PTF preparations contained the polypeptide of 36 kDa, this polypeptide is likely a degradation product of one of the PTF subunits (see also Discussion). When the mU6 PSE sequence was used as a probe, the same set of polypeptides were detected in the PTF · DNA complex (Fig. 3B, lane 9). These results strongly suggested that the four polypeptides were bona fide PTF subunits.

Native size of PTF. To determine the native molecular mass of the PTF complex and to test whether 180-, 55-, 45-, and 44-kDa polypeptides were associated as a single complex in solution, affinity-purified PTF was subjected to sedimentation through glycerol gradients and monitored by gel shift assay (Fig. 4). PTF activity was detected in fractions 16 to 20, with a peak (fraction 18) corresponding to a sedimentation coefficient of 9S; assuming a globular conformation, this corresponds to estimated molecular mass of 200 kDa. Importantly, SDS-PAGE analysis of fraction 18 demonstrated the presence of all four polypeptides (180, 55, 45, and 44 kDa) in that fraction (data not shown). These results indicate these four polypeptides are subunits of PTF.

Highly purified PTF activates transcription from both RNA Pol II- and RNA Pol III-dependent snRNA genes. We have previously shown that partially purified PTF specifically binds to the PSE sequence of both RNA Pol II- and RNA Pol III-transcribed snRNA genes (31). In addition, other laboratories have reported that fractions containing PSE-binding activities activate Pol II-dependent transcription from U1 and U2 snRNA genes as well as the Pol III-dependent U6 gene (3, 38, 41). To investigate whether the same protein species is responsible for transcription from both Pol II- and Pol III-transcribed snRNA genes, we assayed the transcription activity of highly purified PTF in human U1 gene transcription. As shown in Fig. 5, the specific transcription of the U1 snRNA gene is dependent on the PSE site, since a PSE mutant abol-

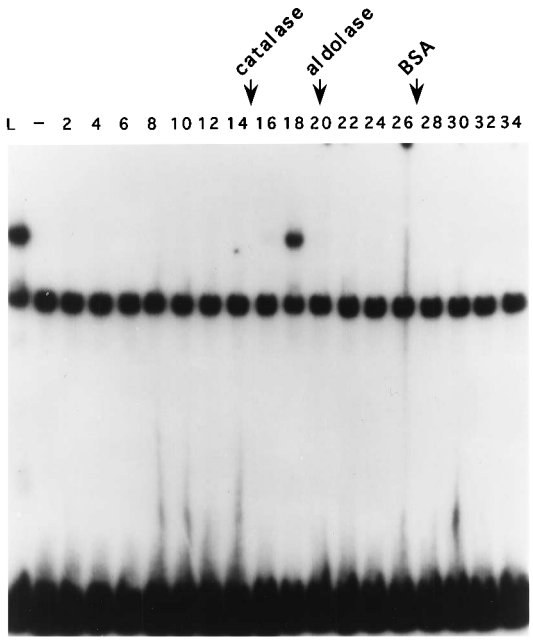

FIG. 4. Glycerol gradient analysis of the purified PTF. Affinity-purified PTF was loaded onto a 15 to 40% glycerol gradient. Molecular size markers were sedimented in a parallel gradient. After centrifugation for 20 h at 45,000 rpm in a Beckman SW60 rotor, fractions were collected dropwise from the bottom and measured for PTF activity by EMSA using the end-labeled *Hin*dIII restriction fragment of O^+P^+ as a probe. Lane L, loading material for the gradient; lane –, EMSA with recombinant Oct-1 alone. The remaining lanes are gradient fractions from the bottom (fraction 2) to the top (fraction 34). The peaks of size marker proteins (catalase, 240 kDa; aldolase, 158 kDa; and BSA, 68 kDa) are indicated.

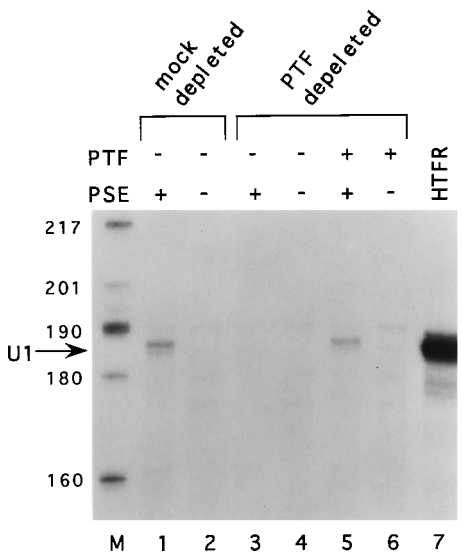

FIG. 5. Affinity-purified PTF activates U1 snRNA gene transcription. In vitro transcription reactions were performed as described in Materials and Methods with either the wild-type U1 plasmid (U1G-; lanes 1, 3, and 5) or the mutant U1 plasmid (U1G-repPSE; lanes 2, 4, and 6) in either mock-depleted (lanes 1 and 2) or PTF-depleted (lanes 3 to 6) extract. Purified PTF (1 μl of fraction in Fig. 2A) was added to transcription reaction mixtures in lanes 5 and 6. Lane 7 is a control using a human transferrin receptor (HTFR) promoter G-less cassette template designed to generate similar-size transcripts. Marker lane M is derived from ^{32}P end labeling of *Msp*I-digested pBR322, with the sizes listed in nucleotides at the left. The arrow indicates the position of U1 transcripts. The U1 transcript was sensitive to 2 μg of α-amanitin per ml (data not shown).

