MOLECULAR AND CELLULAR BIOLOGY, July 1992, p. 3247–3261
0270-7306/92/073247-15$02.00/0

Vol. 12, No. 7

Oct-1 and Oct-2 Potentiate Functional Interactions of a Transcription Factor with the Proximal Sequence Element of Small Nuclear RNA Genes

SHONA MURPHY,† JONG-BOK YOON, THOMAS GERSTER,‡ AND ROBERT G. ROEDER*

Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, New York 10021

Received 5 March 1992/Accepted 4 May 1992

The promoters of both RNA polymerase II- and RNA polymerase III-transcribed small nuclear RNA (snRNA) genes contain an essential and highly conserved proximal sequence element (PSE) approximately 55 bp upstream from the transcription start site. In addition, the upstream enhancers of all snRNA genes contain binding sites for octamer-binding transcription factors (Octs), and functional studies have indicated that the PSE and octamer elements work cooperatively. The present study has identified and characterized a novel transcription factor (designated PTF) which specifically binds to the PSE sequence of both RNA polymerase II- and RNA polymerase III-transcribed snRNA genes. PTF binding is markedly potentiated by Oct binding to an adjacent octamer site. This potentiation is effected by Oct-1, Oct-2, or the conserved POU domain of these factors. In agreement with these results and despite the independent binding of Octs to the promoter, PTF and Oct-1 enhance transcription from the 7SK promoter in an interdependent manner. Moreover, the POU domain of Oct-1 is sufficient for significant in vitro activity in the presence of PTF. These results suggest that essential activation domains reside in PTF and that the potentiation of PTF binding by Octs plays a key role in the function of octamer-containing snRNA gene enhancers.

The small nuclear RNA (snRNA) genes can be divided into two classes on the basis of their structure and the type of RNA polymerase (II or III) responsible for their transcription. The promoters of the RNA polymerase II-dependent snRNA genes (e.g., U1 to U5) contain both an upstream (−220) enhancer with a functional octamer element (often in association with sites for other factors) and an essential proximal (−55) sequence element (PSE) which fulfills the start site selection role played by the TATA box of many mRNA-encoding genes (for reviews, see references 5 and 45). The promoters of snRNA genes transcribed by RNA polymerase III (e.g., 7SK and U6) have the same enhancer-PSE structure but also contain a TATA box (at position −25) which is involved in both start site selection and determination of RNA polymerase specificity (29, 31, 32; for reviews, see references 22, 40, 44, and 60). We and others have presented evidence that octamer-binding transcription factors (Octs) and other DNA-binding proteins active in RNA polymerase II-dependent transcription also participate in transcription of the 7SK and U6 genes via the enhancer sequences (2, 17, 41). We also have shown that in vitro activation of the 7SK promoter by purified Octs requires an intact PSE in addition to Oct-binding sites, suggesting that a factor(s) binding to the PSE interacts functionally with the Octs (41). The PSE thus performs a critical role in the transcription of both classes of snRNA genes and, as the only common essential promoter element, may coordinate their activities. Thus far, however, no transcriptionally active proteins which interact with the PSEs of both class II and class III snRNA genes have been identified.

Using DNA probes with an Oct-binding site adjacent to the PSE, both mobility shift and DNase footprint assays have revealed a factor(s) which binds to the PSE of the 7SK gene in conjunction with Octs. This factor is designated PTF (for PSE transcription factor). We show that adjacent binding of either the ubiquitous Oct-1 or the lymphoid-enriched Oct-2 greatly potentiates binding of PTF to the PSE and results in a supershifted complex consisting of Oct, PTF, and DNA in the mobility shift assay. We have used the supershift assay to partially purify transcriptionally active PTF from HeLa cells. We show that PTF can interact with the PSEs of a variety of RNA polymerase II- and RNA polymerase III-transcribed mammalian snRNA genes, that the POU domain of either Oct-1 or Oct-2 is sufficient to potentiate PTF binding to the PSE, and that the cooperative binding of Oct and PTF is also reflected in a functional cooperativity between the purified factors in vitro.

MATERIALS AND METHODS

Plasmid constructions. O^+P^+ was made by cloning an oligonucleotide of the sequence 5′-GATCCCTTCACCTT ATTTGCATAAGCGAATTCTGCA-3′ into the *Pst*I site of the 5′ Δ−70 deletion mutant (41). H_2b1 and H_2b2 are described by Murphy et al. (41). $O_2{}^+P^+$ was constructed by cloning an oligonucleotide of the sequence 5′-GGATCCACC TTATTTGCATATGCAAATAAGCGAATTCTGCA-3′ into the *Pst*I site of the 5′ Δ−70 deletion mutant. In O^+P^- and $O_2{}^+P^-$ the PSE sequence between −65 and −49 was replaced with the sequence 5′-GTCAAGCCTGTGCCCT-3′ by site-directed mutagenesis (24).

Mobility shift and footprinting analyses. Probes for mobility shift analysis were prepared by end labelling gel-purified restriction fragments or synthetic oligonucleotides with the Klenow enzyme and α-^{32}P-labelled deoxynucleoside triphosphates. Each 20 μl of reaction mixture contained 5 to 10 fmol

* Corresponding author.
† Present address: Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, England.
‡ Present address: Abteilung Zellbiologie, Biozentrum der Universitat Basel, 4056 Basel, Switzerland.

of probe, 0.05 to 2 μg of poly(dI-dC), 1 mM spermidine, 0.03% Nonidet P-40, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.9), 50 mM KCl, 4% Ficoll, 0.5 mg of bovine serum albumin (BSA) per ml, 5% glycerol, and 0.5 to 1 mM dithiothreitol. Reactions were initiated by the addition of protein, and mixtures were incubated for 30 min at 30°C. The whole reaction mixture was electrophoresed on a 4% (37.5:1, acrylamide-bisacrylamide) polyacrylamide gel, with 0.5× Tris-borate-EDTA and 0.03% Nonidet P-40, after a 30-min prerun. For DNase protection studies the Asp-718 sites of the O^+P^+ and O^+P^- probes were labelled either with [α-^{32}P]dGTP and the Klenow enzyme (noncoding strand) or with T4 polynucleotide kinase and [α-^{32}P]ATP after dephosphorylation (coding strand). Ten femtomoles of probe was used in a 20-μl reaction mixture containing 20 mM HEPES (pH 7.9), 50 mM KCl, 1 mM spermidine, 25 μg of poly(dI-dC), 2 mM $MgCl_2$, 0.03% Nonidet P-40, and 0.1 mg of BSA per ml.

After incubation at 30°C for 30 min, DNase was added to 0.2 μg/ml and digestion was carried out for 30 s at 30°C. Double-stranded oligonucleotide competitors used in the mobility shift and footprint experiments had the following sequences: OCTA, 5'-TGCAACTCTTCACCTT**ATTTGCA**TAAGCGATTCTACTGC-3'; OCTA$^-$, 5'-TGCAACTCTTCACCTT**CGGGTACG**AAGCGATTCTACTGC-3'; SP1, 5'-GCCAAGCGACACC**GGGGCGGGGCGGGGCGGGGC**TGC-3'; 7SK PSE, 5'-AGCTCCAACT**TGACCTAAGTGTAAAGTT**GAGT-3'; U1 PSE, 5'-CTAAG**TGACCGTGTGTGTAAAGA**TGAGGATCC-3'; and mouse U6 PSE, 5'-AAGGAAAC**TCACCCTAACTGTAAAGT**AATTGTG-3'. The double-stranded probes used for the experiments shown in Fig. 2 were made by annealing primers to single-stranded oligonucleotides and extending them with the Klenow enzyme in the presence of [α-^{32}P]dCTP.

Purification of PTF. The PTF employed for the experiments shown in Fig. 4 to 6 was prepared as follows. HeLa cell S100 extract (500 ml, 14 mg of protein per ml) prepared by the method of Dignam et al. (7) was precipitated with 40% ammonium sulfate. The precipitate was resuspended in BC buffer (20 mM HEPES [pH 7.9], 20% [vol/vol] glycerol, 0.2 mM EDTA, 0.03% Nonidet P-40, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride), dialyzed to BC buffer–100 mM KCl, and loaded onto a 100-ml phosphocellulose (P11) column. After a BC buffer–300 mM KCl wash, most of the PTF eluted in the BC buffer–500 mM KCl step fraction, giving an approximately 10-fold purification and 2- to 4-fold concentration with respect to the input. The BC buffer–500 mM KCl eluate (180 mg of protein) was dialyzed to BC buffer–100 mM KCl and loaded onto a 20-ml DEAE-cellulose (DE-52) column. Application of a linear KCl gradient from 100 to 400 mM in BC buffer resulted in PTF elution at approximately 170 mM KCl, effecting a 10-fold purification and 4- to 8-fold concentration of PTF. The PTF-containing fractions were pooled (19 mg), dialyzed to BC buffer–100 mM KCl, and applied to a second (3.8-ml) P11 column. Application of a 20-ml linear gradient of 100 to 600 mM KCl resulted in PTF elution in a broad peak from 250 to 450 mM KCl. This step gave only a twofold purification but removed some of the nonspecific DNA-binding proteins detected by the gel retardation analysis.

For preparation of an Oct-PTF oligonucleotide affinity column, the following oligonucleotides were annealed, ligated, and coupled to Sepharose 4B: POct 1, 5'-CTGCAGCTT**ATTTGCATATGCAAAT**AAGGTGGATCCTGC-3'; POct 2, 5'-TTGGACGTGCAGGATCCACCTT**ATTTGCATATGCAAAT**AAGCTGCAG-3'; POct 3, 5'-AGCTCCAACT**TGACCTAAGTGTAAAGTT**GAGT-3'; and POct 4, 5'-CTGCAGTCGACTC**AACTTTACACTTAGGTCAA**G-3'.

A 100-μl DNA affinity column with approximately 100 pmol of binding sites per ml was loaded slowly with 5 pmol of active recombinant Oct-1 in BC buffer–50 mM KCl with 0.1 mg of BSA per ml. The PTF-containing fractions from the second P11 column were pooled (8 mg of protein), dialyzed to BC buffer–50 mM KCl, and loaded at 1 column volume per h onto the Oct-1-saturated DNA affinity column. The column was washed with BC buffer–200 mM KCl and developed with a 1-ml linear gradient of 200 to 800 mM KCl. PTF eluted from 270 to 400 mM KCl, while Oct-1 eluted from 350 to 450 mM KCl. PTF was purified greater than 40-fold and concentrated 5-fold by this step. BSA was added to 0.1 mg/ml to the PTF-containing fractions. The early-eluting, PTF-containing fractions which were essentially free of Oct-1 contamination were transcriptionally active (data not shown) and were used for footprinting studies (see Fig. 4). The peak fractions containing Oct-1 and PTF were pooled, dialyzed to BC buffer–200 mM KCl, and slowly loaded onto a 100-μl DNA affinity column coupled with annealed oligonucleotides POct-1 and POct-2 (OCTA DNA affinity column). The column was washed with BC buffer–200 mM KCl, and the PTF-containing flowthrough and wash fractions were pooled and adjusted to 0.1 mg of BSA per ml. Only this last preparation of PTF was checked for the presence of Ku antigen, by using an autoimmune anti-Ku antibody (kindly supplied by Westley Reeves) to probe Western blots (immunoblots). The input fraction to the OCTA DNA affinity column showed a strong antibody reactivity with two polypeptides corresponding to the Ku subunits (48), while the PTF-containing flowthrough fractions showed no reactivity. This Ku-free fraction was used for the functional studies in Fig. 5 and for PTF–Oct-1 DNA-binding studies cited below.

