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The Oct-1 POU-Specific Domain Can Stimulate Small Nuclear RNA Gene Transcription by Stabilizing the Basal Transcription Complex $SNAP_c$

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The RNA polymerase II and III human small nuclear RNA promoters have a common basal element, the proximal sequence element, which binds the TATA box-binding protein-containing complex $SNAP_c$. They also contain an enhancer characterized by a highly conserved octamer sequence, which constitutes a binding site for the broadly expressed POU domain transcription factor Oct-1. The POU domain is a bipartite DNA-binding domain consisting of a POU-homeo (POU_H) domain and a POU-specific (POU_S) domain joined by a flexible linker. Here, we show that the Oct-1 POU domain but not the related Pit-1 POU domain can facilitate the binding of $SNAP_c$ to the proximal sequence element, and activate transcription. The effect is probably mediated by protein-protein contacts, and 1 of 30 amino acid differences between the Oct-1 and Pit-1 POU_S domains is the key determinant for the differential interaction with $SNAP_c$ and the ability to activate transcription. These results show that a function that is the hallmark of activation domains, namely, recruitment of a basal transcription complex resulting in activation of transcription, can be performed by a DNA-binding domain. In this case, subtle changes between activator DNA-binding domains, as subtle as a single amino acid difference, can profoundly affect interaction with the basal transcription machinery.

Transcription is stimulated by the binding of various activators to specific sites in the DNA. These activators are modular, typically containing separable DNA-binding and activation domains (see reference 38 for a review) that target the activator to the correct promoter and activate transcription, respectively. Transcription activation apparently results from the facilitation of several steps during complex assembly, including recruitment of TATA-binding protein (TBP) (or the TBP-containing complex TFIID), TFIIB, and, in later steps, other members of the initiation complex, through direct contacts between activation domains and various members of the basal transcription machinery including the TBP-associated factors present in the TFIID complex. The discovery of the holoenzyme, which contains RNA polymerase II and all or nearly all the general transcription factors, in yeast (18, 20) and mammalian (35) cells raises the possibility that in vivo, the general transcription factors and RNA polymerase II are recruited to the promoter in one step as part of a preassembled complex (4, 21). This possibility does not, however, change the view that one of the roles of activation domains is to facilitate the recruitment of general transcription factors through direct protein-protein interactions.

Activation domains, then, play a major role in transcription activation. The role of DNA-binding domains is not, however, always limited to just targeting the activator to the DNA. Indeed, DNA-binding domains such as the POU domain have been implicated in the recruitment of regulatory cofactors to promoters. The POU domain is a bipartite DNA-binding domain consisting of two helix-turn-helix-containing DNA-binding structures: an amino-terminal POU-specific (POU_S) domain and a carboxy-terminal POU-homeo (POU_H) domain joined by a flexible linker (1, 8, 16, 19; see reference 15 for a review). It is the hallmark of a large family of transcription factors including the closely related ubiquitous Oct-1 and B-cell Oct-2 octamer sequence (ATGCAAAT)-binding transcription factors and the pituitary Pit-1 transcription factor. The Oct-1 and Oct-2 POU domains can recruit a B-cell-specific cofactor, variously termed OCA-B, OBF-1, and Bob1, to octamer motif-containing immunoglobulin promoters (11, 31, 32, 42). In addition, the Oct-1 but not the Oct-2 POU domain can recruit the herpes simplex virus protein VP16 to *cis*-acting elements in the herpes simplex virus immediate-early promoters (10, 22, 41). In this case, the interaction is directed by the POU_H domain (41) and a single amino acid difference between the Oct-1 and Oct-2 POU_H domains is the primary determinant for selective association (26, 36).

In addition to factors involved in transcription activation, the POU domains of Oct-1 and Oct-2 recruit a basal transcription factor complex called PTF to the human 7SK and small nuclear RNA (snRNA) promoters (34). The RNA polymerase III 7SK promoter and the RNA polymerase II and III snRNA promoters, which direct the synthesis of short RNAs mostly involved in RNA processing, contain a basal regulatory element called the proximal sequence element (PSE) (reviewed in references 14 and 30). By itself, the PSE is sufficient to direct basal levels of RNA polymerase II transcription, whereas in RNA polymerase III snRNA promoters, the PSE works in concert with a downstream TATA box. Several PSE-binding factors, referred to as PBP (48), PTF (34, 49), and $SNAP_c$ (40), have been described. Both PTF and $SNAP_c$ correspond to multisubunit complexes required for transcription of RNA polymerase II and III snRNA genes (13, 40, 49), and it is now clear that they are related or even identical, because they have at least two common subunits as determined by the characterization of corresponding cDNAs (13, 39, 50). Because purified $SNAP_c$ preparations contain variable amounts of TBP and TBP can be coimmunoprecipitated with an antibody directed against

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SNAP43, one of the $SNAP_c$ subunits (13), we have described $SNAP_c$ as a TBP-containing complex. TBP is, however, less tightly associated with the SNAP complex than are the other subunits (13), and pure PTF does not contain TBP (49).

In addition to basal elements, the snRNA promoters contain an upstream enhancer (the distal sequence element [DSE]) characterized by the presence of an Oct-1 and Oct-2 binding site (see references 14 and 30 for reviews). Interestingly, Murphy et al. (34) showed that both the Oct-1 and Oct-2 POU DNA-binding domains alone can enhance transcription of the 7SK gene in vitro and can recruit PTF to the 7SK PSE, indicating a potentially important interaction between an activator and basal transcription-regulatory complex. Here, we show that the Oct-1 and Oct-2 POU domains but not the Pit-1 POU domain enhance the binding of $SNAP_c$ to a human PSE by increasing its on-rate. The effect is mediated by a protein-protein interaction that depends largely on one of many amino acid differences between the Oct-1 and Pit-1 POU_S domains (residue 7). Remarkably, our studies show that the abilities of POU domains to enhance snRNA gene transcription in vitro correlate completely with their abilities to recruit $SNAP_c$ to the PSE. Thus, like an activation domain, a DNA-binding domain can activate transcription by facilitating promoter recruitment of a basal transcription complex.

MATERIALS AND METHODS

Constructs. (i) Constructs for PCR probes. Plasmids containing all possible combinations of wild-type and mutant octamer and PSE elements were generated with the oligonucleotides histone H2B octamer (5′ ATTCGAGCTCGCT T**ATGCAAAT**AAGGTACCTAGAGTCGAGCT 3′), mutated H2B octamer (5′ ATTCGAGCTCGCTT**cgGCAAAT**AAGGTACCTAGAGTCGAGCT 3′), human U6 PSE (3′ ATGGATCTCAGCTCGAACG**AATGGCATTGAACTTTCA**T GAGCTCGACG 5′), mutated mouse U6 PSE (3′ ATGGATCTCAGCTCGAT TG**AGTGGGATTGACATTTCA**TGAGCTCGACG 5′), and inverted histone H2B octamer (5′ ATTCGAGCTCGCTT**ATTTGCAT**AAGGTACCTAGAGTC GAGCT 3′). Oligonucleotides containing octamer and PSE sequences were annealed and filled in with Klenow fragment, and the resulting fragment was cut with *Sac*I and *Xho*I and inserted into pUC118 cleaved with *Sac*I and *Sal*I. The probes used in electrophoretic mobility shift assays (EMSAs) were generated by PCR amplification of these constructs with the universal and the reverse sequencing primers, one of which was 5′-end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Because the same labeled primer was used to generate all the probes, they all had the same specific activity.