ished U1 transcription in a mock-depleted extract (lanes 1 and 2). Depletion of PTF with the mU6 PSE resin reduced the level of transcription from the U1 promoter (lanes 1 and 3). U1 transcription was restored by addition of highly purified PTF (fraction 8 in Fig. 2A), and transcriptional activation by PTF was dependent on the intact PSE site (lanes 5 and 6). These results strongly suggest that the same PTF complex is used for both Pol II- and Pol III-dependent transcription of snRNA genes.

The largest subunit of PTF is in close contact with PSE. To determine which subunit of PTF is directly involved in specific binding to PSE, UV cross-linking studies were performed. A PTF fraction was incubated with a radiolabeled, BrdU-substituted DNA fragment containing either a wild-type (O^+P^+) or mutant (O^+P^-) 7SK PSE sequence adjacent to an octamer site. Some reactions also included a 50-fold molar excess of competitor DNA containing either a wild-type (O^+) or a mutant (O^-) octamer site. Following binding, the reaction mixture was irradiated with UV light. One portion of the reaction mixture was analyzed by gel shift assay (Fig. 6A), and the rest was subjected to SDS-PAGE analysis after treatment with DNase I and micrococcal nuclease (Fig. 6B). The formation of an Oct-1 · PTF · DNA supershift complex with the cross-linker-substituted probe was as efficient as that with the nonsubstituted probe and was unaffected by UV irradiation (Fig. 6A, lane 3). Formation of the supershift complex was dependent on both PTF and the PSE, since the complex was not detected in the absence of the PTF fraction (lane 2) or with the O^+P^- probe (lane 8). Both Oct-1 and Oct-1 · PTF gel shift complexes were efficiently competed for with an oligonucleotide containing the wild-type octamer (O^+) but not with one containing the mutant site (O^-) (lanes 4 and 5). As shown in Fig. 5B, a polypeptide with an apparent molecular mass of 180 kDa was cross-linked to the O^+P^+ probe (Fig. 6B, lane 3). Under the conditions used, Oct-1 was not efficiently cross-linked (lane 2). The cross-linking of 180-kDa polypeptide correlated well with the formation of the supershifted complex. Thus, it was dependent on the presence of PTF and the PSE (lanes 2 and 8) and was competed for only with an excess of a specific competitor (lanes 4 and 5). Cross-linking studies using the mU6 PSE probe gave similar results (data not shown). These results indicate that the 180-kDa polypeptide of PTF is the major, if not the exclusive, DNA-binding subunit.

TBP requirements for Pol III snRNA gene transcription. Recent studies have shown that TBP is involved in transcription by all three RNA polymerases as a component of SL1, TFIID, and TFIIIB, which are distinct TBP–TBP-associated factor (TAF) complexes necessary for transcription from Pol I, Pol II, and Pol III promoters, respectively (reviewed in references 12 and 16). These TBP-containing complexes have been recently isolated from HeLa cells by epitope-tagging methods (6, 48). As a first step toward understanding the mechanism of differential RNA polymerase selection in snRNA gene transcription, we determined which TBP complex participated in transcription from the class III snRNA promoters. TBP was depleted from HeLa nuclear extracts with polyclonal antibodies raised against human TBP. TBP and TBP-containing complexes were then tested for the ability to restore 7SK and VAI transcription to depleted extracts. As shown in Fig. 7A, depletion of TBP was confirmed by Western blotting (immunoblotting) analysis. Titration of depleted extracts indicated that about 90% of TBP was removed in TBP-depleted nuclear extracts compared with mock-depleted ones (data not shown). Depletion of TBP almost completely abolished transcription of both 7SK and VAI genes (Fig. 7B, lanes 1 and 2). Addition of