The PTF preparation used for experiments shown in Fig. 1 to 3 was prepared by chromatography of S100 extract from HeLa cells on P11 and DE-52 resins as described above, with the following changes: the ammonium sulfate precipitation step was omitted, PTF was recovered by this step elution in the first DE-52 chromatography step, and a second DE-52 chromatography step was carried out after the second P11 column step, with PTF elution by a linear gradient of 100 to 300 mM KCl. None of these fractions were checked for the presence of Ku antigen.

In vitro and in vivo transcription. 7SK maxigenes were constructed by site-directed mutagenesis to introduce the sequence 5'-CTAAGCTTGT-3' between positions +27 and +28 of the 7SK coding region. In vitro transcription with normal and factor-depleted extracts (see below) was carried out as described by Murphy et al. (41), with 0.1 μg of the virus-associated RNA I (VAI) gene per ml and without inclusion of a radiolabelled nucleotide. Following incubation, 125 μl of stop mix (0.4 M ammonium acetate, 0.5% sodium dodecyl sulfate, 1 mM EDTA, 40 μg of tRNA per ml) was added, and the mixture was extracted with phenol-chloroform and ethanol precipitated. The pellet was resuspended in 100 μl of DNase buffer (0.1 M Na acetate [pH 6.0], 10 mM $MgCl_2$) with 100 U of RNase-free DNase I per ml and incubated at room temperature for 30 min. The mixture was extracted with phenol-chloroform, and the nucleic acids were precipitated with 250 μl of ethanol containing 330 fmol each of the 7SK primer and the VAI primer (this amount was found to be saturating in preliminary experiments). After precipitation, the pellets were processed for primer extension as described in Murphy et al. (39). The primer for the

7SK maxigene transcripts was a 39-nucleotide oligonucleotide complementary to the last (3′) 5 nucleotides of the maxigene insert and the 34 nucleotides from +28 to +61 of the 7SK coding region. The primer for the VAI transcript was complementary to nucleotides +37 to +56. The primer-extended cDNA products were 71 nucleotides for the 7SK RNA and 56 nucleotides for the VAI RNA.

For in vitro transcription HeLa nuclear extracts (7) were used intact or after depletion of factors. Oct-1 was removed by incubation of the extract with Sepharose 4B (Pharmacia) coupled with annealed oligonucleotides POct-1 and POct-2 as described by Scheidereit et al. (54). Oct-1 and PTF were removed with Sepharose 4B coupled with annealed and ligated oligonucleotides POct-1, POct-2, POct-3, and POct-4. In the experiment of Fig. 6, 1 U of Oct-1^B was equivalent to 7.5 fmol of binding activity and 1 U of PTF was equivalent to 5 fmol of binding activity. One femtomole of binding activity is the amount of protein which shifts 1 fmol of O^+P^+ probe under the gel shift conditions described above. The amount of Oct-1^B calculated by binding units corresponded closely to the amount estimated by Coomassie blue staining.

For analysis in cultured HeLa cells, transfections were carried out essentially as described by Gerster et al. (10). The test 7SK plasmid (10 μg) was cotransfected with 10 μg of the VAI plasmid, and RNA was prepared as described by Chomczynski and Sacchi (3). Quantitative primer extension analysis was carried out as described for the in vitro transcription analysis. Quantitation of in vitro transcription and mobility shift experiments was carried out with a Betagen radioimage analyzer.

Production of Oct-1 and Oct-2. Recombinant Oct-1 was produced in bacteria by using the entire coding sequence for Oct-1 present in pBS-oct-1+ (64). The end-filled *Xba*I-*Hin*dIII fragment isolated from pBS-oct1+ was ligated with the end-filled *Bam*HI site of PET-11b (63). The resulting plasmid was introduced into BL21 (DE3) containing pLysS. After induction with isopropyl-β-D-thiogalactopyranoside (IPTG), the extract was prepared by sonication in BC buffer–100 mM KCl containing 10% glycerol. After centrifugation at 10,000 × *g* for 15 min, the pellet was washed twice in the same buffer and dissolved in BC buffer–100 mM KCl containing 6 M guanidine-HCl. Soluble material was loaded onto a Superose 12 (25-ml) column (Pharmacia) equilibrated with BC buffer–100 mM KCl and eluted at a flow rate of 0.3 ml/min. Active fractions were dialyzed to BC buffer–50 mM KCl and loaded onto a Mono S (1-ml) column. The column was eluted with a linear gradient of 50 to 500 mM KCl. Oct-1 eluted at approximately 100 mM KCl and was stored at −70°C. To produce the POU domain of Oct-1 in bacteria, the *Hin*dII-*Ava*II fragment of pBS-oct-1+ was inserted into the *Xho*I site of the 6HisT-pET11 vector (14). The resulting plasmid was introduced into BL21 (DE3) containing pLysS. After induction with IPTG, the extract was prepared by sonication in BC buffer–500 mM KCl. After centrifugation at 10,000 × *g* for 15 min, the supernatant was adjusted to 5 mM imidazole and loaded onto a Ni^{2+}-nitrilotriacetic acid column (Qiagen). The column was then washed with 10 column volumes of BC buffer–500 mM KCl containing 20 mM imidazole before being eluted with BC buffer–500 mM KCl containing 100 mM imidazole. The eluate was dialyzed against BC buffer–100 mM KCl and loaded onto a heparin column. The column was eluted with a linear gradient of 100 to 700 mM KCl. POU eluted at 300 mM KCl and was apparently homogenous.

The truncated Oct-1 used for gel shift studies (see Fig. 3, Oct-1p) was produced by deleting the amino terminus of Oct-1 in the pBS-oct-1+ clone with *Bst*XI and *Hin*cII as described by Sturm et al. (64). The resulting plasmid was cut with *Pfl*MI and used as a template to make RNA which was translated into protein with rabbit reticulocyte lysate. The truncation is identical to ΔBSTXI-HincII/PflMI described by Sturm et al. (64), and the protein contains little more than the POU domain. Full-length Oct-2 was made either by translating RNA made from clone OO (10) with rabbit reticulocyte lysate or by expression in HeLa cells with a vaccinia virus vector (11a). The truncated Oct-2 (see Fig. 3, Oct-2p) was produced by expression of clone N154 C376 (10) in transfected HeLa cells. Uncapped RNA was produced by T3 or T7 RNA polymerase, and the in vitro translations were carried out as described by Promega, the supplier of the rabbit reticulocyte lysate. Oct-1^H was purified from HeLa nuclear extracts as described by Pierani et al. (46).

RESULTS

Octs potentiate binding of a protein(s) to the PSE of the human 7SK gene. Although the natural promoter of the 7SK gene contains no consensus octamer elements, a number of degenerate octamer sequences function in vitro to mediate activation by Oct-1 (41). We have shown that a single consensus octamer sequence (ATTTGCAT) placed immediately 5′ to the PSE also effectively mediates Oct-dependent transcription of the 7SK gene in vitro. Our observation that the PSE is essential for this activation to occur in vitro (41) suggested that transcription factors were binding to this element. However, binding assays with DNA fragments containing the 7SK PSE sequence failed to detect PSE-specific binding proteins in transcriptionally active extracts. Reasoning that we might be able to exploit the indicated functional interactions of Octs with such a factor, we tested a probe containing closely juxtaposed octamer and PSE sequences in a mobility shift assay. The structures of the probes used are shown in Fig. 1A; O^+P^+ consists of a single consensus octamer sequence 23 bp upstream from the PSE; O^+P^- is similar except for mutations in the PSE (see Materials and Methods). An activity capable of supershifting the prominent Oct-DNA complexes was detected in transcriptionally active nuclear extracts from both HeLa and Namalwa cells and was only seen when the PSE was present (data not shown). This activity was also detected in S100 extracts from HeLa cells and further concentrated and separated from Oct-1 by chromatography on phosphocellulose and DEAE-cellulose (see Materials and Methods). Figure 1B shows a representative mobility shift analysis of this fraction with the probes shown in Fig. 1A. The O^+P^+ probe (lane 2) resulted in the appearance of two relatively weak but distinct bands (marked by arrows at the left) which were absent when the O^+P^- probe was used (lane 12). These complexes are clearly different from Oct-DNA complexes (marked by O at the right) formed when the same probe was incubated with Oct-1 purified from HeLa cells (Oct-1^H) (lane 6). Addition of 20 ng (100-fold molar excess) (lane 3) or 40 ng (lane 4) of an oligonucleotide containing the 7SK PSE (positions −70 to −45) effectively abolished the lower complex and reduced the upper one. Addition of 40 ng of a nonspecific competitor had no effect on either complex (lane 5). Therefore, the lower of the two PTF-dependent complexes seems to be specific, while the upper may contain both specific and nonspecific components, suggesting that PTF alone can bind weakly to the 7SK PSE.

Incubation of the O^+P^+ probe with both Oct-1^H and the PTF-enriched fraction resulted in the appearance of a prom-