(ii) Templates for transcription. The templates $OCTA^+$ and $OCTA^-$ were generated by replacing the *Sac*I-*Xho*I promoter fragment of pU6/Hae/RA.2 containing the LS5 clustered point mutation (29) with the *Sac*I-*Xho*I fragments used for the EMSAs (see above).

(iii) Expression constructs. The constructs containing the Oct-1/Pit-1 POU domain chimeras, OOPP, PPOO, OPPP, PPOP, and POPP, have been described previously (2). For POU domain mutagenesis, plasmid pET11c.G.POU-1 (26), which encodes the Oct-1 POU domain fused to the glutathione *S*-transferase (GST) gene product, was used as a template to generate forms of the Oct-1 POU domain carrying residues from the Pit-1 POU_S domain. To generate the ΔEEPS form of the Oct-1 POU domain, we used the pET 11c.G.POU-1 plasmid as a template for PCR with the downstream primer OcHBam, which matches the 3′-coding sequences of the Oct-1 POU_H domain (2), and upstream primer SP65′α 1 POU_S, which matches the 5′-coding sequences of α-helix 1 of the POU_S domain. The upstream primer (5′-CTATTTAGGTGACACTATAGAAACAG ACACCATGGACCTTGAGGAGCTTGA-3′) bypasses the first 3 amino acid residues of the Oct-1 POU_S domain and provides in vitro transcription and translational signals. Other substitutions in the Oct-1 POU_S domain were generated by oligonucleotide-mediated site-directed mutagenesis (24, 51). Each mutagenic oligonucleotide contained a new restriction site sequence for mutant identification. The following sequences were created to generate the substituted proteins: Oct-1 EIR-1, AGT **GAg aTc ccG** GAG (*Mbo*I); Oct-1 NEV-1, **AAc gaa** TTC AAA (*Eco*RI); Oct-1 TNE-2, **acc aAT** GTT GGG **gaa** (*Bsl*I); Oct-1 D5E, AGT **GAg** CTc GAG (*Sac*I); Oct-1 L6I, AGT **GAt** aTc GAG (*Eco*RV); and Oct-1 E7R, GAC ctc **cgG** GAG (*Hpa*II). In this notation scheme, the bases that are altered in the wild-type Oct-1 POU-domain sequence are shown in lowercase lettering, the new amino acid codon or codons are shown in boldface type, and the engineered restriction sites for the nucleases indicated in parentheses are underlined. To generate the substituted form of the Pit-1 POU_S domain, a similar approach was taken wherein the plasmid pET11c.G.Pit-1 POU (2), which encodes the Pit-1 POU domain fused to the GST gene product, was used as a template. The oligonucleotide sequence created to generate the mutant protein was Pit-1 R7E, GAg ATC **gaA** GAA (*Mbo*I).

Sources of proteins. (i) $SNAP_c$. The $SNAP_c$ used in these experiments was derived from a Mono Q peak fraction, which corresponds to the fourth step in the purification of $SNAP_c$ (13), and is purified approximately 2,500-fold.

(ii) Expression and purification of POU domain proteins. The wild-type GST–Oct-1 POU, GST–Oct-2 POU, and GST–Pit-1 POU proteins were expressed in *Escherichia coli* BL21 (DE3) cells with the T7 expression system (43) as described previously (2). For transcription in vitro, the GST moiety of the fusion proteins was removed by cleavage with thrombin. Cleaved proteins were eluted from the beads, dialyzed against buffer D (100 mM KCl, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.9], 5 mM $MgCl_2$, 10% glycerol, 0.1 mM EDTA, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride), and stored at −70°C. In each case, protein purity was assessed to be at least 90% by Coomassie blue staining of a sodium dodecyl sulfate (SDS)–15% polyacrylamide gel and the protein concentration was measured by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.).

(iii) In vitro translation. Except for ΔEEPS, in vitro translation of wild-type and substituted forms of the Oct-1 POU domain was carried out as described previously (5). For ΔEEPS, the same approach was used; however, in the PCR amplification of template for in vitro transcription, the oligonucleotide primers described above were used. The in vitro-translated forms of the Pit-1 POU domain were generated in the same manner, except that in the PCR amplification of the template for in vitro transcription, the upstream primer was SP65′PtPOU (5′-CTATTTAGGTGACACTATAGAAACAGACACCATGGC AGACATGGACTCCCCG-3′), which matches the 5′-coding sequences of the Pit-1 POU_S domain and provides in vitro transcription and translational signals, and the downstream primer was PtHBam (5′-AAGGATCCTACGTTTTCAC CCGTTTTTC-3′), which matches the 3′-coding region of the Pit-1 POU_H domain. The extent and quality of synthesis were monitored by SDS-polyacrylamide gel electrophoresis on a 15% acrylamide gel. To normalize amounts of active POU proteins, we adjusted the amounts of protein to obtain equal H2B octamer motif-binding activities in the EMSA.

EMSA. The binding reactions were performed in a total volume of 20 μl containing 5 μl of $SNAP_c$ fraction and final concentrations of 100 mM KCl, 20 mM HEPES (pH 7.9), 5 mM $MgCl_2$, 0.2 mM EDTA, 10% glycerol, 0.2 mg of fetal calf serum per ml as a protein carrier, 1 mM DTT, and 0.25 μg each of poly (dI-dC) · (dI-dC) and pUC118. The amounts of POU domain proteins added are indicated in the figure legends. The reaction mixtures were incubated for 30 min at 4°C before addition of the radiolabeled probe followed by a 15-min incubation at room temperature. The reaction products were then electrophoresed through a 5% nondenaturing polyacrylamide gel (acrylamide/bisacrylamide ratio, 39:1) in 1× TGE running buffer (50 mM Tris base, 380 mM glycine, 2 mM EDTA) at 140 V for 4 h at room temperature. The gel was dried and autoradiographed. The intensities of the signals were measured by phosphorimaging with a Fuji BAS1000 phosphorimager.

In vitro transcriptions. (i) Immunodepletions of nuclear extracts. Ascites YL15 and YL8 (2.5 ml each) were incubated with protein A-agarose beads (packed-bead volume 1 ml) for 2 h at room temperature with constant mixing. The beads were centrifuged once at 3,000 rpm in an Eppendorf centrifuge (model 5415C) for 5 min, and the supernatant was removed. The beads were then washed five times with 3 packed-bead volumes of RIPA buffer (12) without SDS and three times with buffer D containing 0.01% Nonidet P-40 and 0.01% Tween 20. The beads were then stored at 4°C in buffer D containing 0.01% sodium azide. Just before use, they were washed three times in 10 packed-bead volumes of buffer D and resuspended in 2 packed-bead volumes of the same buffer.

HeLa cell nuclear extract (9) (1.5 ml, 12 mg of protein per ml) was diluted 1:1 in buffer D, mixed with 2 ml of the above bead suspension, and incubated with gentle mixing for 75 min at 4°C. Mock-depleted nuclear extracts were processed identically but with beads containing no antibodies. The beads were centrifuged at 3,000 rpm for 5 min, and the supernatants were used in transcription assays.

(ii) In vitro transcription reactions. Transcription reactions with mock- or Oct-1-depleted nuclear extracts were carried out in a total volume of 30 μl containing 60 mM KCl, 37 mM HEPES (pH 7.9), 12% glycerol, 5 mM $MgCl_2$, 1 mM spermidine, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid mM (EGTA), 0.3 mM phenylmethylsulfonyl fluoride, 0.5 mM each ATP, UTP, CTP, and GTP, 0.5 mM dithiothreitol, 0.3 mM EDTA, 0.5 μg of supercoiled template, and 12 μl (about 70 μg of protein) of depleted or mock-depleted extract (1:1). Purified POU domain proteins (obtained by cleavage of GST-POU fusion proteins produced in *E. coli* as described above) were added as indicated in the figure legends, and the transcription reactions were allowed to proceed for 40 min at 30°C. The reactions were terminated by the addition of stop mix (0.2 M NaCl, 0.1% SDS, 1 mM EDTA, 10 μg of tRNA, 0.6 mg of proteinase K per ml) and incubated at 37°C for another 30 min. The products were analyzed by RNase T_1 protection with the probe U6/RA.2/143 as described previously (29).