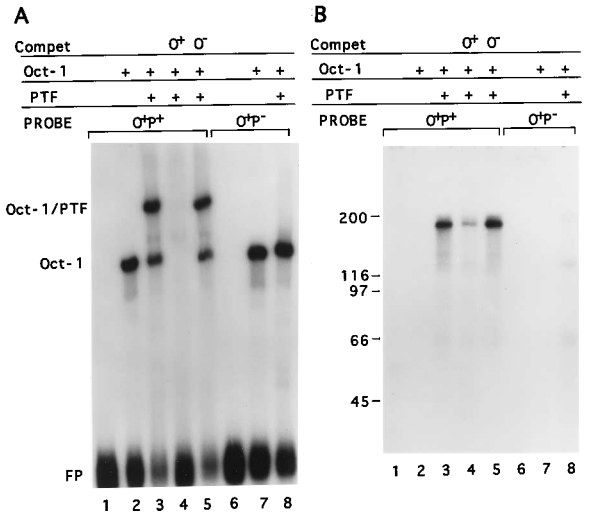

FIG. 6. The largest subunit of PTF directly contacts DNA. (A) EMSA following UV irradiation of binding reactions with BrdU-substituted probes. DNA probes homogeneously labeled and substituted with BrdU were prepared as described in Materials and Methods. The probe (O^+P^+ or O^+P^-) and factor (PTF or Oct-1) included in the reaction mixture are indicated above each lane. The binding reaction mixtures in lanes 4 and 5 also contained the unlabeled oligonucleotide competitors corresponding to the wild-type (O^+) and the mutant (O^-) octamer sequences, respectively. The binding reaction mixtures (20 μl each) were UV irradiated as described in Materials and Methods, and 5 μl of each reaction mixture was subjected to EMSA. (B) The rest (15 μl of each) of the binding reaction mixtures were treated with DNase I and micrococcal nuclease and subjected to SDS-polyacrylamide gel analysis. UV cross-linked proteins were visualized by autoradiography. The positions (in kilodaltons) of molecular size markers are indicated at the left.

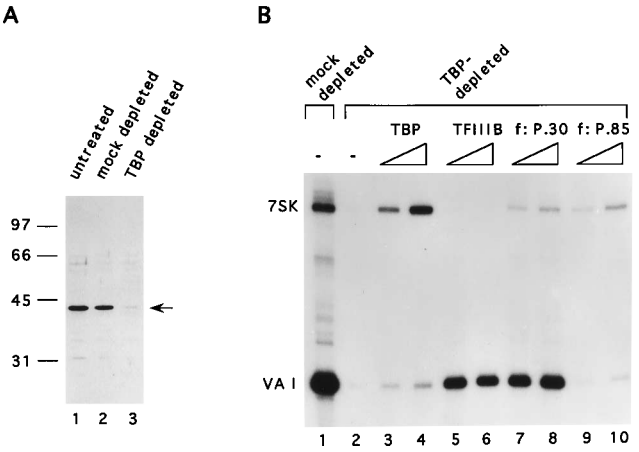

FIG. 7. Distinct TBP complex requirements for VAI and 7SK genes. (A) Immunodepletion of TBP from HeLa nuclear extracts. HeLa nuclear extracts (3 μl of each, 24 μg of protein) treated as indicated above each lane were separated by electrophoresis on an SDS–10% polyacrylamide gel and transferred to nitrocellulose. The blot was incubated with a 1:2,000 dilution of a rabbit antiserum raised against the recombinant human TBP. Antigen-antibody complexes were visualized as described in Materials and Methods. The positions (in kilodaltons) of protein molecular size markers are indicated at the left. The arrow indicates the position of TBP. (B) In vitro transcription of VAI and 7SK genes in a TBP-depleted HeLa nuclear extract. In vitro transcription reactions were performed with human 7SK (400 ng) and adenovirus VAI (4 ng) templates in either mock-depleted (lane 1) or TBP-depleted (lanes 2 to 10) HeLa nuclear extracts. Reaction mixtures in lanes 3 to 10 were supplemented with recombinant human TBP or TBP-containing complexes (2 ng of TBP in lanes 3, 5, 7, and 9; 6 ng of TBP in lanes 4, 6, 8, and 10) as indicated above the lanes. Recombinant human TBP was purified as described previously (18). Purification of epitope-tagged TBP complexes from 0.3 M (f: P.30) and 0.85 M (f: P.85) KCl P11 fractions was described previously (6). TFIIIB used in lanes 5 and 6 was purified from the HeLa S100 extract through five chromatographic steps (43). The positions of 7SK and VAI transcripts are indicated at the left.

increasing amounts of recombinant human TBP fully restored 7SK transcription but had a minimal effect on VAI transcription (lanes 3 and 4). In contrast, highly purified TFIIIB restored transcription from the VAI promoter but not transcription from the 7SK promoter (lanes 5 and 6). Similar results were obtained when each template was separately tested (data not shown). We have also tested transcription activity of epitope-tagged TBP-containing complexes derived from P11 0.3 M (f: P.30) and 0.85 M (f: P.85) KCl fractions (lanes 7 to 10). f: P.30, which contained TFIIIB and B-TFIID, restored transcription from the VAI promoter but not from the 7SK promoter; f: P.85, containing TFIID activity, did not restore transcription for either template. Residual transcription from the 7SK promoter by f: P.30 or f: P.85 is likely to be derived from either free epitope-tagged TBP in the preparations or from dissociation of TBP-containing complexes, since TFIID prepared by epitope tagging of one of the Pol II-specific TAFs was much less active in restoring 7SK transcription (data not shown). Similar results were obtained when the mU6 promoter was used (data not shown). These results indicate that neither TFIIIB nor TFIID alone can supply the TBP requirement for the 7SK and U6 genes.