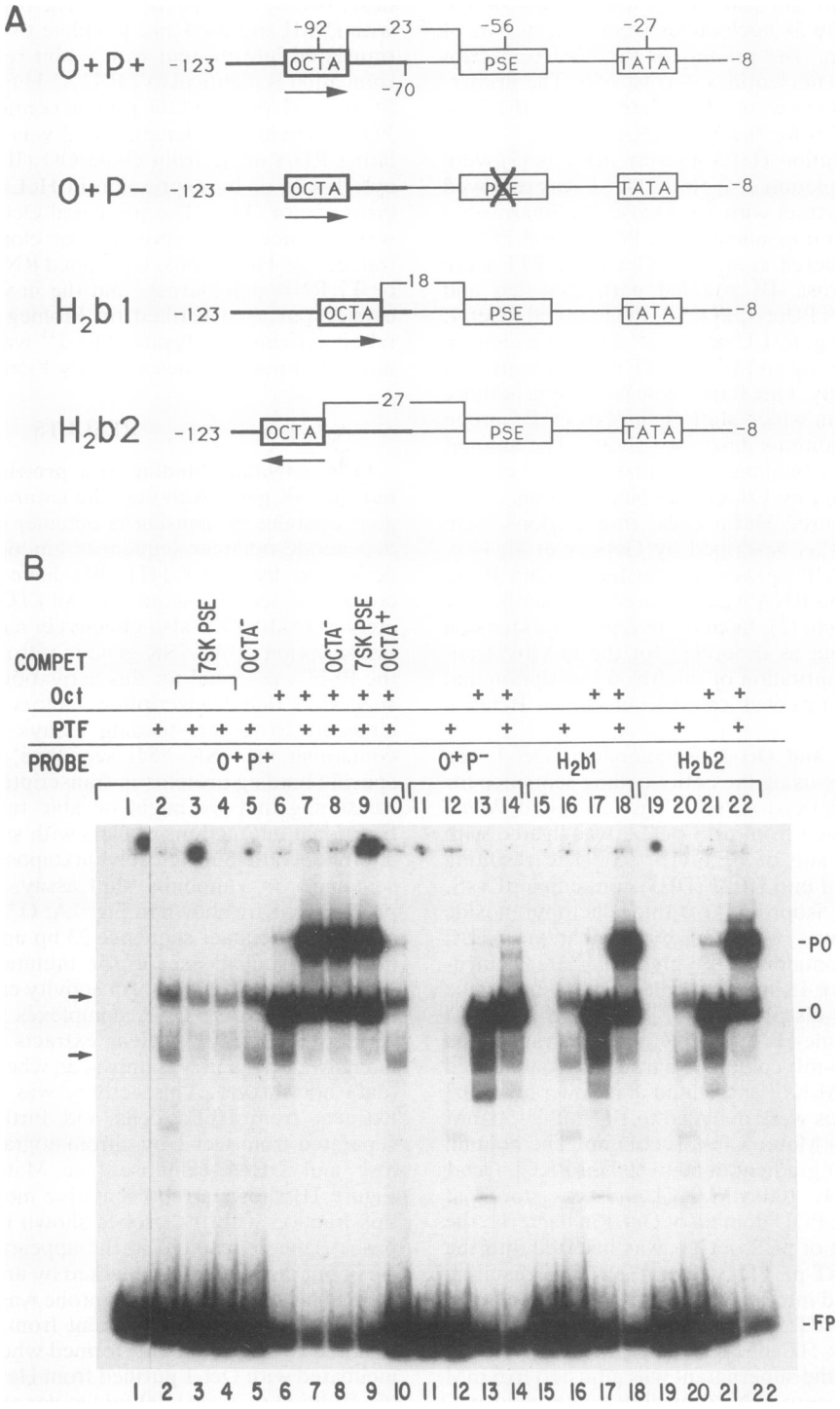


FIG. 1. Octs potentiate binding of a protein to the PSE of the human 7SK gene. (A) Structure of the probes used for gel retardation assays. These probes were prepared from the plasmids indicated at the left (see Materials and Methods). The position of the midpoint of the functional elements upstream of the transcription start site is indicated (×). The distances between the 5′ border of the PSE and the 3′ border of the octamer sequence (in base pairs) are noted by the brackets. The arrows indicate the direction of the octamer sequence with respect to the octamer site in the histone H_2b promoter (25). Thin lines and thick lines indicate the sequences derived from the human 7SK gene and the human H_2b gene, respectively. (B) Mobility shift assay using the probes shown in panel A. The probes, the factors (Oct or PTF), and the cold competitors (COMPET) added to the reaction mixture are indicated above each lane. The amounts of cold competitors added to the reaction mixture were 20 ng (lane 3) or 40 ng (lanes 4, 5, 8, 9, and 10). PO indicates complexes that contain Oct and PTF. O indicates complexes that contain Octs. FP indicates the position of the free probe. The arrows indicate PTF-dependent complexes.

inent low-mobility complex (marked PO at the right) (lane 7) which was not seen when either PTF (lane 2) or Oct-1 (lane 6) alone was incubated with the O^+P^+ probe or when both factors were incubated together with the O^+P^- probe (lane 14). Nor was this complex produced when an O^-P^+ probe (−70 to −8) was used (data not shown). Since this complex was dependent on the presence of Oct-1, PTF, and the octamer and PSE sequences, we conclude that it represents a PSE-PTF-dependent supershift of the Oct-1–DNA complex. Addition of 40 ng of the 7SK PSE oligonucleotide to the reaction mixtures containing Oct and PTF (lane 9) resulted in a slight reduction of the amount of the supershift complex, whereas addition of 40 ng of an oligonucleotide containing a consensus octamer sequence (lane 10) restored the pattern seen when PTF was added alone (lane 2). Under the same conditions, addition of 40 ng of a nonspecific oligonucleotide had no detectable effect (lane 8). Assuming that all complexes visible in lane 2 are PSE specific, there was at least a 10-fold difference between the amount of DNA complexed by Oct plus PTF and that complexed by PTF alone. Quantitation of the Oct-containing complexes (O and PO) in lanes 6 and 7 (and in lanes 2 and 3 of Fig. 3) indicated no increase of Oct binding upon addition of PTF. No increase of PTF binding was observed upon addition of Oct-1 unless the octamer-binding sequence was present (data not shown). These results indicate that Oct-1 bound to the O^+P^+ probe effectively potentiates binding of a protein(s) to the adjacent PSE. In several similar experiments the extent of potentiation varied from 10- to 20-fold.

Using PTF-enriched fractions, we also investigated the effect of changes in the position of the octamer sequence (relative to the PSE) on the potentiation of PTF binding (Fig. 1B, lanes 15 to 22). In probe H_2b1 the distance between the octamer and the PSE has been reduced from 23 to 18 bp, whereas in probe H_2b2 the octamer is in the opposite orientation and 27 bp from the PSE (Fig. 1A). Each of these probes gave essentially the same result as the O^+P^+ probe in the Oct-DNA supershift assay. Specifically, addition of both Oct-1 and PTF to either probe resulted in marked potentiation (greater than 10-fold) of PTF binding (compare lane 16 to lane 18 and lane 20 to lane 22). These results show that the position of the octamer sequence relative to the PSE is not critical (in this assay) and suggest that the interaction between the two factors is quite flexible.

PTF interacts with the PSEs of both RNA polymerase II- and RNA polymerase III-transcribed snRNA genes. We next investigated interactions of the PTF preparation with the PSE sequences of other human snRNA genes transcribed by both RNA polymerases II (U1 and U2) and III (U6), as well as the mouse and *Xenopus tropicalis* U6 genes (Fig. 2A). We designed oligonucleotides with an octamer sequence upstream from these PSEs to determine the effect of adjacent binding of Octs on protein interactions with the PSE (Fig. 2A) (see references 5, 40, and 47 for comparative diagrams of these different PSEs). Figure 2B shows the results of this investigation. As expected, all probes gave a single complex (O) with Oct-1 alone (lanes 3, 7, 11, 15, 19, and 23). In contrast, for all except the mouse U6 probe (lane 18), there was little detectable binding by PTF alone (lanes 2, 6, 10, 14, and 22). However, for all except the *Xenopus* U6 probe (lane 24) there was a strong supershift band (PO) when PTF was present with Oct-1 (lanes 4, 8, 12, 16, and 20). Thus, all of the mammalian PSEs tested are recognized by proteins in the PTF preparation, but Oct-1 binding to adjacent sequences greatly increases PTF binding to the PSEs. The failure to observe a supershift band with the *Xenopus* probe suggests that the human PSE-binding factors in this fraction do not bind efficiently to the amphibian PSE sequence. We cannot rule out the possibility that PTF would bind to this sequence under different assay conditions and/or in the context of the natural octamer and PSE in the amphibian gene. However, we have shown above that the relative positions of the two elements are quite flexible (Fig. 1B).

In contrast to the results obtained with the other mammalian snRNA genes, incubation of the mouse U6 PSE with the PTF fraction resulted in the appearance of three relatively strong complexes (lane 18). This result suggests that PTF may have a higher affinity for the mouse U6 PSE than for the other PSEs. In an analysis of the specificity of these bands (Fig. 2C), two (upper and lower) were inhibited by addition of 20 ng (100-fold molar excess) of an oligonucleotide containing the mouse U6 PSE (lane 9) but not by the same amount of an unrelated oligonucleotide (lane 7). The two specific complexes had the same mobility as the two bands marked with arrows in Fig. 1B (lane 2) and probably represent complexes containing the same protein(s). Addition of 20 ng (100-fold molar excess) of the 7SK PSE oligonucleotide (lane 8) had much less effect on these two specific shifts than did addition of the U6 PSE, further indicating that PTF binds more strongly to the mouse U6 PSE. The mouse U6 PSE oligonucleotide also effectively competed for the Oct-PTF-DNA supershift complexes (PO) formed on all of the mammalian PSEs in the presence of both PTF and Oct-1 (lanes 5, 12, 14, 16, and 18). An equivalent molar amount of oligonucleotide comprising either the U1 (lane 3) or the 7SK (lane 4) PSE did not compete as efficiently as the mouse U6 PSE (lane 5) for the Oct-PTF-DNA supershift complex formed on the 7SK PSE. Since the PSE-dependent supershift complexes observed on the reconstructed templates containing PSE sequences from both RNA polymerase II- and RNA polymerase III-transcribed snRNA genes have the same apparent specificity, we consider it likely that these complexes contain the same protein species and that we are not detecting RNA polymerase II- and RNA polymerase III-specific PSE-binding proteins in the PTF preparation.

The conserved POU domain of both Oct-1 and Oct-2 is sufficient to potentiate PTF binding. The ubiquitous Oct-1 and the B-cell-specific Oct-2 bind identically to the same octamer sequence and stimulate transcription of mRNA-encoding genes both in vitro and in vivo (9, 10, 38, 46, 54, 55, 66; reviewed in reference 51). These factors have been cloned (37, 53, 61, 64) and are closely related members of the POU family of transcription factors, which contain a DNA-binding region composed of a POU-specific domain and a POU-homeo domain (13; reviewed in reference 51). Since both Oct-1 and Oct-2 stimulate transcription of the 7SK gene in vitro (41), we tested various preparations of intact or truncated Oct-1 and Oct-2 to determine whether they were equally capable of Oct-mediated potentiation of PTF binding and which parts of the proteins were required. Figure 3 shows the results of testing natural Oct-1 purified from HeLa cells ($Oct\text{-}1^H$, lanes 2 to 4), recombinant Oct-1 produced in bacteria ($Oct\text{-}1^B$, lanes 5 to 7), recombinant Oct-2 produced by translation in rabbit reticulocyte lysate ($Oct\text{-}2^R$, lanes 8 to 10), recombinant Oct-2 produced in vaccinia virus-infected cells ($Oct\text{-}2^V$, lanes 11 to 13), truncated Oct-1 composed of the POU domain and produced by translation in rabbit reticulocyte lysate (Oct-1p, lanes 14 to 16; see Materials and Methods), and a truncated Oct-2 composed of little more than the POU domain (construct N154/C376 described in reference 10) produced by expression in transfected HeLa cells (Oct-2p, lanes 17 to 19). All of these preparations