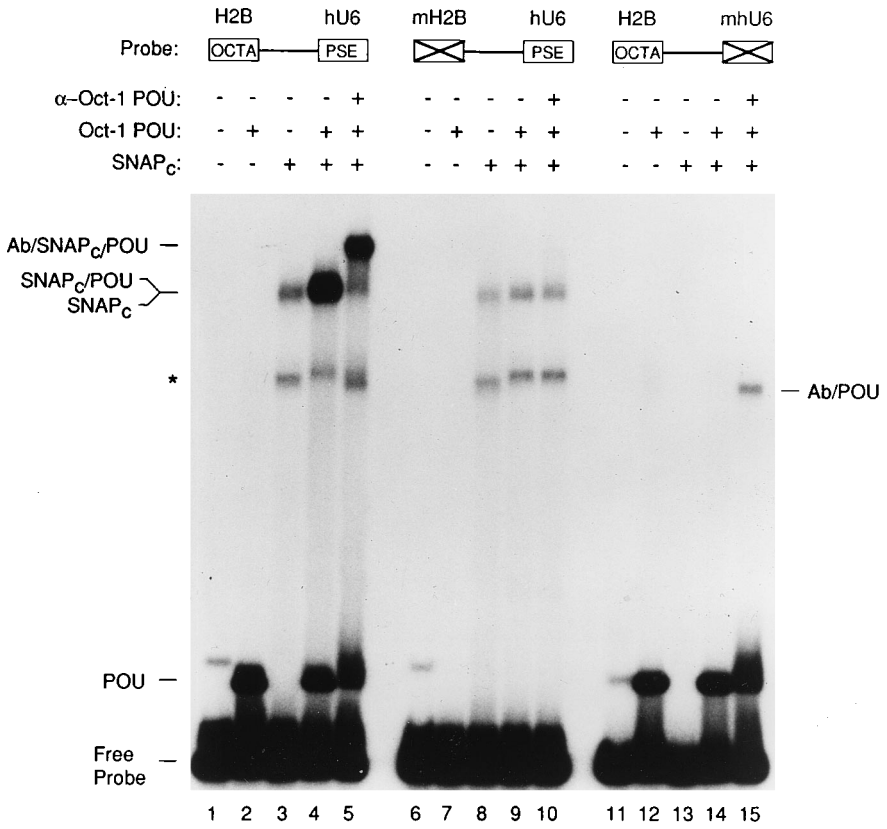

FIG. 1. The POU domain of Oct-1 binds cooperatively with SNAP$_c$ to a probe containing an octamer sequence and a PSE. The EMSA was performed with the probes and protein factors indicated above the lanes. SNAP$_c$ was obtained from a partially purified Mono Q fraction (13), whereas the Oct-1 POU domain was obtained by in vitro translation. The anti-Oct-1 antibody used in lanes 5, 10, and 15 (α-Oct-1 POU) was the YL15 MAb (25, 27), which recognizes the linker region in the Oct-1 POU domain. The positions of the free probe and the complexes formed on the probes and containing the factors Oct-1 POU (POU), α-Oct-1 POU and Oct-1 POU (Ab/POU), SNAP$_c$ alone (SNAP$_c$), SNAP$_c$ and Oct-1 POU (SNAP$_c$/POU), and α-Oct-1 POU, SNAP$_c$, and Oct-1 POU (Ab/SNAP$_c$/POU) are indicated. The asterisk indicates a partial or degraded SNAP$_c$.

RESULTS

Murphy et al. (34) showed that the PSE-binding factor PTF can be recruited to the PSE of the 7SK promoter as well as to the PSEs of the human U1, U2, and U6 promoters by binding of the Oct-1 or Oct-2 POU domains to an adjacent octamer site. Both the Oct-1 and Oct-2 POU domains stimulated 7SK transcription in vitro, but because no Oct-1 (or Oct-2) mutant incapable of interacting with PTF was tested, it was not clear whether transcription activation actually resulted from recruitment of PTF. It was, therefore, of interest to determine whether the Oct-1 POU domain can recruit SNAP$_c$ to a PSE and, if so, whether this event results in transcription activation.

The Oct-1 POU domain enhances SNAP$_c$ binding to the human U6 PSE. To monitor the binding of SNAP$_c$ to the PSE in the presence and absence of an octamer-bound Oct-1 POU domain, we used the three probes shown at the top of Fig. 1, which contain different combinations of intact or mutated histone H2B octamer sequence and human U6 PSE. We chose the H2B octamer sequence (derived from the human H2B promoter) because the structure of the Oct-1 POU domain bound to this site has been determined (19). In addition, this particular octamer sequence is a high-affinity binding site for the Oct-1 and Oct-2 and the distantly related Pit-1 POU domains (2). As detailed below, this characteristic allowed us to test Pit-1 and Oct-1/Pit-1 POU domain chimeras for their effect on SNAP$_c$ binding. The human U6 PSE is a low-affinity binding site for SNAP$_c$, which enhances the effect of the Oct-1 POU domain on SNAP$_c$ binding to the DNA (data not shown). The spacing between the octamer sequence and the PSE in our experiments (23 bp) is arbitrary, but Murphy et al. (34) showed that the potentiation of PTF binding by Oct-1 is unaffected by the precise distance between the octamer site and the PSE.

As shown in Fig. 1, when the Oct-1 POU domain was incubated alone with the probe containing intact octamer and PSE sites, a strong protein-DNA complex was formed (POU; lane 2). This complex was dependent on an intact octamer site (lane 7) but not on an intact PSE (lane 12). When a fraction highly enriched in SNAP$_c$ (Mono Q fraction [13]) was incubated alone with a probe containing an intact PSE, two weak complexes were formed (lane 3). The more slowly migrating complex (labeled SNAP$_c$) corresponds to the intact SNAP complex, whereas the faster-migrating complex (labeled with an asterisk) arises upon storage of the SNAP$_c$ fraction and most probably corresponds to a partial (or degraded) SNAP complex. As expected, formation of both complexes was dependent on an intact PSE (lane 13) but not on an intact octamer motif (lane 8). In the presence of both SNAP$_c$ and the Oct-1 POU domain, the binding of SNAP$_c$ to the human U6 PSE was enhanced 8- to 10-fold (compare lane 4 with lane 3). This enhanced binding was dependent on the presence of both an intact PSE (lane 14) and an intact octamer motif (lane 9), suggesting that it resulted from the simultaneous binding of SNAP$_c$ and the Oct-1 POU domain to their respective binding

sites. In contrast to $SNAP_c$, the partial SNAP complex was not enhanced by the Oct-1 POU domain (lane 4).