TFIIIC1, but not TFIIIC2, is required for Pol III-dependent snRNA transcription. We further investigated factor requirements for 7SK transcription by using heat-treated nuclear extracts. Mild heat treatment of nuclear extracts has been used to inactivate components of both the Pol II and Pol III transcription machinery (22, 32). As shown in Fig. 8, preincubation of the extract at 47°C for 15 min completely abolished transcription from both the 7SK and VAI promoters (lanes 1 and 2). Complementation of the heat-treated extract with transcription factors necessary for 7SK (Oct-1, PTF, and TBP) and VAI (TFIIIC1, TFIIIC2, and TFIIIB [43, 47]) transcription partially restored the activity of both promoters (lane 3). To identify heat-labile components among the added transcription factors, individual factors were in turn omitted from the complementation assays (lanes 4 to 9). Under the conditions used, PTF, TFIIIC1, and TFIIIC2 activities proved to be heat labile, since deletion of each abolished or greatly reduced transcription from the 7SK and/or VAI genes (lanes 5, 8, and 9). Heat inactivation of PTF and TFIIIC2 selectively abolished transcription of the 7SK and VAI promoters, respectively (lanes 5 and 9), confirming our previous indications that TFIIIC2 is not involved in 7SK transcription (24) and that PTF is not involved in VAI transcription (31). Most importantly, depletion of the TFIIIC1 activity by heat treatment greatly reduced both 7SK and VAI transcription (lane 8). These results suggest that TFIIIC1 (43, 47) is a basic Pol III transcription factor that is essential for both external and internal promoter-containing class III genes, whereas PTF and TFIIIC2 are gene-specific factors for each subclass of genes. It may be noted that while the indicated factors (Fig. 8) could complement the heat-treated extract for 7SK transcription, they were not sufficient in themselves (data not shown); thus, there are still other factors, residual in the heat-treated extract, necessary for 7SK transcription.

DISCUSSION

PTF is a complex of four polypeptides. To better understand the mechanisms of snRNA gene transcription, we have purified an snRNA gene-specific transcription factor, PTF, to near

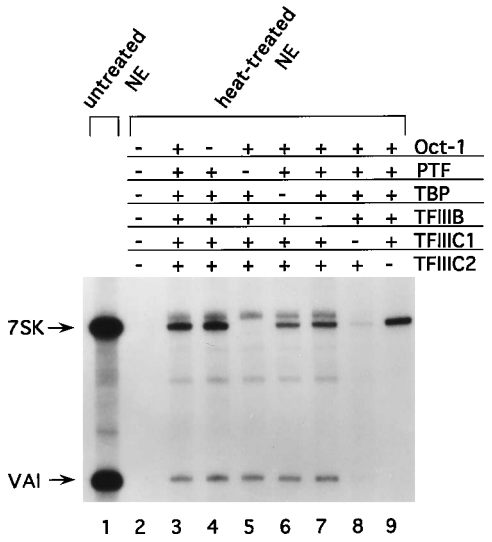

FIG. 8. Human 7SK gene transcription requires TFIIIC1 but not TFIIIC2. In vitro transcription reactions were performed with human 7SK (400 ng) and adenovirus VAI (4 ng) templates in either untreated (lane 1) or heat-treated (lanes 2 to 9) HeLa nuclear extracts (NE). Transcription reaction mixtures in lanes 3 to 9 were complemented with factors indicated above the lanes. TFIIIC1 was purified from the HeLa S100 extract by using P11 and Mono Q columns (43, 47). TFIIIC2 was purified as described previously (22). The positions of 7SK and VAI transcripts are indicated at the left.