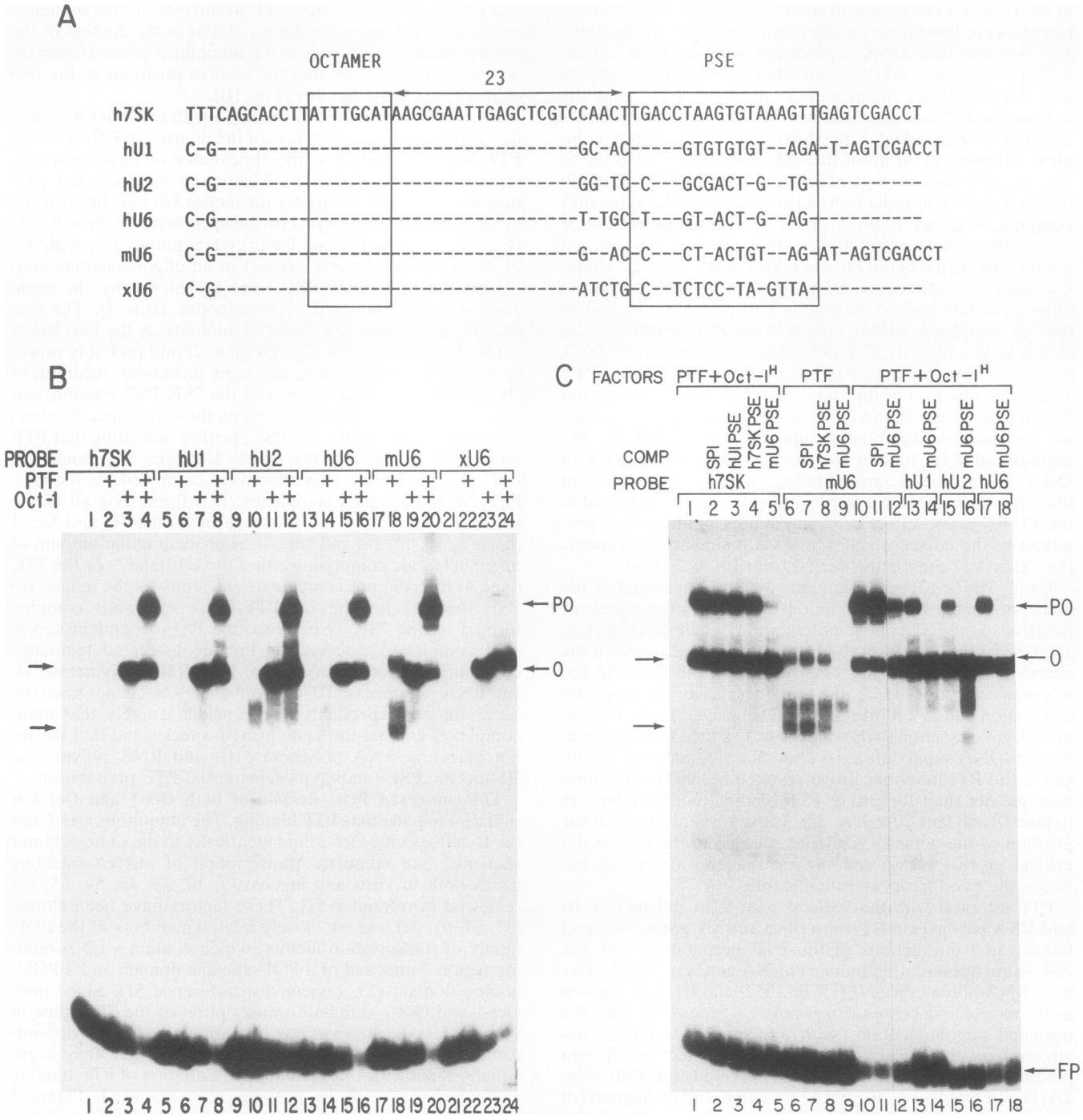


FIG. 2. PTF interacts with the PSE of polymerase II- and III-transcribed genes. (A) Sequences of the probes used for gel retardation assays. The gene corresponding to each PSE is indicated at the left. The sources of the PSE sequences are human U6 (23), human U1 (58), human U2 (1), mouse U6 (6), and *Xenopus* U6 (21). The octamer elements and PSEs are boxed. Dashes indicate that the base is the same as in the 7SK sequence. (B) Mobility shift assay using the probes shown in panel A. The probe used and the factors added to the reaction mixture are indicated above the lanes. The positions of the Oct-1 promoter complex (O) and the PTF-Oct-retarded complexes (PO) are indicated at the right. The arrows at the left indicate putative PTF-retarded complexes that are visible in lanes 2 (after longer autoradiographic exposure) and 18. (C) Competition for PTF binding by different PSE sites from different genes. The type of probe used and the factors added to the reaction mixtures are indicated above the lanes. The competitor (COMP) (20 ng in each case) added to the reaction mixture is indicated above each lane. FP indicates the position of the free probe.

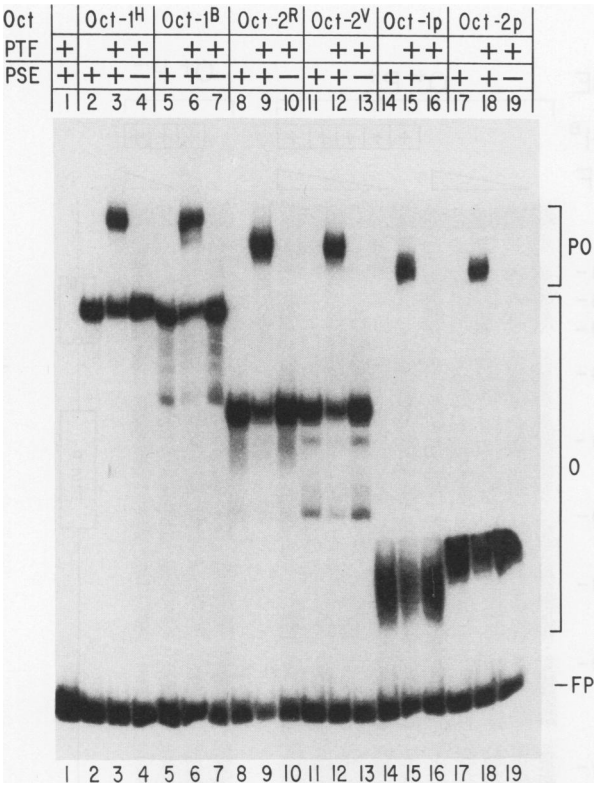


FIG. 3. The conserved POU domain of both Oct-1 and Oct-2 is sufficient to potentiate PTF binding. Shown is a gel retardation assay using various Oct-1 and Oct-2 preparations in conjunction with PTF. The preparation of Oct added to the reaction mixture is indicated above the lanes: Oct-1^H, Oct-1 purified from HeLa cells; Oct-1^B, recombinant Oct-1 produced in bacteria; Oct-2^R, recombinant Oct-2 translated in rabbit reticulocyte lysate; Oct-2^V, recombinant Oct-2 produced by vaccinia virus infection of HeLa cells; Oct-1p, the POU domain of recombinant Oct-1 translated in rabbit reticulocyte lysate; Oct-2p, the POU domain of recombinant Oct-2 produced by overexpression in transfected HeLa cells. See Materials and Methods for detailed descriptions of the proteins used. The probes used were O^+P^+ and O^+P^- (Fig. 1). O, PO, and FP are as defined in the legend to Fig. 2.

potentiated binding of PTF to the 7SK PSE, and no supershift complexes were observed when the PSE was mutated (lanes 4, 7, 10, 13, 16, and 19). Furthermore, the extent of this potentiation was approximately the same in all cases (at least 10- to 15-fold when normalized to the faint bands seen in lane 1). We conclude from these results that the potentiation of PTF binding does not require any mammalian cell-specific modifications of Oct-1, that Oct-1 and Oct-2 are equally effective in potentiation, and, in fact, that the POU DNA-binding domain of either Oct is sufficient for effective potentiation.

PTF interacts with a 30-bp region of the 7SK promoter centered on the PSE. Having determined that bacterially produced recombinant Oct-1 can potentiate binding of PTF to the 7SK PSE, we utilized Oct-1^B to further purify PTF on an oligonucleotide affinity column with a double-octamer site adjacent to the PSE. This column was preloaded with Oct-1^B to saturate the Oct-binding sites and then loaded with a preparation of PTF which had been partially purified on P11 and DE-52. About 90% of the PSE binding activity was retained on this affinity column and subsequently eluted with a linear gradient of KCl (see Materials and Methods). Since PTF began to elute just before Oct-1, some fractions devoid of Oct-1 were obtained. These Oct-free PTF fractions were used for DNase I footprinting studies in conjunction with both HeLa cell-derived and recombinant Oct-1. Figure 4A shows strong interactions of either Oct-1^H alone (lane 3) or Oct-1^B alone (lane 4) over the octamer site of the coding strand of the O^+P^+ probe. Incubation of the probe with PTF alone resulted in little change of the DNase I digestion pattern apart from the appearance of a hypersensitive site at position −43 (lane 5 versus lane 2). However, the same amount of PTF gave very clear protection from positions −44 to −74 in conjunction with either Oct-1^H (lane 6) or Oct-1^B (lane 7). The presence of 40 ng (200-fold molar excess) of a nonspecific competitor oligonucleotide had little effect on the footprints (lane 8), whereas addition of the same amount of an oligonucleotide containing the consensus octamer site resulted in a pattern indistinguishable from that observed upon addition of PTF alone (lane 9). The footprint of Oct-1^B on the O^+P^- and O^+P^+ probes was the same (lanes 4 and 12), but as expected, PTF along with Oct-1^B gave no protection of the mutated PSE on the O^+P^- probe (lane 13). Since we could detect no difference in binding between Oct-1^H and Oct-1^B in this assay, we used Oct-1^B for subsequent footprinting studies.

PTF binding to the noncoding strand of the O^+P^+ probe was examined in a titration analysis in the absence (Fig. 4B, lanes 2 to 5) and presence (lanes 7 to 10) of Oct-1^B. On this strand addition of PTF alone had no obvious effect on the DNase I digestion pattern, whereas incubation of the probe with both PTF and Oct-1 resulted in a clear protection over both the octamer and PSE regions. Under the same conditions no protection was observed over the mutated PSE by PTF (lanes 13 and 14). With these experiments we have mapped the site of interaction of PTF on both strands of the 7SK PSE and have confirmed that the potentiation effect of Oct-1 can be observed in a DNase protection assay as well as in a mobility shift assay. A schematic summary of these data is shown in Fig. 4C.

We also investigated PTF binding to the wild-type 7SK promoter containing sequences extending to −243 (41). Recombinant Oct-1 gave the same extended footprint over the region from −90 to −243 (Fig. 4D, lanes 3 to 5) that was previously observed by using Oct-1 purified from HeLa cells (41). Most importantly, as with the synthetic O^+P^+ construct, incubation with PTF alone resulted in little change of the DNase pattern, apart from the appearance of a hypersensitive site at −43 (lane 7). Addition of both Oct-1 and PTF resulted in a clear footprint over the PSE (lane 6). This result indicates that in vitro the cooperative interactions of these two factors are similar on both the synthetic and natural templates. However, it is unlikely that Oct-1 interacts in exactly the same way with this wild-type template in vivo (see Discussion).