Addition of the Oct-1 POU domain did not visibly retard the migration of the $SNAP_c$-PSE complex, probably because of the small size of the Oct-1 POU domain (18 kDa). Therefore, to confirm that the Oct-1 POU domain is present in the enhanced complex, we tested the effect of the anti-Oct-1 monoclonal antibody (MAb) YL15, which recognizes the linker region of the Oct-1 POU domain (25, 27), on complex formation. This antibody retarded the migration of the Oct-1 POU-octamer complex, as expected (lane 15, complex labeled Ab/POU, which serendipitously comigrates with the partial SNAP complex). Significantly, the anti-Oct-1 antibody also retarded the migration of the enhanced complex formed in the presence of both $SNAP_c$ and the Oct-1 POU domain on a wild-type probe (lane 5, complex labeled Ab/$SNAP_c$/POU), confirming that the enhanced complex contains Oct-1 POU. We also found that the anti-peptide antibody αCSH375, which recognizes the carboxy terminus of the $SNAP_c$ subunit SNAP43 (13), retarded the migration of both the $SNAP_c$-PSE complex and the enhanced complex, confirming that both complexes contain $SNAP_c$ (data not shown). Thus, together, these results suggest that the binding of the Oct-1 POU domain to the octamer motif dramatically enhances the binding of $SNAP_c$ to the PSE.

The Oct-1 POU domain enhances the on-rate of $SNAP_c$ binding to DNA. To determine how the Oct-1 POU domain enhances the binding of $SNAP_c$ to the PSE, we first examined the rate of association of $SNAP_c$ in the presence and absence of the Oct-1 POU domain. As summarized at the top of Fig. 2B, we mixed the probe containing the wild-type octamer site and human PSE with the $SNAP_c$ fraction either alone or together with the Oct-1 POU domain and tested aliquots of the binding-reaction mixture, after increasing incubation times, by EMSA. The results are shown in Fig. 2A and plotted in Fig. 2B. $SNAP_c$ alone bound to the PSE slowly, such that the levels of DNA-protein complex were still increasing after 1 h (lanes 1 to 9). In sharp contrast, in the presence of the Oct-1 POU domain, the formation of the complex was more rapid and reached a plateau by 15 to 30 min (lanes 10 to 18).

To determine whether the Oct-1 POU domain affected the off-rate of the complex, we performed the experiment summarized at the top of Fig. 2C: we allowed the complex to form in the presence or absence of a GST–Oct-1 POU domain fusion protein for 30 min and then added a 50-fold excess of cold competitor containing a wild-type PSE before loading aliquots of the binding-reaction mixture at increasing time intervals thereafter. We used a GST–Oct-1 POU fusion protein because it is large enough to show a clear supershift of the $SNAP_c$-PSE complex upon binding to the octamer motif (see Fig. 3), and thus its dissociation from the trimeric complex can be easily monitored. The results of this analysis are shown in the graph in Fig. 2C. The trimeric complex dissociated quite slowly in both the presence and absence of GST–Oct-1, as determined by phosphorimager quantitation of the retarded complex. Thus, the Oct-1 POU domain can considerably increase the on-rate of $SNAP_c$ binding to the PSE without significantly affecting its off-rate.

$SNAP_c$ binding is enhanced by the Oct-1 and Oct-2 POU domains but not by the Pit-1 POU domain. In contrast to VP16, which is effectively recruited to the DNA by the Oct-1 but not the Oct-2 POU domain as part of the VP16-induced complex (41), PTF is recruited to the 7SK PSE by both the Oct-1 and Oct-2 POU domains (34). We wanted to determine whether this is also true for $SNAP_c$ binding to the human U6 PSE and whether a more distantly related POU domain, that of Pit-1, could mediate the same effect. Although the Pit-1 POU domain is only 50% identical to the Oct-1 POU domain, it, like the Oct-1 POU domain, binds efficiently to the H2B octamer motif as a monomer (2). As shown in Fig. 3, we compared the abilities of the Oct-1, Oct-2, and Pit-1 POU domains as GST-fusion proteins to facilitate the binding of $SNAP_c$ to the PSE, using amounts of the three proteins that resulted in equal binding to the octamer motif (lanes 2 to 4). All three GST-POU fusion proteins supershifted the $SNAP_c$-PSE complex (compare lanes 6 to 8 with lane 5), but only the GST–Oct-1 POU and GST–Oct-2 POU proteins enhanced the binding of $SNAP_c$ to the PSE (compare lanes 6 and 7 with lane 8, complex labeled $SNAP_c$/GST-POU). These results show that like PTF, $SNAP_c$ is recruited to the PSE by both the Oct-1 and Oct-2 POU domains. They also indicate that residues specific to the Pit-1 POU domain prevent the interaction with $SNAP_c$.

The differential abilities of the Oct-1 and Pit-1 POU domains to enhance the binding of $SNAP_c$ to the PSE map to the amino-terminal region of the Oct-1 POU domain. The observation that the Pit-1 POU domain is unable to facilitate the binding of $SNAP_c$ to the PSE allowed us to use Oct-1–Pit-1 POU-domain chimeras (2) to locate a region of the Oct-1 POU domain that is involved in interaction with $SNAP_c$. The top of Fig. 4 illustrates the structures of the Oct-1–Pit-1 chimeras used here. To construct these chimeras, the Oct-1 and Pit-1 POU domains were divided into four regions: amino- and carboxy-terminal halves of the POU_S domain, the linker, and the POU_H domain. OOOO and PPPP designate the wild-type Oct-1 and Pit-1 POU domains, respectively, whereas OOPP, for example, corresponds to a chimera containing the entire POU_S domain from Oct-1 and the linker domain and homeodomain from Pit-1.

OOOO but not PPPP facilitated the binding of $SNAP_c$ to the PSE, as expected (compare lanes 2 and 8 with lane 1). The chimeras POPP, PPOP, and PPOO were not active (lanes 5 to 7), but OOPP and, to a large extent, OPPP were both active (lanes 3 and 4). Thus, exchange of the carboxy-terminal half of the Pit-1 POU_S domain, the Pit-1 linker, and the Pit-1 POU_H domains for their Oct-1 counterparts had little or no effect, but exchange of just the amino-terminal half of the Pit-1 POU_S domain for its Oct-1 counterpart imparted to Pit-1 the ability to enhance the binding of $SNAP_c$ to the PSE. Strikingly, in this region of the Oct-1 protein, all the residues that differ from Pit-1 are solvent exposed in the octamer-bound Oct-1 POU domain (19). This observation suggests that the Oct-1 POU domain recruits $SNAP_c$ to the PSE by a direct protein-protein contact involving the POU_S domain, rather than by induction of a change in the conformation of the DNA. Thus, like the POU_H domain, which mediates protein-protein interactions with VP16 in the VP16-induced-complex, the POU_S domain can also mediate protein-protein interactions with heterologous factors that are not POU-domain proteins.

Exchange of a single amino acid in the Oct-1 POU_S domain for its Pit-1 counterpart debilitates the Oct-1 POU-domain interaction with $SNAP_c$. Figure 5A shows the differences between Pit-1 and Oct-1 in the amino-terminal half of the POU_S domain and summarizes the results of the binding studies described here. To determine which of these differences are responsible for the differential effects on binding of $SNAP_c$, we tested the deletion and several exchanges of Oct-1 sequences for Pit-1 sequences depicted in Fig. 5A. The construct ΔEEPS lacks the first four amino acids of the Oct-1 POU domain, which are apparently unstructured in the octamer-bound Oct-1 POU domain (19). In each of the EIR-1, NEV-1, and TNE-2 mutations, three Oct-1 amino acids were exchanged for their Pit-1 counterparts. Thus, for example, in the EIR-1 mutation, the Oct-1 DLE sequence was replaced by the Pit-1 EIR se-