homogeneity. Although PSE-binding activities have been reported by several laboratories, this is the first time that specific polypeptides could be associated with the binding activity. Purified PTF has all of the activities ascribed to the transcription factor binding to the PSE: it binds specifically to PSEs, interacts with Oct-1 bound to an octamer sequence present in the snRNA enhancer (DSE), and activates transcription in vitro from promoters of both Pol II- and Pol III-transcribed snRNA genes. Our purification of a functional PTF has demonstrated that PTF is composed of four nonidentical subunits. In a number of preparations of PTF, these four polypeptides have consistently coeluted with both DNA-binding and transcriptional activities of PTF. In addition, several other observations strongly indicate that these four polypeptides are bona fide subunits of PTF. First, coupled EMSA/SDS-PAGE analysis (Fig. 3) showed that all four polypeptides are present in both Oct-1 · PTF · 7SK PSE and PTF · mU6 PSE complexes. Since the presence of these polypeptides in the protein · DNA complexes is dependent on the intact PSE, and since these polypeptides bind to two divergent PSE probes, the four polypeptides are most likely PTF subunits and not a DNA-binding protein recognizing an unrelated sequence fortuitously present in the probes. Second, all four polypeptides cosediment during glycerol gradient centrifugation analysis. Although the combined molecular mass of four subunits (320 kDa) is larger than the mass (200 kDa) estimated from the sedimentation coefficient and the assumption that PTF is a globular protein, it is known that both size and shape of the protein contribute to sedimentation velocity during centrifugation, and estimates from gel filtration have indicated a natural size of circa 500 kDa for PTF (46). Remarkably, the protein-protein interactions between PTF subunits survive sedimentation through a glycerol gradient in the presence of 4 M urea, suggesting that PTF is a very tightly associated complex (46). Third, the size of the peptide UV cross-linked specifically to the PSE correlates with that of the large subunit of PTF, suggesting that the 180-kDa polypeptide is the DNA-binding subunit of PTF. Finally, the peptide profiles of Lys C digestion products have demonstrated that the four putative PTF subunits are not related to each other (46). Some PTF preparations contain a 36-kDa polypeptide whose Lys C peptide profile and derived peptide sequences suggest that it is related to the 45-kDa subunit of PTF.

Relationship between PTF and other PSE-binding activities. In addition to PTF, other PSE-binding activities have been reported by several laboratories. Knuth et al. (21) have shown that PSE1, which is immunologically related to autoimmune antigen Ku, binds to the PSE of the U1 gene and activates U1 transcription in vitro. PTF is clearly unrelated to PSE1/Ku by both size and immunogenicity. In light of recent data suggesting that Ku is the DNA-binding component of a DNA-dependent protein kinase (11, 13) and that many of its substrates are transcription factors, it remains possible that PSE1/Ku may have an important function in controlling gene expression. In contrast to PSE1/Ku, other PSE-binding factors, PBP and SNAPc, share certain properties with PTF. Their chromatographic behavior on some columns is compatible with that of PTF, they all bind specifically to PSEs, with the highest affinity for the mU6 PSE, and they activate transcription from both Pol II and Pol III class snRNA genes. Furthermore, like PTF, PBP is heat labile (42). However, the reported apparent molecular mass of PBP (90 kDa) is considerably smaller than that of PTF (200 kDa) (41), while SNAPc has the same apparent size as PTF in glycerol gradient analysis (38). SNAPc has been recently reported to consist of TBP complexed with several additional polypeptides. Purified PTF, however, does not contain any detectable TBP, as measured by a Western blotting assay; conversely, TBP complexes purified from HeLa cells by an epitope-tagging method do not contain any PTF activity (46). Since we observe the same mobility of the PTF · PSE complex in gel shift assays during the course of purification, and even in glycerol gradients with 4 M urea, it is unlikely that TBP is stripped from the PTF complex during purification. Although PTF may well interact with TBP in solution, considering the fact that TBP is known to interact with a variety of transcription factors (including activators, repressors, cofactors, and basic factors) (15), we do not think that TBP is an integral part of PTF. Since we have consistently observed one major PSE-binding activity during multiple preparations obtained by using various chromatographic schemes, it is likely that these PSE-binding factors are identical or highly related to each other. However, since the molecular compositions of PBP and SNAPc are not known, the precise relationship of these factors remains to be determined.

Factor requirements in transcription of Pol III class snRNA genes. Transcriptional initiation by RNA Pol III has been characterized best with genes containing an internal control region. Promoter elements of the prototypical VAI and tRNA genes consist of box A and box B present entirely within the coding region. Fractionation of nuclear extracts has revealed that in addition to RNA Pol III, at least two transcription factors, TFIIIB and TFIIIC, are required for in vitro transcription of this class of genes. TFIIIB is a TBP-containing complex distinct from SL1 and TFIID (26, 39, 40, 45), which are required for transcription by Pol I and Pol II, respectively. Recently, a TBP requirement was demonstrated for transcription of the U6 gene, which contains an external promoter. However, contradictory results were obtained regarding the involvement of specific factors, probably because of contamination with TBP of the factors. For example, Lobo et al. (26) have shown that TBP, TFIIIB (0.38 M KCl eluate from Mono Q), or TFIID can independently activate U6 transcription in TBP-depleted ex-