Given reports (19) of functional interactions of an abundant nuclear protein designated Ku antigen (48) with PSE elements, we further purified the peak PTF fraction from the OCTA-PSE affinity column by chromatography on a second DNA affinity column containing Oct-1-binding sites. This effectively separated PTF (which flows through the column under the conditions used) from the Oct-1 which binds. This procedure also separated PTF from contaminating Ku antigen, which was monitored with anti-Ku autoimmune sera and remained bound to the column (data not shown; see Materials and Methods). Importantly, this Ku-free PTF was found to interact cooperatively with both Ku-free purified

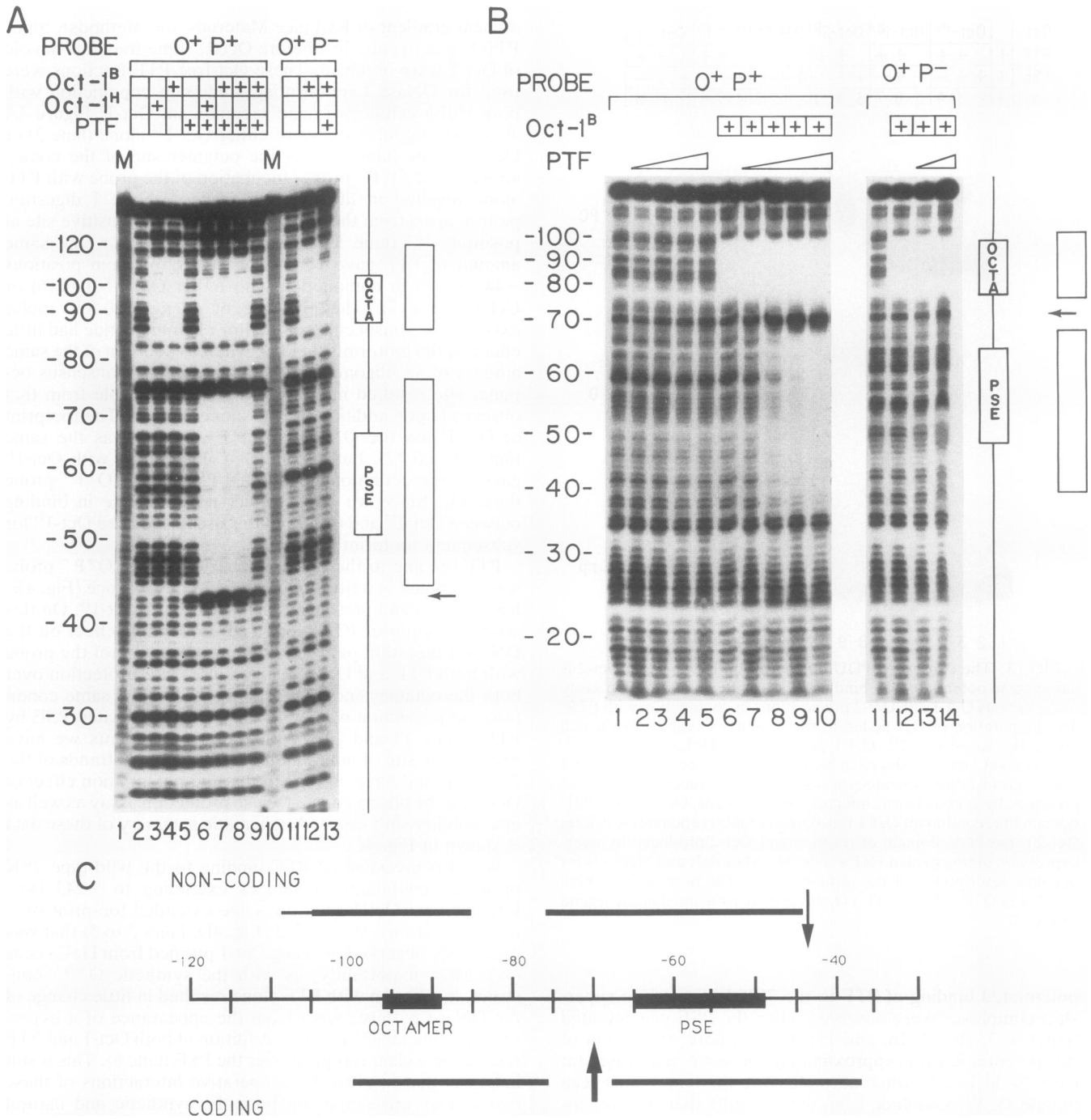
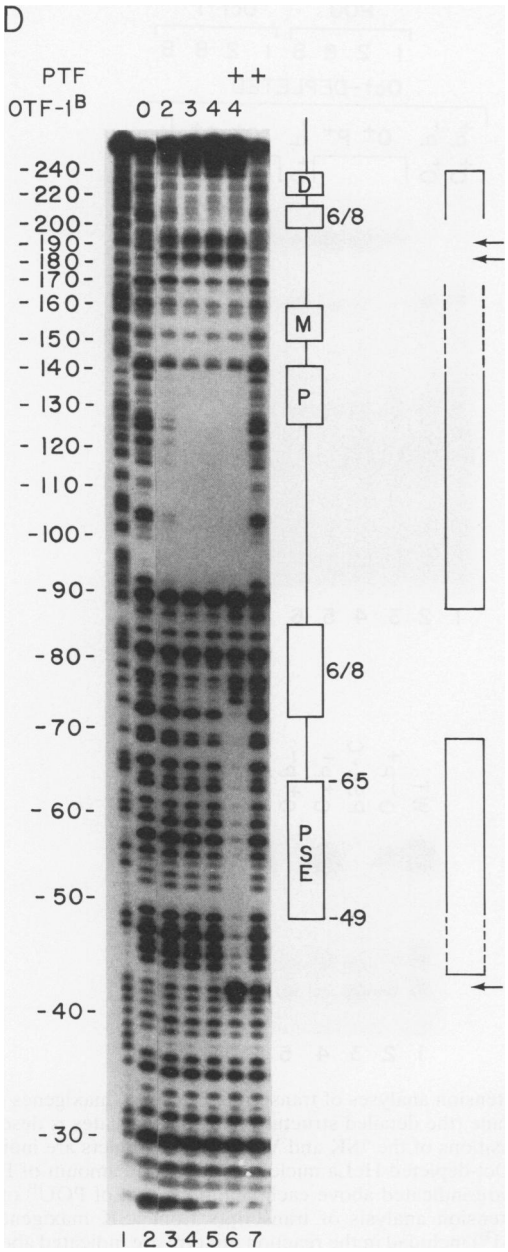


FIG. 4. PTF interacts with a 30-bp region of the 7SK promoter centered on the PSE. (A) DNase protection of the coding strand of O^+P^+ and O^+P^- probes by Oct-1 and PTF. The type of probe used is indicated above each lane. Additions of Oct-1^H (20 fmol), Oct-1^B (20 fmol), and PTF (10 to 20 fmol) were as indicated above the lanes. The reaction mixture in lane 9 contained a 200-fold molar excess of an oligonucleotide (OCTA) containing a consensus octamer element, while the reaction mixture in lane 8 contained a 200-fold molar excess of a related oligonucleotide ($OCTA^-$) with a mutated octamer. The positions of the octamer sequence and the PSE are indicated at the right, while open boxes indicate the extent of the footprints. The arrow indicates a hypersensitive site. M (lanes 1 and 10) indicates size markers. The numbers at the side indicate the distance upstream of the transcription start site. (B) DNase protection of the noncoding strand of O^+P^+ and O^+P^- probes with Oct-1 and PTF. The probe used and the scheme of Oct-1^B and PTF addition are indicated above the lanes. The triangles indicate additions of increasing amounts of PTF to the reactions mixtures: lanes 2 and 7, 2.5 fmol; lanes 3 and 8, 5 fmol; lanes 4, 9, and 13, 10 fmol, lanes 5, 10, and 14, 15 fmol. The positions of the octamer sequence and PSE are indicated at the right. Open boxes indicate the extent of the footprints. The arrow indicates a hypersensitive site. (C) Schematic representation of footprints on the O^+P^+ probe by Oct-1 and PTF. The numbers indicate the distance upstream from the transcription start site. The positions of the octamer sequence and PSE are indicated. The extent of the footprints is shown above (noncoding strand) and below (coding strand) the line. Arrows indicate hypersensitive sites. (D) DNase protection of the coding strand of the wild-type 7SK gene promoter from −243 to −30 with Oct-1^B and PTF. Addition of Oct-1^B (relative amounts, 2 to 20 fmol) and PTF (10 fmol) to the reaction mixture is indicated above each lane. The positions of the elements within the promoter are shown schematically at the side (41), and the boxes at the far right indicate the extent of the footprints. Arrows indicate hypersensitive sites.



Oct-1 and recombinant Oct-1 (data not shown), indicating that Ku antigen is not required for Oct-PTF interaction under the conditions used.

PTF is active in transcription of the 7SK gene in vitro. To establish an assay for testing purified PTF, 7SK maxigenes containing octamer, PSE, and TATA elements (Fig. 1A) in their normal context were transcribed in HeLa cell nuclear extracts and the products were analyzed by quantitative primer extention. The RNA polymerase III-dependent adenovirus VAI gene was included as an internal control. In agreement with the results of previous studies with a Namalwa cell extract (41), the template containing intact octamer and PSE elements was transcribed more efficiently (14-fold in this experiment) than templates with one or both of these elements mutated (Fig. 5A, lane 1 versus lanes 2, 3, and 4). Significantly, the fact that the O^+P^- (lane 2) and O^-P^+ (lane 3) templates were transcribed at essentially the same level as the O^-P^- template (lane 4), which retains only an intact TATA box, indicates that the PSE and octamer elements function in an interdependent manner.

Transcription was then analyzed in extracts in which Oct-1 was selectively depleted with an Oct-specific DNA affinity matrix (Fig. 5B). In this extract, the level of transcription was low and equivalent for the O^+P^+ (lane 1) and the O^+P^- (lane 2) templates, indicating that Oct-1 is necessary for transcriptional activation of the O^+P^+ template. Addition of increasing amounts of the Oct-1 POU domain, produced in bacteria, stimulated transcription of the O^+P^+ template to a maximum of fivefold (lanes 3 to 5), but this protein had little effect on transcription of O^+P^- (lane 6). Addition of bacterially produced Oct-1 ($Oct\text{-}1^B$) stimulated transcription of the O^+P^+ template to a maximum of 12-fold (lanes 7 to 9) but did not increase transcription of the O^+P^- template. These results show that bacterially produced Oct-1 is transcriptionally active in vitro and that the DNA-binding (POU) domain alone is sufficient to stimulate transcription, albeit to a lesser extent than the larger Oct-1 protein.

Transcription was then examined in an extract in which both Oct-1 and PTF were depleted with an OCTA-PSE-DNA affinity matrix (Fig. 5C). As expected, the O^+P^+ (lane 1) and O^+P^- (lane 2) templates were transcribed at the same low (basal) level in the Oct-PTF-depleted extract. Addition of a saturating amount of $Oct\text{-}1^B$ alone resulted in a slight stimulation (about two- to threefold) of transcription from the O^+P^+ template (lane 3) relative to the O^+P^- template (lane 4), perhaps because PTF was not completely depleted or possibly as a result of an effect of Ku antigen in the extract (see Discussion). Addition of 10 fmol of PTF alone had little effect on transcription of either the O^+P^+ (lane 5) or the O^+P^- (lane 6) template. However, addition of either half (lane 7) or the same amount (lane 8) of this PTF in conjunction with saturating amounts of $Oct\text{-}1^B$ resulted in a marked stimulation of transcription from the O^+P^+ template (15-fold in lane 7 and 21-fold in lane 8) relative to that observed with either the O^+P^- (lane 9) or the O^-P^+ (lane 10) template. Indeed, under these conditions the difference in transcription efficiency between the O^+P^+ and O^+P^- templates exceeded that seen in an undepleted nuclear extract (21-fold in this experiment compared to 14-fold in the extract analyzed in Fig. 5A), suggesting that PTF is limiting in the HeLa nuclear extract. We conclude from these results that PTF purified on the basis of its ability to bind to the 7SK PSE is transcriptionally active in vitro and that it functions in a cooperative (interdependent) fashion with purified Oct-1.