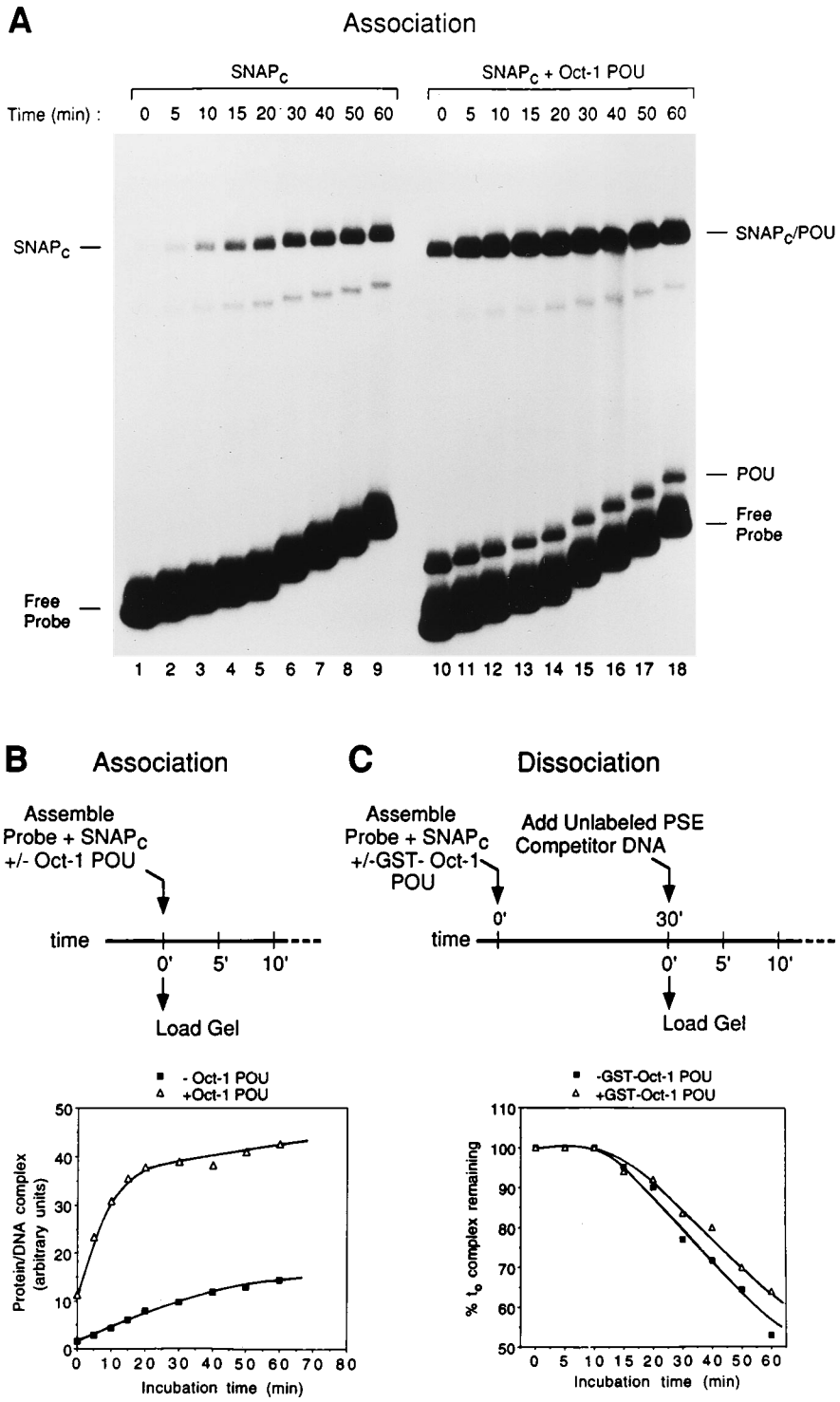

FIG. 2. Oct-1 POU accelerates the on-rate of SNAP$_c$. (A) Association rates of SNAP$_c$ with the human U6 PSE in the absence and presence of Oct-1 POU. The EMSA was performed with the probe containing wild-type octamer sequence and PSE and the proteins indicated above the lanes. The sources of SNAP$_c$ and Oct-1 POU were as for Fig. 1. In lanes 1 to 9, unprogrammed rabbit reticulocyte lysate was added to normalize protein concentrations in all lanes. The binding reactions were performed as described in Materials and Methods, except that the reaction products were loaded on a running gel at the times indicated above the lanes after addition of the radiolabeled probe. The signals obtained were quantitated by phosphorimager analysis. (B) Strategy used to measure association rates in the presence and absence of Oct-1 POU and graphical representation of the results shown in panel A. (C) Strategy used to measure dissociation rates in the presence and absence of GST–Oct-1 POU, and graphical representation of the results. The EMSA was performed with the probe containing a wild-type octamer sequence and PSE and either SNAP$_c$ alone or SNAP$_c$ plus GST–Oct-1 POU. The source of SNAP$_c$ was as for Fig. 1. GST–Oct-1 POU was produced in *E. coli*. The binding reactions were performed as described in Materials and Methods, except that they were allowed to proceed for 30 min at room temperature after addition of the radiolabeled probe, after which a 50-fold excess of unlabeled competitor (containing a wild-type PSE) was added, and aliquots of each reaction product were loaded on a running gel at the times indicated after addition of the unlabeled competitor. The signals obtained were quantitated by phosphorimager analysis. The amounts of binding for SNAP$_c$ alone and SNAP$_c$ together with GST–Oct-1 POU obtained after the 30-min incubation were plotted as 100%.

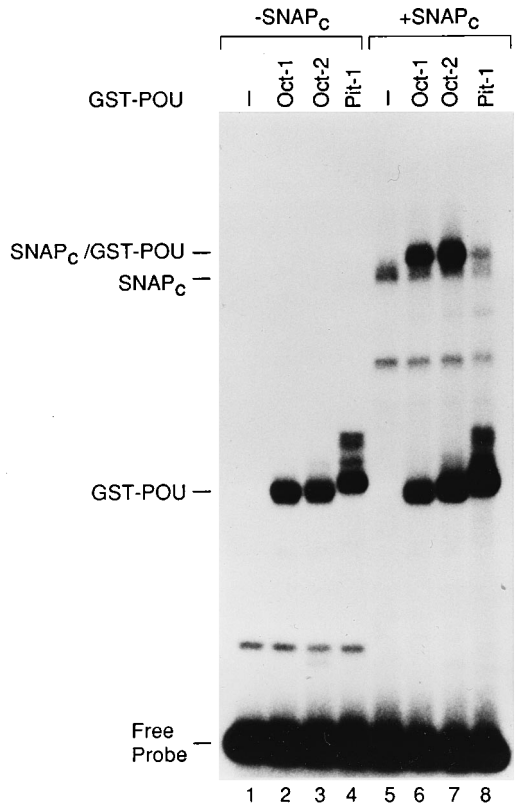

FIG. 3. The Oct-1 and Oct-2 POU domains but not the Pit-1 POU domain facilitate the binding of $SNAP_c$ to the PSE. The EMSA was performed with a probe containing a wild-type octamer sequence and PSE and the proteins indicated above the lanes. The GST-POU fusion proteins were expressed in *E. coli* and purified as described in Materials and Methods.

quence. As shown in Fig. 6A, whereas the ΔEEPS, NEV-1, and TNE-2 mutants enhanced $SNAP_c$ binding as well as the wild-type Oct-1 POU domain did (compare lanes 10, 12, and 13 with lane 9), the EIR-1 mutant had largely lost this ability (lane 11), thus defining the DLE region in the Oct-1 POU-specific domain as the main determinant for the selective interaction with $SNAP_c$.