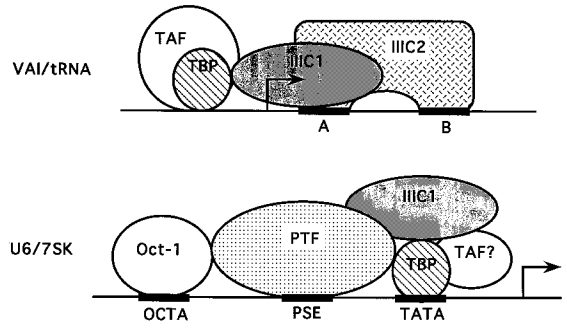

FIG. 9. Model for RNA polymerase III transcription. The B box in the internal promoters of VAI and tRNA genes is recognized by TFIIIC2. TFIIIC1 interacts with TFIIIC2, resulting in strong binding over both the A box and the B box. TFIIIC1 recruits the TBP-containing complex TFIIIB. Finally, Pol III-specific TAFs (in TFIIIB) and TFIIIC1 recruit RNA Pol III. On the external promoters of U6 and 7SK genes, Oct-1 bound to the octamer in the DSE helps the binding of PTF to the PSE. TFIIIC1 interacts with PSE-bound PTF and TATA-bound TBP. TFIIIC1 and hypothetical TAFs loosely associated with TBP recruit RNA Pol III. In this model, TFIIIC1 itself can be considered as a Pol III-specific TAF loosely associated with TBP.

tracts. In contrast, Bernues et al. (3) have reported that both TBP and TFIIIB are involved in U6 transcription. Our data, however, clearly indicate for the first time that the TBP requirement for the U6 and 7SK promoters cannot be fulfilled by highly purified TFIIIB or TFIID preparations that are sufficient for transcription of VAI and typical class II promoters, respectively. Instead, TBP alone can fully support U6 and 7SK transcription. However, it remains possible that additional Pol III-specific TAFs are present in excess in nuclear extracts and that they can interact with ectopic TBP to support U6 and 7SK transcription; alternatively such TAFs may be only weakly associated with TBP and readily dissociated by the nuclear extract preparation conditions or by the anti-TBP antibodies used.

Human TFIIIC has been chromatographically resolved into two components, TFIIIC1 and TFIIIC2, that are jointly required for transcription of 5S, tRNA, and VAI RNA genes (43, 47). The multisubunit complex TFIIIC2 binds to the B box of the internal control region of VAI and tRNA promoters with high affinity, while TFIIIC1 binds weakly, at best, to the A box. TFIIIC1 and TFIIIC2 together, however, create an extended DNase I footprint over both the A box and the B box (43, 47). Our finding that TFIIIC1 is also required for an external promoter-containing class III gene (7SK) suggests that TFIIIC1 has a more general function in Pol III transcription than merely facilitating TFIIIC2 binding to DNA. It is tempting to speculate that TFIIIC1 may interact with PSE-bound PTF and/or TATA-bound TBP, just as it binds to TFIIIC2 in genes with internal promoters. In this regard, it is noteworthy that addition of an internal B box increases transcription of a promoter with the PSE/TATA configuration (34), which is exemplified by the *Xenopus* selenocysteine $tRNA^{(Ser)Sec}$ gene (5). This finding suggests that TFIIIC1 is a common target for TFIIIC2 and PTF/TBP and that it may help to recruit RNA Pol III. So far, TFIIIC1 is the least well characterized of the class III transcription factors. Our data also demonstrate that (like TFIIIC2 and PTF) TFIIIC1 is heat labile, which could be useful for its further characterization.

Although human TFIIIB is not fully characterized, yeast TFIIIB is composed of TBP, a 70-kDa subunit (TFIIB-related factor [BRF]/PCF4/TDS4) with homology to Pol II transcription factor TFIIB (4, 7, 27), and a 90-kDa subunit (2, 20). UV cross-linking studies have indicated that intricate conformational changes result from protein-protein interactions between promoter-bound TFIIIB and TFIIIC and that TBP unmasks a cryptic DNA-binding domain of BRF (2, 20), whereas other studies have indicated interactions of BRF with an RNA Pol III-specific subunit (44). In the case of U6 and 7SK transcription, direct binding of TBP to the TATA box might circumvent the need of some of the normal TFIIIB TAFs. On the basis of existing data, we propose a model for transcription of class III genes that is depicted in Fig. 9. In this model, PTF and TFIIIC2 function as gene-specific factors for PSE- and B-box-containing promoters, respectively, while TFIIIC1 may interact with both PTF/TBP and TFIIIC2 in the corresponding promoters. By analogy to other promoters requiring specific TBP-TAF complexes, additional Pol III-specific TAFs loosely associated with TBP may also be required for U6 and 7SK transcription. Finally, it is suggested that TFIIIC1 and the hypothetical TAFs recruit RNA Pol III to the promoter complex.