Since the templates used for the above-described studies were not identical to the wild-type 7SK gene, we compared their transcription efficiencies following transfection into HeLa cells (Fig. 5D). The single-octamer O^+P^+ template employed above was transcribed (lane 4) about half as well as a wild-type promoter with sequences extending to −243 (lane 1), while an $O_2{}^+P^+$ template containing duplicate octamer elements adjacent to the PSE (lane 6) was transcribed slightly more efficiently than the wild-type template. Mutation of the octamer in the O^+P^+ template reduced transcription sevenfold (lane 1 versus lane 2), consistent with observations (17, 39a) that efficient transcription of the wild-type template in vivo requires DNA sequences between −243 and −200 that contain Oct-1-binding sites (41) (Fig. 4D). Significantly, mutations in the PSE of the wild-type template (lane 3), the O^+P^+ template (lane 5), and the $O_2{}^+P^+$ template (lane 7) reduced transcription in each case

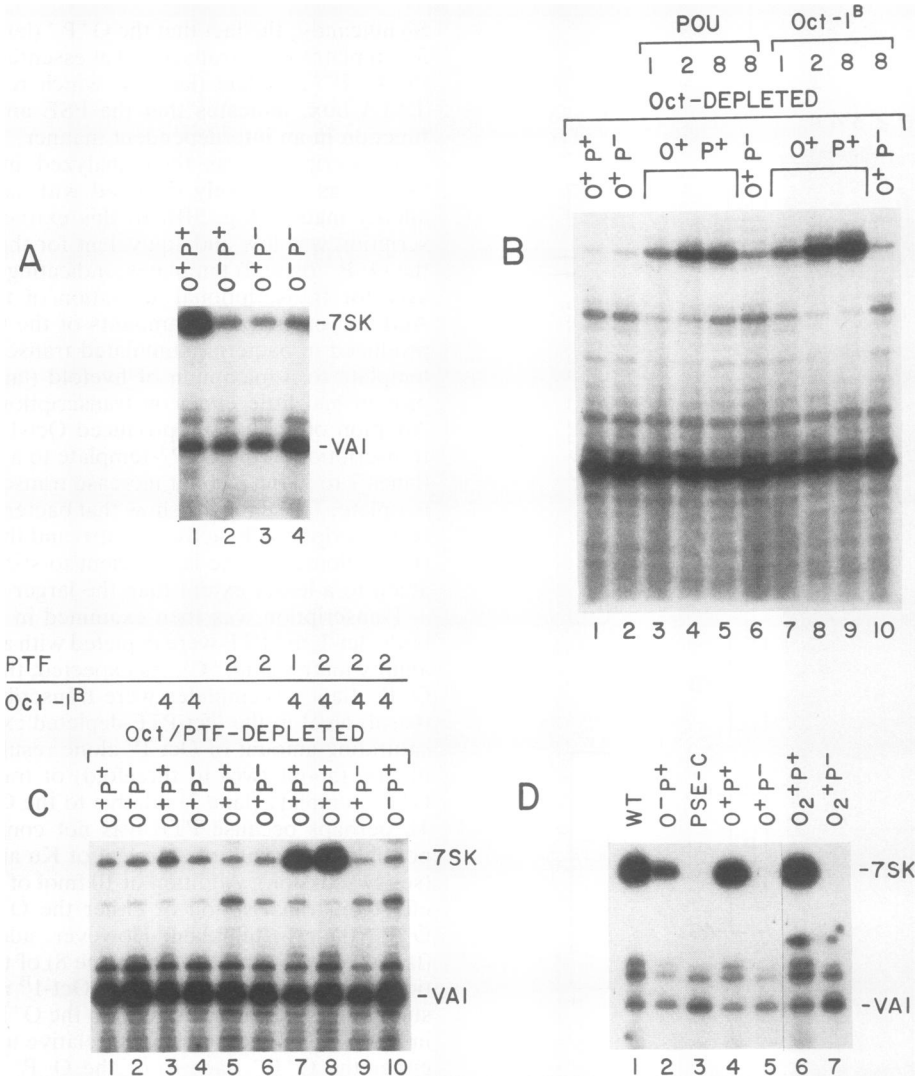


FIG. 5. PTF is active in transcription of the 7SK gene in vitro. (A) Primer extension analyses of transcripts from 7SK maxigenes in HeLa nuclear extract. The template present in each reaction is indicated above each lane (the detailed structure of these templates is described in Materials and Methods). A control VAI gene was included in each lane. The positions of the 7SK and VAI cDNA products are indicated at the right. (B) Primer extension analysis of transcripts from 7SK maxigenes in Oct-depleted HeLa nuclear extract. The amount of POU^B or $Oct\text{-}1^B$ added (in units) and the test template included in the reaction mixture are indicated above each lane. One unit of POU^B or $Oct\text{-}1^B$ represents approximately 3.75 fmol of DNA-binding activity. (C) Primer extension analysis of transcripts from 7SK maxigenes in an Oct-PTF-depleted HeLa nuclear extract. The templates and factors (PTF or $Oct\text{-}1^B$) included in the reaction mixture are indicated above each lane. One unit of PTF and 1 U of $Oct\text{-}1^B$ correspond to approximately 5 and 7.5 fmol of protein, respectively. (D) Primer extension analysis of transcripts from 7SK maxigenes transfected into HeLa cells. The template transfected is indicated above each lane. $O_2{}^+P^+$ and $O_2{}^+P^-$ contains two octamer elements with an intact or mutated PSE, respectively (see Materials and Methods for details). PSE-C represents the natural wild-type (WT) template except for mutations in the PSE site (41).

to undetectable levels. These results indicate that the placement of octamer sites close to the TATA sequence does not simply overcome the need for a PSE sequence in vivo, and they suggest that the reconstructed O^+P^+ templates faithfully mimic at least some of the functional properties of the natural promoter.

DISCUSSION

The PSE is the one critical control element common to both RNA polymerase II- and RNA polymerase III-transcribed snRNA genes and has several potential roles: to coordinate the activities of these genes, to effect stimulation of the basal transcriptional machinery in response to enhancer elements, and, in a related role, to help select the basal transcription system (RNA polymerase II or RNA polymerase III) employed. Our documentation of a factor (PTF) that interacts conditionally with a variety of PSEs and exhibits transcriptional activity through at least one (7SK) is thus of considerable significance. Even if PTF is not directly involved in all of these processes, it will be required, along with other enhancer and general initiation factors, to elucidate the mechanisms involved. Waldschmidt et al. (67) recently reported the identification of a factor (PBP) which bound to the PSE of the mouse U6 gene. This factor was transcriptionally active in vitro and showed some character-

istics similar to those of PTF. Both proteins bind readily to the PSE of the mouse U6 PSE in the absence of Oct-1, and both have similar chromatographic behavior, in that they bind to both phosphocellulose and DEAE resins. However, identity between these two proteins remains to be determined, especially since the PBP studies were restricted to an analysis of the mouse U6 gene and since it is possible that several distinct proteins bind to the PSE of snRNA genes. For example, Knuth et al. (19) have reported that the autoimmune antigen Ku (48) interacts with the PSE of the human U1 gene and is transcriptionally active in vitro (12). We have determined that PTF and Ku are distinct proteins by chromatographic separation (see Results), and although Oct-1 and PTF interact cooperatively in the absence of Ku antigen, the involvement of this highly abundant nuclear protein in transcription of class III snRNA genes remains a possibility.

Functional interactions of PTF with the 7SK PSE. The demonstration of a functional interdependency between the 7SK PSE and an adjacent Oct-binding site in vitro prompted the utilization of an Oct-PSE mobility shift supershift assay which led to the detection and partial purification of a PSE-binding factor designated PTF. Although a partially purified PTF displayed weak binding to the PSE, binding was enhanced markedly by simultaneous interactions of either Oct-1 or Oct-2 with an adjacent octamer. Moreover, even though Octs readily bind to the octamer elements in the absence of PTF, preliminary studies have indicated that these interactions are markedly stabilized in the Oct-PTF-DNA complex. Both of these results indicate direct cooperative interactions between Octs and PTF. It was also observed that the conserved Oct-1 and Oct-2 POU domains involved in DNA recognition (reviewed in reference 51) suffice to enhance PTF binding, indicating primary interactions of PTF with conserved residues and/or structural features therein.

Functional studies in reconstituted transcription systems further showed that PTF is transcriptionally active but that stimulation of the basal promoter activity is dependent upon both PTF and either intact or truncated Oct-1. The inability of either factor alone to efficiently stimulate transcription has important implications for the mechanism of action of these factors (see below). It was also found that natural and recombinant Oct-1 and Oct-2 (39c) are equally active for PSE-dependent transcription by RNA polymerase III. These results confirm previous findings that Oct-1 and Oct-2, usually thought of as activators for RNA polymerase II-dependent promoters, also enhance in vitro transcription by RNA polymerase III—in this case by potentiating the binding of a second factor (PTF) to promoter sequences. However, it remains to be determined whether Oct-2 can stimulate transcription of class III snRNA genes in vivo. Possibly relevant is a recent report (66) suggesting that transcription of the human U2 gene by RNA polymerase II is activated by Oct-1-specific domains and not by Oct-2a (but see reference 69 discussed below).

Is PTF another factor commonly used by RNA polymerases II and III? Apart from Oct-1 and Oct-2 (see above) there are indications that other upstream factors (ATF, SP1, and the CACCC factor) implicated in the activation of class II genes are also involved in transcription by RNA polymerase III (15–17). Surprisingly, more recent work has provided direct evidence that TFIID, long known as a general factor for transcription of TATA-containing promoters by RNA polymerase II (42), also is involved in transcription by RNA polymerases I and III (4, 30, 31, 57, 68; reviewed in reference 56). In the case of the human U6 promoter this involves TFIID interactions with the functional U6 TATA element (30, 22, 57), and, paradoxically, the TATA box of vertebrate class III snRNA genes is a major determinant of RNA polymerase specificity (29, 32). Recombinant human TFIID also binds to and protects from DNase digestion a 25-bp region centered on the TATA element of the human 7SK promoter (39b). Ironically, a role for TFIID in transcription of the TATA-less class II snRNA promoters has not yet been shown, but the apparent TFIID requirement for some mRNA-encoding genes lacking TATA elements (59) leaves open this possibility.

Related to these observations on general and gene-specific factors, it is shown here that PTF binds avidly to the PSEs of human U1 and U2 promoters by an Oct-dependent mechanism indistinguishable from that described for the 7SK and U6 promoters. Our data are therefore consistent with earlier suggestions that the enhancer of the polymerase II-dependent snRNA genes functions by facilitating stable association of a limiting factor with the promoter (1, 33), and it seems likely that PTF is also involved in the transcription of snRNA genes by RNA polymerase II. This further complicates the puzzle regarding involvement of an array of factors normally utilized by RNA polymerase II in the transcription of snRNA genes by RNA polymerase III. As discussed by Lescure et al. (28), one key to RNA polymerase specificity may be the particular spatial juxtaposition of the various promoter elements and the resulting constraints imposed on various factor-factor and factor-polymerase interactions. For example, Oct-PTF-TFIID interactions may generate complexes recognized uniquely by RNA polymerase III and its accessory factors. As a related possibility, the 38-kDa human TFIID subunit that binds the TATA element may be a common subunit of larger RNA polymerase-specific complexes that can selectively recognize, in the proper context, not only class II and class III TATA elements but also class I, II, and III promoters lacking TATA elements (4, 30, 31, 56, 57, 68). Relevant to this idea, recent studies have identified factors that are tightly bound to the TATA-binding polypeptide in natural TFIID (8) as well as soluble factors that bind to the recombinant TFIID polypeptide to influence transcription (35, 36).