We then exchanged each of the three amino acids mutated in EIR-1 individually with their Pit-1 counterparts to generate the Oct-1 POU domains D5E, L6I, and E7R shown in Fig. 5A. These single amino acid exchanges do not affect Oct-1 POU-domain binding to the octamer sequence (data not shown). As shown in Fig. 6B, the D5E and L6I Oct-1 POU mutants were as active as wild-type Oct-1 POU in facilitating binding of $SNAP_c$ to the PSE (compare lanes 4 and 5 with lane 3). In sharp contrast, like the Pit-1 POU domain, the E7R mutant was inactive (compare lanes 6 and 7). Thus, the glutamic acid at position 7 of the Oct-1 POU_S domain is a key determinant for the interaction with $SNAP_c$.

Exchange of a single Pit-1 amino acid for its Oct-1 counterpart confers upon Pit-1 the ability to facilitate the binding of $SNAP_c$ to the PSE. Exchange of the glutamic acid at position 7 of the Oct-1 POU domain for its Pit-1 counterpart causes a loss of function, in which the Oct-1 POU domain is no longer able to facilitate the binding of $SNAP_c$ to the PSE. To determine whether we could impart to Pit-1 a gain-of-function phenotype, we substituted the arginine at position 7 in the Pit-1 POU domain with the glutamic acid present at the corresponding position in the Oct-1 POU domain (Fig. 5A, bottom, mutant R7E). Strikingly, as shown in Fig. 6C, this single amino acid exchange imparted to the Pit-1 POU domain the ability to facilitate the binding of $SNAP_c$ to the PSE to nearly the same level as the wild-type Oct-1 POU domain (compare lane 4 to lanes 3 and 5). Thus, the differential abilities of the Oct-1 and Pit-1 POU domains to interact with the basal transcription complex $SNAP_c$ can be attributed largely to a single amino acid difference in the POU_S domains of the two proteins. Figure 5B shows the position of this single amino acid difference on a model of the Oct-1 POU_S domain bound to the histone H2B octamer motif (19).

An anti-Oct-1 MAb that recognizes the amino terminus of the Oct-1 POU_S domain disrupts the interaction between the Oct-1 POU domain and $SNAP_c$. As shown in Fig. 5B, the critical glutamic acid at position 7 lies on an exposed surface of the Oct-1 POU_S domain, facing away from the DNA, suggesting that it is involved in a protein-protein contact with $SNAP_c$. To examine this possibility further, we asked whether the anti-Oct-1 MAb YL21, which recognizes the DLE sequence (Fig. 5A) at the amino terminus of the POU_S domain (references 25

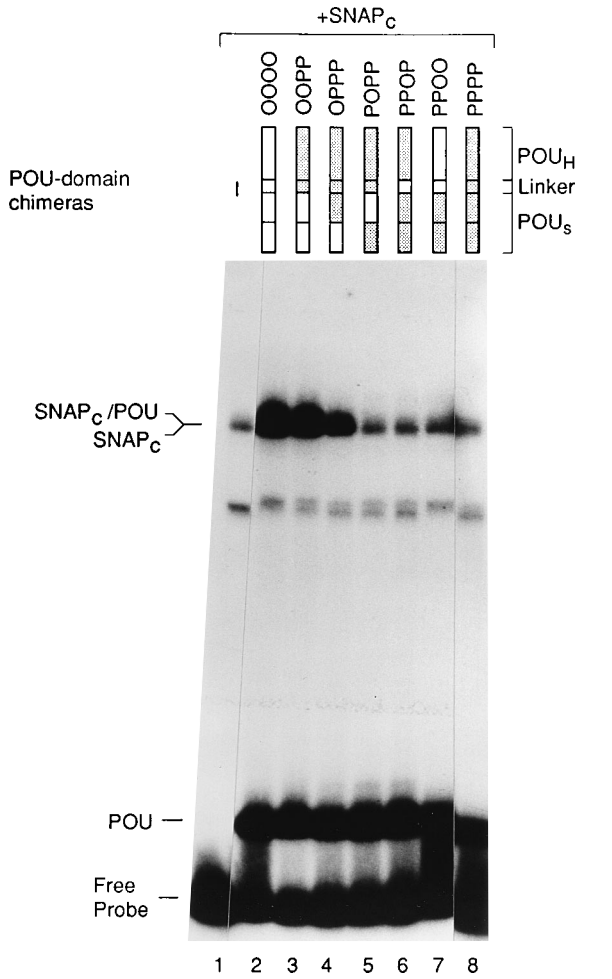

FIG. 4. The amino-terminal half of the Oct-1 POU_S domain is required to facilitate the binding of $SNAP_c$ to the PSE. The EMSA was performed with a probe containing a wild-type octamer sequence and PSE and the protein factors indicated above the lanes. The POU domain chimeras were produced by translation in vitro, and their structures are indicated schematically above the lanes. White boxes indicate segments derived from Oct-1 POU, stippled boxes indicate segments derived from Pit-1 POU. Lanes 1 and 8 are from different regions of the same gel.

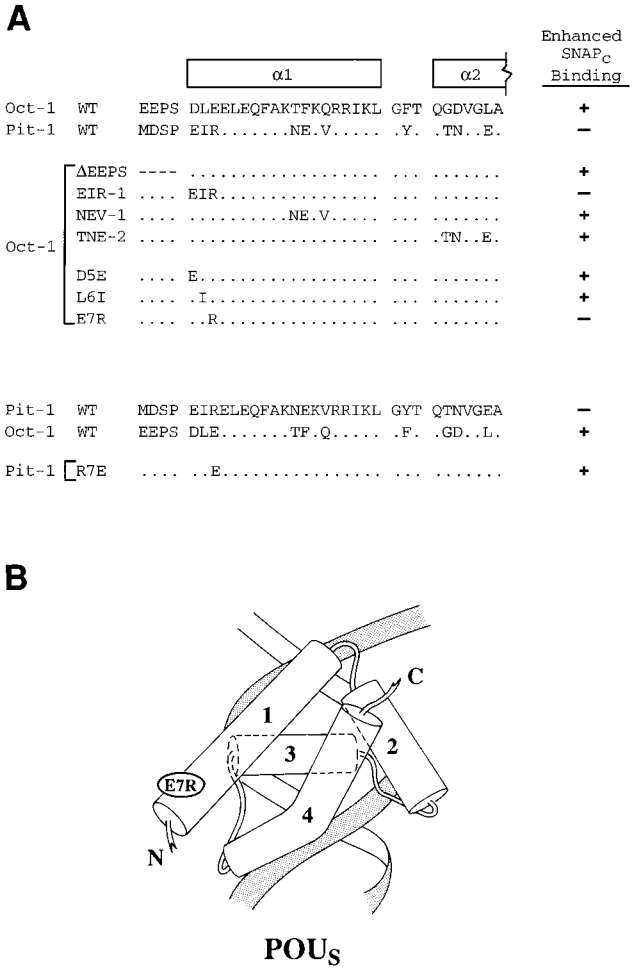

FIG. 5. (A) Amino acid sequence comparison between the Oct-1 and Pit-1 POU$_S$-domain amino-terminal regions. ΔEEPS is a derivative of Oct-1 POU lacking the first four amino acids. EIR-1, NEV-1, TNE-2, D5E, L6I, and E7R are derivatives of Oct-1 POU containing the indicated amino acid changes. R7E is a derivative of Pit-1 POU containing the indicated amino acid change. The abilities of each of the wild-type (wt) and mutant POU domains to enhance the binding of SNAP$_c$ to the PSE are indicated by (+) and (−) on the right. (B) Location of the E7R exchange on the three-dimensional structure of the Oct-1 POU$_S$ domain bound to the H2B octamer sequence. The four α-helices are labeled 1 to 4. N, amino terminus; C, carboxy terminus. Adapted from reference 15.