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REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1993. Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York.
2. **Bartholomew, B., G. A. Kassavetis, and E. P. Geiduschek.** 1991. Two components of *Saccharomyces cerevisiae* TFIIIB are stereospecifically located upstream of a tRNA gene and interact with the second largest subunit of TFIIIC. Mol. Cell. Biol. **11:**5181–5189.
3. **Bernues, J., K. A. Simmen, J. D. Lewis, S. I. Gunderson, M. Moncollin, J.-M. Egly, and I. W. Mattaj.** 1993. Common and unique transcription factor requirements of human U1 and U6 snRNA genes. EMBO J. **12:**3573–3585.
4. **Buratowski, S., and H. Zhou.** 1992. A suppressor of TBP mutations encodes an RNA polymerase III transcription factor with homology with TFIIB. Cell **71:**221–230.
5. **Carbon, P., and A. Krol.** 1991. Transcription of the *Xenopus laevis* selenocysteine $tRNA^{(Ser)Sec}$ gene: a system that combines an internal B box and upstream elements also found in U6 snRNA genes. EMBO J. **10:**599–606.
6. **Chiang, C.-M., H. Ge, Z. Wang, A. Hoffmann, and R. G. Roeder.** 1993. Unique TATA-binding protein-containing complexes and cofactors involved in transcription by RNA polymerase II and III. EMBO J. **12:**2749–2762.
7. **Colbert, T., and S. Hahn.** 1992. A yeast TFIIB-related factor involved in RNA polymerase III transcription. Genes Dev. **6:**1940–1949.
8. **Dahlberg, J. E., and E. Lund.** 1988. The genes and transcription of the major small nuclear RNAs, p. 38–70. *In* M. L. Birnsteil (ed.), Structure and function of major and minor small nuclear ribonucleoprotein particles. Springer-Verlag KG, Berlin.
9. **Das, G., D. Henning, D. Wright, and R. Reddy.** 1988. Upstream regulatory elements are necessary and sufficient for transcription of a U6 RNA gene by RNA polymerase III. EMBO J. **7:**503–512.
10. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
11. **Dvir, A., S. R. Petereon, M. W. Knuth, H. Lu, and W. S. Dynan.** 1992. Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. Proc. Natl. Acad. Sci. USA **89:**11920–11924.
12. **Goodrich, J. A., and R. Tjian.** 1994. TBP-TAF complexes: selectivity factors for eukaryotic transcription. Curr. Opin. Cell Biol. **6:**403–409.
13. **Gottlieb, T. M., and S. P. Jackson.** 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell **72:**131–142.
14. **Gunderson, S. I., M. W. Kunth, and R. R. Burgess.** 1990. The human U1 snRNA promoter correctly initiates transcription in vitro and is activated by PSE1. Genes Dev. **4:**2048–2060.
15. **Hernandez, N.** 1992. Transcription of vertebrate snRNA genes and related