Transcriptional activation involving Octs. The present studies with isolated factors show that the 7SK promoter is activated by PTF and Oct-1 in an interdependent fashion, even though Oct-1 binds efficiently in the absence of PTF. In the simplest model, the Oct has little or no intrinsic activation potential (activating surfaces that contact the basic transcription machinery) but merely recruits the potent activator PTF to the promoter via the potentiation of PTF binding. In a variation of this model, neither Oct nor PTF alone is sufficient to efficiently activate transcription and an activation domain either is induced (exposed) by the action of one of these factors on the other or is formed by joint contributions from each. The finding that the POU domain of Oct-1 stimulates 7SK transcription in vitro (with PTF) indicates either that activation domains reside within this region (and therefore do not correspond to those mapped in the transcription of mRNA genes; see below) or that interactions between the POU domain and PTF and the resulting potentiation of PTF binding are sufficient for activation. However, stimulation in vitro is more effective with the larger recombinant Oct-1^{B}, suggesting that activation by Oct proteins may be a multistep process in this case, with activation domains outside the DNA-binding domain (see below) required for full activity.

How do the present results and the proposed mechanisms relate to the role of Octs in the regulation of mRNA-encoding genes through octamer elements? Three examples are relevant.

(i) Histone H2B promoter. S-phase induction is effected by an octamer element, indicating regulation via Oct-1 (26). Although Oct-1 stimulates transcription through this element in vitro (9, 46), the constant level and DNA-binding activity of Oct-1 during induction (25) suggest the involvement of a second regulatory factor, which could either activate Oct-1 by covalent modification (50) or contribute an activation domain by stable interaction with Oct-1.

(ii) Herpesvirus immediate-early promoters. Induction by the viral activator VP16 involves distal control elements with Oct-1-binding sites and contiguous sequences for VP16 recognition (11, 20). Although VP16 alone may bind weakly (20), Oct-1 binding stimulates markedly the formation of a stable complex involving Oct-1, VP16, and another cellular factor(s) (11, 34, 43). Since the potent activation domain of VP16 functions efficiently when covalently linked to the DNA-binding domains of heterologous factor (52), Oct-1 and the other factors appear to have a role as tethering factors. Whether this is the sole function in the context of the natural promoters (with distal regulatory elements) remains to be determined. Relevant to this question is the observation that the POU domain of Oct-1 is sufficient for VP16 recruitment to the promoter (20), whereas activation domains outside the POU domain have been mapped in other assays (65, 66).

(iii) Ig promoters. B-cell-specific activation is mediated via the octamer element, and the existence of lymphoid-specific Oct-2 has led to the presumption that it plays the major regulatory role for immunoglobulin (Ig) promoters (reviewed in reference 46). Consistent with this, purified Oct-2 was shown to activate Ig promoters in vitro (54) and ectopic expression of Oct-2 in HeLa cells activated artificial lymphoid-specific promoters and allowed mapping of N- and C-terminal activation domains (10, 38, 65). However, transcriptional activation of natural B-cell-specific (Ig) promoters by ectopic Oct-2 in HeLa cells requires high concentrations of Oct-2 and is very inefficient compared with octamer-dependent expression in B cells (10). More recently it has been shown that purified Oct-1 and Oct-2 have equal potential for octamer-dependent activation of Ig and H2B promoters in vitro (27, 46), whereas efficient B-cell-specific transcription of Ig promoters requires a novel non-DNA-binding B-cell-specific accessory factor that works efficiently with either Oct-1 or Oct-2 (46). This factor has been separated from Oct-2 and Oct-1, with which it interacts, and appears to be a more important determinant of tissue-specific Ig promoter function than is Oct-2 (30a). Thus, in these cases there is direct or indirect evidence that activation via Oct recognition sites involves not only interacting Octs but also equally important secondary factors whose interactions depend both on Oct binding and, in some cases, on additional DNA sequence elements. In light of these results, the relationship found for Octs and PTF on the snRNA genes is not surprising. However, this does provide the first clear demonstration of Octs interacting with cell-encoded proteins other than themselves (see below).

Protein-protein interactions involving POU-domain factors is not uncommon (reviewed in reference 51). The best-studied case is the heterologous Oct-1–VP16 interaction (see above), which has features in common with the Oct-PTF interactions. These include recruitment of an essential secondary factor into a promoter complex with the Oct and the direct recognition of DNA sequences by the secondary factor (see above). However, in the case of the Oct-PTF interaction, the two binding sites are clearly separable, whereas the VP16–Oct-1 complex forms directly over the target TAATGARAT. Furthermore, PTF binding is potentiated equally well by the Oct-1 and Oct-2 POU domains, implicating conserved residues in the interaction. In contrast, Oct-2 is remarkably inefficient relative to Oct-1 in promoting the binding of VP16 (9), and the basis for this difference has been mapped to a few nonconserved residues in the Oct-1 POU-homeo domain (62) that is otherwise sufficient for complex formation (20). Possibly relevant to this and in agreement with past observations that Oct-1 and Oct-2 are both proficient in 7SK promoter activation in vitro (41), Yang et al. (69) have reported that a hybrid Oct-2 protein can activate transcription from the U2 promoter by RNA polymerase II and that the POU-homeo domain (replaced with the GAL4 DNA-binding domain) is not required (but see also reference 66 discussed above). Thus, the regions of the POU domains involved in PTF versus VP16 interactions are clearly not identical and might be distinct. In view of the proposed mechanisms (see above), key questions for the future are the exact POU domain structural determinants for PTF binding and how regions outside the POU domains contribute to activation of snRNA promoters.

How does the 7SK gene enhancer work in vivo? We showed earlier that degenerate octamer sequences between −200 and the PSE could mediate Oct-dependent activation of 7SK promoter in vitro and that the region between −240 and −200 is not required (41). It was further shown here that recombinant Oct-1 can bind to these degenerate octamer sequences and potentiate PTF binding to the PSE (Fig. 4D), which could account for the activation of transcription observed in vitro. However, in contrast to these results and in agreement with Kleinert and coworkers (17, 18), we find that removal of the region of the 7SK gene between −240 and −200 results in the loss of enhancer function in vivo (39a). Interestingly, this region contains two degenerate octamer-binding sites (at −236 and −218) with a CACCC sequence between them (17, 18, 41). Kleinert et al. (17, 18) showed that the −236 octamerlike sequence of the 7SK promoter had no effect on enhancer activity, while mutation of the CACCC sequence severly impaired enhancer function. However, the possible involvement of octamer-binding sequences was not ruled out, since mutation of the 6/8 octamer-like sequence at position −218 was necessary to completely eliminate enhancer activity in these studies (17, 18). We have shown that both purified (41) and recombinant (Fig. 4D) Oct-1 can bind to this sequence, which is reminiscent of the functional 6/8 octamer-like sequence at position −219 of the human U1 gene (58). In addition, the sequence at −218 of the 7SK gene corresponds closely to the consensus noted by Dahlberg and Lund (5) for human snRNA gene enhancers. It appears then that both the CACCC sequence and the adjacent 6/8 octamer are required for full enhancer activity, which is similar to the case of the U2 gene enhancer, in which an SP1 site just upstream of the consensus octamer is required but not sufficient for efficient expression of the gene (16, 69). Thus, the 7SK gene may have the same general structure as the other mammalian snRNA genes, which have octamer or octamerlike sequences in the enhancer region located around −220, approximately 160 bp upstream from the PSE. Indeed, in agreement with our own published studies, Kleinert et al. (17) showed that a consensus octamer sequence placed at −230 could functionally replace the natural enhancer of the 7SK gene. We have also shown here that a single octamer placed upstream of the 7SK

PSE efficiently activates transcription of the 7SK gene in vivo, indicating that the sequences between −220 and −70 are not required in vivo for activation via an octamer sequence placed at −90 and emphasizing that this relatively simple arrangement functionally mimics the more complex wild-type promoter structure.

One possibility to account for the discrepancy between the in vivo and in vitro results is that binding of factors to sequences within the enhancer is highly favored by cooperative interactions between the proteins binding in this region (i.e., Octs and the CACCC-binding protein). Alternatively, the region between the enhancer and the PSE may be occluded in vivo. For instance, the distance between the enhancer and the PSE could accommodate one nucleosome (49), and a structure in which the enhancer and the PSE are positioned at the exit and entrance to the coil can be envisaged. This would allow Octs and other proteins bound to the enhancer to easily contact proteins bound to the PSE. Such an arrangement would allow another level of control over snRNA gene activity and could account for the strict positioning of the mammalian snRNA gene enhancer.

In summary, we propose that potentiation of PTF binding by Octs plays a key role in snRNA gene transcription in vivo by both RNA polymerase II and RNA polymerase III. Recruitment of a strong activation domain to the transcription complex through cooperative interactions would provide a simple mechanism of octamer-mediated enhancer function and would allow fine tuning of snRNA gene transcription. Protein-protein contacts between PTF and Oct-1 might stabilize the PTF-DNA complex by lowering the off rate. Alternatively, Oct-1 interactions could induce a PTF conformational change that increases the affinity for the PSE (and perhaps at the same time exposes an activation domain). Detailed dissection of this mechanism will be of considerable importance to our understanding of how activators in general and Octs in particular act to set up a functional transcription complex.

ACKNOWLEDGMENTS

We thank Jeff DeJong and Akiko Hata for kindly providing protein fractions from S100 extracts after ammonium sulfate precipitation and P11 chromatography and Westley Reeves for the donation of anti-Ku autoimmune serum and for carrying out Western blotting on fractions from preliminary chromatography. We are grateful to Richard Sturm, Gokul Das, and Winship Herr for kindly providing the pBSoct1+ clone and to Kathy Scotto for help in quantitating data with the Betagen radioimage analyzer. We are indebted to many members of the Roeder lab for scientific and technical advice and especially to Philippe Pognonec for help with Western blotting and to Yan Luo for an Oct preparation. We also thank Merlin Crossley, Helen Mardon, and Susan Mallet for critical reading of the manuscript and N. J. Proudfoot for his patience.

J.-B.Y. is a Leukemia Society of America Inc. Special Fellow. T.G. was supported by the Schweizerischer Nationalfonds. This work was further supported by grants from the U.S. Public Health Service to R.G.R. (CA42567 and RR05869) and from the Pew Charitable Trusts to The Rockefeller University.

ADDENDUM IN PROOF

Recently, Simmens et al. (J. Mol. Biol. **223**:873–884, 1992) have compared the independent binding of a human factor (PBP) to human, mouse, and *Xenopus* PSE elements, with results consistent with those reported here for PTF.

REFERENCES

1. **Ares, M., Jr., M. Mangin, and A. M. Weiner.** 1985. Orientation-dependent transcriptional activator upstream of a human U2 snRNA gene. Mol. Cell. Biol. **5**:1560–1570.
2. **Carbon, P., S. Murgo, J. P. Ebel, A. Krol, G. Tebb, and I. W. Mattaj.** 1987. A common octamer motif binding protein is involved in the transcription of the U6 snRNA by RNA polymerase III and U2 snRNA by RNA polymerase II. Cell **51**:71–79.
3. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolated by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:156–159.
4. **Comai, L., N. Tanese, and R. Tjian.** 1992. The TATA-binding protein and associated factors are integral components of the RNA polymerase I transcription factor, SL1. Cell **68**:965–976.
5. **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.
6. **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.
7. **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.
8. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66**:1–20.
9. **Fletcher, C., N. Heintz, and R. G. Roeder.** 1987. Purification and characterization of Oct-1, a transcription factor regulating cell cycle expression of a human histone H2b gene. Cell **51**:773–781.
10. **Gerster, T., C.-G. Balmeceda, and R. G. Roeder.** 1990. The cell type-specific octamer transcription factor Oct-2 has two domains required for the activation of transcription. EMBO J. **9**:1635–1643.
11. **Gerster, T., and R. G. Roeder.** 1988. A herpes virus transactivating protein interacts with transcription factor Oct-1 and other cellular proteins. Proc. Natl. Acad. Sci. USA **85**:6347–6351.