and 27 and data not shown), would interfere with stabilization of SNAP$_c$ on the PSE. As a control, we used another anti-Oct-1 POU-domain MAb, the YL15 MAb, which is directed against the linker that connects the POU$_S$ and POU$_H$ domains. As shown in Fig. 7, both antibodies retarded the migration of a GST–Oct-1 POU–octamer complex (lanes 3 and 4, complex labeled Ab/GST-POU). Thus, neither antibody interferes with binding of the Oct-1 POU domain to DNA. As expected, neither antibody affected the SNAP$_c$/PSE complex (lanes 6 and 7). However, when the YL15 and YL21 MAbs were added before assembly of the GST–Oct-1 POU/SNAP$_c$/DNA complex, they had very different effects. The control YL15 antibody did not inhibit complex formation and retarded the migration of the complex, suggesting that the Oct-1 POU domain bound by this antibody can still enhance recruitment of SNAP$_c$ to the PSE (lane 10, complex labeled Ab/SNAP$_c$/GST-POU). In sharp contrast, the YL21 antibody, which, as shown above, does not affect binding of the Oct-1 POU domain alone to DNA, inhibited formation of the GST-POU/SNAP$_c$/DNA complex (lane 9; the amount of SNAP$_c$/GST-POU complex decreases and the level of SNAP$_c$ complex increases). Indeed, no YL21-containing Ab/SNAP$_c$/GST-POU complex was formed, indicating that the Oct-1 POU domain bound by YL21 cannot cooccupy the probe with SNAP$_c$. These results suggest that the Oct-1 POU domain recruits SNAP$_c$ to the PSE through a protein interaction involving the very region recognized by YL21.

Oct-1 POU domain recruitment of SNAP$_c$ to the PSE is independent of octamer site orientation. The observation that the Oct-1 POU domain probably recruits SNAP$_c$ to the DNA through a direct protein-protein contact suggests that the orientation of the Oct-1 POU domain on the DNA should be important. Indeed, the tertiary structure of the Oct-1 POU domain bound to an octamer site (19) indicates that on our probes, the amino-terminal region of the Oct-1 POU$_S$ domain faces toward SNAP$_c$. To determine whether the Oct-1 POU domain could also recruit SNAP$_c$ when bound to an octamer sequence in the reverse orientation, we generated the probe shown in Fig. 8, in which the octamer site is inverted relative to the orientation of the PSE. Remarkably, on this probe, as on probes with an octamer site facing toward the PSE, the Oct-1 POU domain but not the Pit-1 POU domain is capable of enhancing SNAP$_c$ binding to the PSE (lanes 6, 7, and 9). Moreover, this ability could be suppressed by the E7R mutation (lane 8) and imparted to the Pit-1 POU domain by the R7E mutation (lane 10). Thus, the Oct-1 POU domain can enhance binding of SNAP$_c$ to the PSE regardless of the orientation of the octamer site, through protein-protein contacts that involve the same region of the POU$_S$ domain. Together, these results suggest a surprisingly high level of flexibility in the interaction between the DNA-binding domain of a sequence-specific activator and a basal transcription complex.

The abilities of different POU domains to facilitate the binding of SNAP$_c$ to the PSE correlate with their abilities to activate transcription in vitro. The Oct-1 POU domain has been shown to activate RNA polymerase III transcription from the 7SK promoter in vitro (34). This observation suggested that we could use an in vitro transcription system to examine the role, if any, of the protein-protein contact between SNAP$_c$ and the Oct-1 POU domain in transcription activation. We therefore tested the abilities of different POU-domain mutants to activate RNA polymerase III transcription from the human U6 promoter. As templates, we used derivatives of the U6 promoter containing the original arrangement and orientation of octamer sequence and human U6 PSE used for the binding studies described above (see Materials and Methods for details). In Fig. 9, OCTA$^+$ and OCTA$^-$ refer to templates containing wild-type and mutant octamer sequences, respectively.

Figure 9 shows that in a mock-depleted extract, the OCTA$^+$ template was about fourfold more active than the OCTA$^-$ template (compare lanes 1 and 2), suggesting that, as with the human 7SK promoter (33), endogenous Oct-1 present in these extracts can activate transcription of the human U6 promoter. Indeed, in extracts immunodepleted of endogenous Oct-1, the OCTA$^+$ (lane 3) and OCTA$^-$ (lane 4) promoters were nearly equally active. When such a depleted extract was supplemented with the Oct-1 POU domain, transcription was stimulated threefold (compare lanes 3 and 5) and the effect was dependent on the presence of a wild-type octamer site (compare lanes 5 and 9). Strikingly, however, the Oct-1 POU domain with the E7R mutation had little effect on transcription (compare lane 3 with lane 6). Reciprocally, addition of the Pit-1 POU domain did not stimulate transcription (lane 7) whereas addition of the Pit-1 POU domain with the R7E mutation, which imparts to Pit-1 the ability to facilitate the binding of SNAP$_c$ to the PSE, stimulated transcription to the