genes, p. 281–313. *In* S. McKnight and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
16. **Hernandez, N.** 1993. TBP, a universal eukaryotic transcription factor? Genes Dev. **7:**1291–1308.
17. **Hernandez, N., and R. Lucito.** 1988. Elements required for transcription initiation of the human U2 snRNA gene coincide with elements required for snRNA 3′ end formation. EMBO J. **7:**3125–3134.
18. **Hoffman, A., and R. G. Roeder.** 1991. Purification of his-tagged proteins in non-denaturing conditions suggests a convenient method for protein interaction studies. Nucleic Acids Res. **19:**6337–6338.
19. **Kadonaga, J. T., and R. Tjian.** 1986. Affinity purification of sequence-specific DNA binding proteins. Proc. Natl. Acad. Sci. USA **83:**5889–5893.
20. **Kassavetis, G. A., C. A. P. Joazeiro, M. Pisano, E. P. Geiduschek, T. Colbert, S. Hahn, and J. A. Blanco.** 1992. The role of the TATA-binding protein in the assembly and function of the multisubunit yeast RNA polymerase III transcription factor, TFIIIB. Cell **71:**1055–1064.
21. **Knuth, M. W., S. I. Gunderson, M. E. Thompson, L. A. Stransheim, and R. R. Burgess.** 1990. Purification and characterization of proximal sequence element-binding protein 1, a transcription activating protein related to Ku and TREF that binds the proximal sequence element of the human U1 promoter. J. Biol. Chem. **265:**17911–17920.
22. **Kovelman, R., and R. G. Roeder.** 1992. Purification and characterization of two forms of human transcription factor IIIC. J. Biol. Chem. **267:**24446–24456.
23. **Kunkel, G. T.** 1991. RNA polymerase III transcription of genes that lack internal control regions. Biochim. Biophys. Acta **1088:**1–9.
24. **Lagna, G., R. Kovelman, J. Sukegawa, and R. G. Roeder.** 1994. Cloning and characterization of an evolutionarily divergent DNA-binding subunit of mammalian TFIIIC. Mol. Cell. Biol. **14:**3053–3064.
25. **Lobo, S. M., and N. Hernandez.** 1989. A 7bp mutation converts a human RNA polymerase II snRNA promoter into an RNA polymerase III promoter. Cell **58:**55–67.
26. **Lobo, S. M., M. Tanaka, M. L. Sullivan, and N. Hernandez.** 1992. A TBP complex essential for transcription from TATA-less but not TATA-containing RNA polymerase III promoters is part of the TFIIIB fraction. Cell **71:**1029–1040.
27. **Lopez-de-Leon, A., M. Librizzi, K. Tuglia, and I. Willis.** 1992. *PCF*4 encodes an RNA polymerase III transcription factor with homology to TFIIB. Cell **71:**211–220.
28. **Mattaj, I. W., N. A. Dathan, H. D. Parry, P. Carbon, and A. Krol.** 1988. Changing the RNA polymerase specificity of U snRNA gene promoters. Cell **55:**435–442.
29. **Murphy, S., C. DiLiegro, and M. Melli.** 1987. The in vitro transcription of the 7SK gene by RNA polymerase III is dependent only on the presence of an upstream promoter. Cell **51:**81–87.
30. **Murphy, S., B. Moorefield, and T. Pieler.** 1989. Common mechanisms of promoter recognition by RNA polymerases II and III. Trends Genet. **5:**122–126.
31. **Murphy, S., J.-B. Yoon, T. Gerster, and R. G. Roeder.** 1992. Oct-1 and Oct-2 potentiate functional interaction of a transcription factor with the proximal sequence element of small nuclear RNA genes. Mol. Cell. Biol. **12:**3247–3261.
32. **Nakajima, N., M. Horikoshi, and R. G. Roeder.** 1988. Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA box-promoter interactions of TFIID. Mol. Cell. Biol. **8:**4028–4040.
33. **Neuman de Begvar, H. E., and J. E. Dahlberg.** 1989. Initiation and termination of human U1 transcription requires the concerted action of multiple flanking sequence elements. Nucleic Acids Res. **17:**9305–9318.
34. **Parry, H. D., and I. W. Mattaj.** 1990. Positive and negative functional interactions between promoter elements from different classes of RNA polymerase III-transcribed genes. EMBO J. **9:**1097–1104.
35. **Parry, H. D., G. Tebb, and I. W. Mattaj.** 1989. The *Xenopus* U2 gene PSE is a single, compact, element required for transcription initiation and 3′ end formation. Nucleic Acids Res. **17:**1989.
36. **Pierani, A., A. Heguy, H. Fujii, and R. G. Roeder.** 1990. Activation of octamer-containing promoters by either octamer-binding transcription factor 1 (Oct-1) or Oct-2 and requirement of an additional B-cell-specific component of optimal transcription of immunoglobulin promoters. Mol. Cell. Biol. **10:**6204–6215.
37. **Reddy, R., and R. Singh.** 1991. Synthesis of small nuclear RNAs. Prog. Mol. Subcell. Biol. **12:**1.
38. **Sadowski, C. L., R. W. Henry, S. M. Lobo, and N. Hernandez.** 1993. Targeting TBP to a non-TATA box cis-regulatory element; a TBP-containing complex activates transcription through the PSE. Genes Dev. **7:**1535–1548.
39. **Simmen, K. A., J. Bernues, J. D. Lewis, and I. W. Mattaj.** 1992. Cofractionation of the TATA-binding protein with the RNA polymerase III transcription factor TFIIIB. Nucleic Acids Res. **20:**5889–5898.
40. **Taggart, A. K. P., T. S. Fisher, and B. F. Pugh.** 1992. The TATA-binding protein and associated factors are components of pol III transcription factor TFIIIB. Cell **71:**1015–1028.
41. **Waldschmidt, R., I. Wanandi, and K. H. Seifart.** 1991. Identification of transcription factors required for the expression of mammalian U6 genes in vitro. EMBO J. **10:**2595–2603.
42. **Wanandi, I., R. Waldschmidt, and K. H. Seifart.** 1993. Mammalian transcription factor PBP. J. Biol. Chem. **268:**6629–6640.
43. **Wang, Z., and R. G. Roeder.** Unpublished data.
44. **Werner, M., N. Chaussivert, I. M. Willis, and A. Sentenac.** 1993. Interaction between a complex of RNA polymerase III subunits and the 70-kDa component of transcription factor IIIB. J. Biol. Chem. **268:**20721–20724.
45. **White, R. J., and S. P. Jackson.** 1992. Mechanism of TATA-binding protein recruitment to a TATA-less class III promoter. Cell **71:**1041–1053.
46. **Yoon, J.-B., and R. G. Roeder.** Unpublished data.
47. **Yoshinaga, S. K., P. A. Boulanger, and A. J. Berk.** 1987. Resolution of human transcription factor TFIIIC into two functional components. Proc. Natl. Acad. Sci. USA **84:**3585–3589.
48. **Zhou, Q., P. M. Lieberman, T. G. Boyer, and A. J. Berk.** 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6:**1964–1974.