11a. **Gerster, T., and R. G. Roeder.** Unpublished results.

12. **Gunderson, S. I., M. W. Knuth, 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.
13. **Herr, W., R. A. Sturm, R. G. Clerc, L. M. Corcoran, D. Baltimore, P. A. Sharp, H. A. Ingraham, M. G. Rosenfeld, M. Finney, G. Ruvken, and H. R. Horvitz.** 1988. The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and *Caenorhabditis elegans* unc-86 gene products. Genes Dev. **2**:1513–1516.
14. **Hoffmann, 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.
15. **Howe, J. G., and M.-D. Shu.** 1989. Epstein-Barr virus small RNA (EBER) genes: unique transcription units that combine RNA polymerase II and III promoter elements. Cell **57**:825–834.
16. **Janson, L., and U. Peterson.** 1990. Cooperative interaction between transcription factors Sp1 and OTF-1. Proc. Natl. Acad. Sci. USA **87**:4732–4736.
17. **Kleinert, H., R. Assert, and B.-J. Benecke.** 1991. A single base pair deletion from the inactive octamer-like motif of the 7SK distal sequence element brings full functionality in vivo. J. Biol. Chem. **266**:23872–23877.
18. **Kleinert, H., S. Bredow, and B. J. Benecke.** 1990. Expression of a human 7SK gene in vivo requires a novel pol III upstream element. EMBO J. **9**:711–718.
19. **Knuth, M. W., S. I. Gunderson, M. E. Thompson, L. A. Strasheim, 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.
20. **Kristie, T. M., and P. A. Sharp.** 1990. Interactions of the Oct-1 POU subdomains with specific DNA sequences and with the HSV α-trans-activator protein. Genes Dev. **4**:2383–2396.
21. **Krol, A., P. Carbon, J. P. Ebel, and B. Appel.** 1987. *Xenopus tropicalis* U6 snRNA genes transcribed by pol III contain upstream elements used by pol II dependent U snRNA genes. Nucleic Acids Res. **15**:2463–2478.
22. **Kunkel, G. R.** 1991. RNA polymerase III transcription of genes that lack internal control regions. Biochim. Biophys. Acta **1088**:1–9.
23. **Kunkel, G. R., and T. Pederson.** 1988. Upstream elements required for efficient transcription of a human U6 gene resemble those of U1 and U2 genes even though a different polymerase is used. Genes Dev. **2**:196–204.
24. **Kunkel, T. A.** 1985. Rapid and efficient site specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82**:488–492.
25. **La Bella, F., P. Gallinari, J. McKinney, and N. Heintz.** 1989. Histone H1 subtype-specific consensus elements mediate cell cycle-regulated transcription in vitro. Genes Dev. **3**:1982–1990.
26. **La Bella, F., H. L. Sive, R. G. Roeder, and N. Heintz.** 1988. Cell-cycle regulation of a human histone H2b gene is mediated by the H2b subtype-specific DNA consensus element. Genes Dev. **2**:32–39.
27. **LeBowitz, J. H., T. Kobayashi, L. Staudt, D. Baltimore, and P. A. Sharp.** 1988. Octamer-binding proteins from B or HeLa cells stimulate transcription of the immunoglobulin heavy-chain promoter in vitro. Genes Dev. **2**:1227–1237.
28. **Lescure, A., P. Cabon, and A. Krol.** 1991. The different positioning of the proximal sequence element in the Xenopus RNA polymerase II and III snRNA promoters is a key determinant which confers RNA polymerase III specificity. Nucleic Acids Res. **19**:435–441.
29. **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.
30. **Lobo, S. M., J. Lister, M. L. Sullivan, and N. Hernandez.** 1991. The cloned RNA polymerase II transcription factor IID selects RNA polymerase III to transcribe the human U6 gene in vitro. Genes Dev. **5**:1477–1489.

30a. **Luo, Y., H. Fujii, and R. G. Roeder.** Unpublished observations.

31. **Margottin, F., G. Dujardin, M. Gerard, J.-M. Egly, J. Huet, and A. Sentenac.** 1990. Participation of the TATA factor in transcription of the yeast U6 gene by RNA polymerase C. Science **251**:424–426.
32. **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.
33. **Mattaj, I. W., S. Lienhard, J. Jiricny, and E. M. DeRobertis.** 1985. An enhancer-like sequence within the *Xenopus* U2 gene promoter facilitates the formation of stable transcription complexes. Nature (London) **316**:163–167.
34. **McKnight, J. L. C., T. M. Kristie, and B. Roizman.** 1987. The binding of the virion protein mediating a gene induction in herpes simplex virus 1 infected cells to its cis site requires cellular proteins. Proc. Natl. Acad. Sci. USA **84**:7061–7065.
35. **Meisterernst, M., and R. G. Roeder.** 1991. A family of proteins which interact with TFIID and regulate promoter activity. Cell **67**:557–567.
36. **Meisterernst, M., A. L. Roy, H. M. Lieu, and R. G. Roeder.** 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. Cell **66**:1–20.
37. **Muller, M. M., S. Ruppert, W. Schaffner, and P. Matthias.** 1988. A cloned octamer transcription factor stimulates transcription from lymphoid-specific promoters in non-B cells. Nature (London) **336**:544–551.
38. **Muller-Immergluck, M. M., W. Schaffner, and P. Matthias.** 1990. Transcription factor oct-2A contains functionally redundant activating domains and works selectively from a promoter but not from a remote enhancer position in non-lymphoid (HeLa) cells. EMBO J. **9**:1625–1634.
39. **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.

39a. **Murphy, S., T. Gerster, and R. G. Roeder.** Unpublished observations.

39b. **Murphy, S., A. Hoffman, and R. G. Roeder.** Unpublished observations.

39c. **Murphy, S., Y. Luo, T. Gerster, and R. G. Roeder.** Unpublished observations.

40. **Murphy, S., B. Moorefield, and T. Pieler.** 1989. Common mechanisms of promoter recognition by RNA polymerases II and III. Trends Genet. **5**:122–126.
41. **Murphy, S., A. Pierani, C. Scheidereit, M. Melli, and R. G. Roeder.** 1989. Purified octamer binding transcription factors stimulate RNA polymerase III-mediated transcription of the 7SK gene. Cell **59**:1071–1080.
42. **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.
43. **O'Hare, P., and C. R. Goding.** 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both targets for virus transactivation. Cell **52**:435–445.
44. **Palmer, J. M., and W. R. Folk.** 1990. Unravelling the complexities of transcription by RNA polymerase III. Trends Biochem. **15**:300–304.
45. **Parry, H. D., D. Scherly, and I. W. Mattaj.** 1989. Snurpogenesis: the transcription and assembly of U snRNA components. Trends Biochem. **14**:15–18.
46. **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 for optimal transcription of immunoglobulin promoters. Mol. Cell. Biol. **10**:6204–6215.
47. **Reddy, R., and R. Singh.** 1991. Synthesis of small nuclear RNAs. Prog. Mol. Subcell. Biol. **12**:1.
48. **Reeves, W. H., and Z. M. Sthoeger.** 1989. Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. J. Biol. Chem. **264**:5047–5052.
49. **Richmond, T. J., J. T. Finch, B. Rushton, D. Rhodes, and A. Klug.** 1984. Structure of the nucleosome core particle of 7A resolution. Nature (London) **311**:532–537.
50. **Roberts, S. B., N. Segil, and N. Heintz.** 1991. Differential phosphorylation of the transcription factor Oct1 during the cell cycle. Science **253**:1022–1026.
51. **Rosenfeld, M. G.** 1991. POU-domain transcription factors: pouer-ful developmental regulators. Genes Dev. **5**:897–907.
52. **Sadowski, I., J. Ma, S. Treyenberg, and M. Ptashne.** 1988. Gal4-VP16 is an unusually potent transcriptional activator. Nature (London) **335**:563–564.
53. **Scheidereit, C., J. A. Cromlish, T. Gerster, K. Kawakami, C.-G. Balmeceda, R. A. Currie, and R. G. Roeder.** 1988. A human lymphoid-specific transcription factor that activates immunoglobulin genes is a homeobox protein. Nature (London) **336**:551–557.
54. **Scheidereit, C., A. Heguy, and R. G. Roeder.** 1987. Identification and purification of a human lymphoid-specific octamer binding protein (Oct-2) that activates transcription of an immunoglobulin promoter in vitro. Cell **51**:783–793.
55. **Schreiber, E., P. Matthias, M. M. Muller, and W. Schaffner.** 1988. Identification of a novel lymphoid-specific octamer-binding protein (Oct-2B) by proteolytic clipping bandshift assay (PCBA). EMBO J. **7**:4221–4229.
56. **Sharp, P. A.** 1992. TATA-binding protein is a classless factor. Cell **68**:819–821.
57. **Simmen, K. A., J. Bernues, H. D. Parry, H. G. Stunenberg, A. Berkenstam, B. Cavallini, J.-M. Egly, and I. W. Mattaj.** 1991. TFIID is required for in vitro transcription of the human U6 gene by RNA polymerase III. EMBO J. **10**:1853–1862.
58. **Skuzeski, J. M., E. Lund, J. T. Murphy, T. H. Steinberg, R. R.**

Burgess, and J. E. Dahlberg. 1987. Synthesis of human U1 RNA II: identification of two regions of the promoter essential for transcription initiation at position +1. J. Biol. Chem. **259:**8345–8352.

59. **Smale, S. T., M. C. Schmidt, A. J. Berk, and D. Baltimore.** 1990. Transcriptional activation by Sp1 as directed through TATA or initiator: specific requirement for mammalian transcription factor IID. Proc. Natl. Acad. Sci. USA **87:**4509–4513.
60. **Sollner-Webb, B.** 1988. Surprises in polymerase III transcription. Cell **52:**153–154.
61. **Staudt, L. M., R. G. Clerc, H. Singh, J. H. LeBowitz, P. A. Sharp, and D. Baltimore.** 1988. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. Science **241:**577–580.
62. **Stern, S., M. Tanaka, and W. Herr.** 1989. The Oct-1 homoeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. Nature (London) **341:**624–630.
63. **Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. **185:**60–89.
64. **Sturm, R. A., G. Das, and W. Herr.** 1988. The ubiquitous octamer-binding protein oct-1 contains a POU domain with a homeo box subdomain. Genes Dev. **2:**1582–1599.
65. **Tanaka, M., and W. Herr.** 1990. Differential transcriptional activation by oct-1 and oct-2: interdependent activation domains induce oct-2 phosphorylation. Cell **60:**375–386.
66. **Tanaka, M., J.-S. Lai, and W. Herr.** 1992. Promoter-selective domains in Oct-1 and Oct-2 direct differential activation of an snRNA promoter. Cell **68:**755–767.
67. **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.
68. **White, R., S. P. Jackson, and P. W. Rigby.** 1992. A role for the TATA-box-binding protein component of the transcription factor IID complex as a general RNA polymerase III transcription factor. Proc. Natl. Acad. Sci. USA **89:**1949–1953.
69. **Yang, J., M. M. Muller-Immergluck, K. Seipel, L. Janson, G. Westin, W. Schaffner, and U. Petterson.** 1991. Both Oct-1 and Oct-2A contain domains which can activate the ubiquitously expressed U2 snRNA genes. EMBO J. **10:**2291–2296.