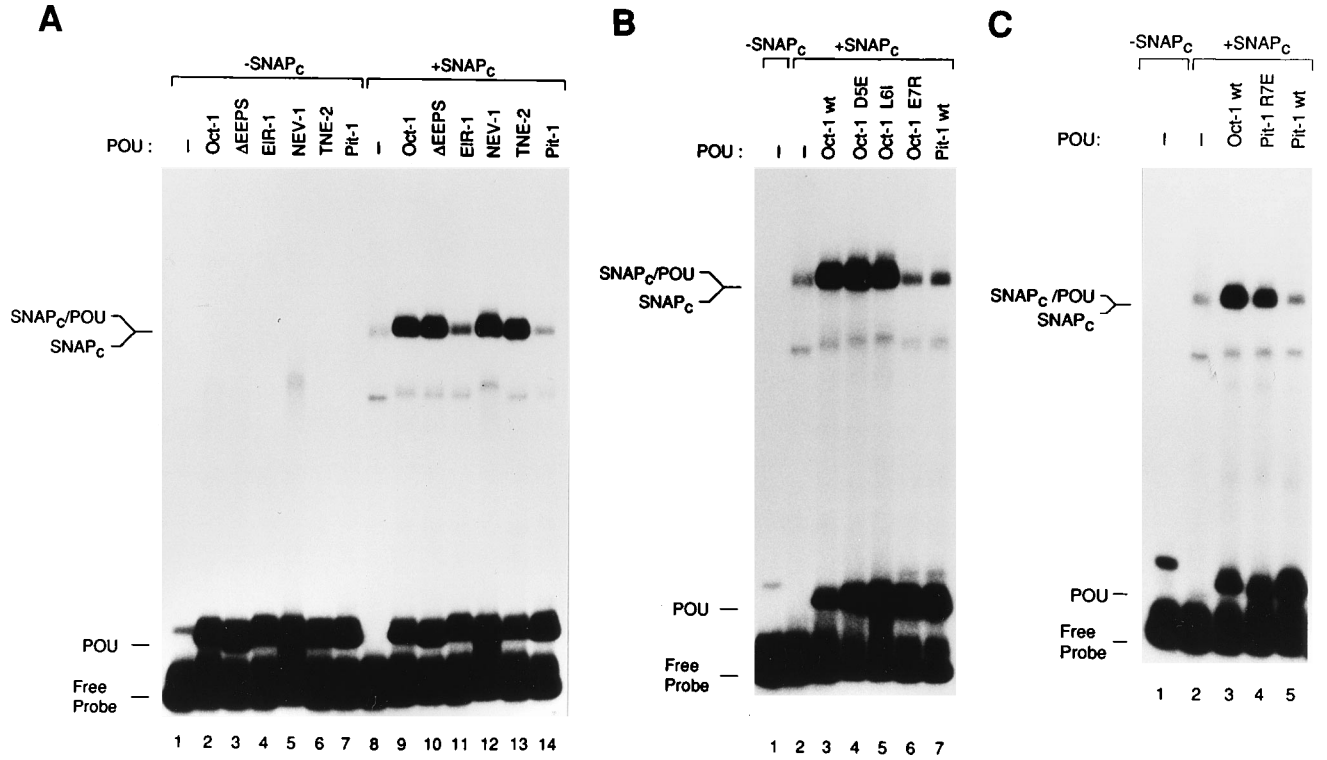

FIG. 6. A single amino acid difference is responsible for the differential effects of the Oct-1 POU and Pit-1 POU domains on the binding of $SNAP_c$ to the PSE. The binding reactions were performed with a probe containing a wild-type (wt) octamer sequence and PSE and the proteins indicated above the lanes. The POU domain derivatives indicated above the lanes were generated by in vitro translation. (A) Effects of a deletion and exchanges of clustered amino acids for their Pit-1 counterparts on the ability of Oct-1 POU to facilitate the binding of $SNAP_c$ to the PSE. (B) A glutamic acid residue at position 7 of the Oct-1 POU_S domain is the key determinant for the selective recruitment of $SNAP_c$ to the PSE by the Oct-1 POU domain. (C) Substitution of the arginine at position 7 in the Pit-1 POU domain with its glutamic acid Oct-1 counterpart confers on the Pit-1 POU domain the ability to enhance the binding of $SNAP_c$ to the PSE.

same level as did addition of the wild-type Oct-1 POU domain (compare lane 8 with lane 5). Thus, the abilities of the different POU domains to stimulate U6 transcription in vitro correlate with their abilities to facilitate the binding of $SNAP_c$ to the PSE. Together, these results suggest that transcription stimulation in vitro results directly from improved recruitment of $SNAP_c$ to the PSE by the Oct-1 POU domain. Thus, the POU DNA-binding domain of Oct-1 not only serves to target Oct-1 to the promoter but also is involved in the transcription activation process.

DISCUSSION

Activation domains play a major role in recruiting basal transcription factors to the initiation complex and thereby activating transcription. We have shown that a DNA-binding domain, the POU domain of the transcription factor Oct-1, can recruit the basal transcription factor $SNAP_c$ to the PSE. The related Pit-1 POU domain is unable to recruit $SNAP_c$ to the PSE, and the differential effects of the Oct-1 and Pit-1 POU domains on binding of $SNAP_c$ to the PSE can be attributed to a single amino acid difference in the POU_S domains of the two proteins. Remarkably, the abilities of the Oct-1 and Pit-1 wild-type and mutant POU domains to recruit $SNAP_c$ to the PSE correlate directly with their abilities to stimulate RNA polymerase III snRNA gene transcription in vitro, indicating that recruitment of $SNAP_c$ to the PSE is transcriptionally relevant.

Although we have not tested transcription from an RNA polymerase II snRNA promoter, we suspect that the result would be the same. Indeed, the PSEs of RNA polymerase II and III promoters can be exchanged with no effect on RNA polymerase specificity (see reference 30 for a review), and $SNAP_c$ is required for transcription of both RNA polymerase II and III snRNA promoters (13, 40). Furthermore, the DSEs of RNA polymerase II and III snRNA promoters are interchangeable (3, 23); indeed, they can both be replaced by synthetic enhancers consisting of reiterated octamer motifs (38a, 44). Thus, it is likely that recruitment of $SNAP_c$ to the PSE by the Oct-1 POU domain results in enhancement of both RNA polymerase II and III snRNA gene transcription.

How does the Oct-1 POU domain recruit $SNAP_c$ to the PSE? Our results show that the Oct-1 POU domain increases the on-rate of $SNAP_c$ to the PSE without significantly altering its off-rate. One possibility is that binding of the Oct-1 POU domain to the octamer motif changes the conformation of the DNA in the nearby PSE, thus creating a higher-affinity binding site for $SNAP_c$. Another possibility is that the effect results from a direct protein contact. We favor the latter hypothesis for two reasons. (i) The DNA-binding properties of the E7R Oct-1 POU-domain mutant, which is unable to recruit $SNAP_c$ to the PSE, are not altered, consistent with the location of the E7R mutation in the first helix of the POU_S domain, facing away from the DNA and thus available for protein contact. (ii) Perhaps even more significantly, prior binding of a protein, the YL21 antibody, to the same region of the POU_S domain does not affect the binding of the Oct-1 POU domain to DNA but disrupts formation of the POU-$SNAP_c$-DNA complex.

Our analysis of Oct-1–Pit-1 POU-domain chimeras can only score the differences between the Oct-1 and Pit-1 POU domains that are responsible for the selective association of the

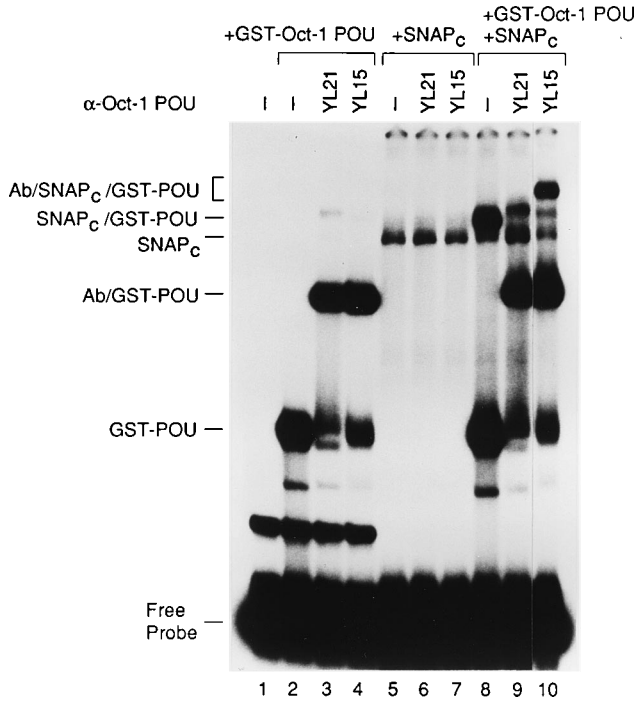

FIG. 7. The recruitment of SNAP$_c$ to the PSE by the Oct-1 POU domain involves a protein-protein contact. The EMSA was performed with the probe containing wild-type octamer and PSE and the protein factors indicated above the lanes. GST–Oct-1 POU was produced in *E. coli* and purified as described in Materials and Methods. MAbs YL15 and YL21 recognize the linker and the amino terminus of the Oct-1 POU domain, respectively (25, 27). The positions of the free probe and the complexes formed on the probe and containing the factors GST–Oct-1 POU (GST-POU), YL21 (lanes 3 and 9) or YL15 (lanes 4 and 10) and GST–Oct-1 POU (Ab/GST-POU), SNAP$_c$ (SNAP$_c$), SNAP$_c$ and GST–Oct-1 POU (SNAP$_c$/GST-POU), and YL21 (lane 9) or YL15 (lane 10), SNAP$_c$, and GST–Oct-1 POU (Ab/SNAP$_c$/GST-POU) are indicated by arrows.

Oct-1 POU domain with SNAP$_c$. Although additional residues, conserved between the two proteins, are likely to be required for association with SNAP$_c$, the importance of the glutamic acid at position 7 of the Oct-1 POU domain implicates the POU$_S$ domain in contacting SNAP$_c$. This observation is interesting, because previous associations of POU domains with heterologous proteins have all been mapped to the POU$_H$ domain (15). For example, VP16 differentiates between Oct-1 and Oct-2 by discriminating an amino acid difference in the POU$_H$ domain; intriguingly, as with the POU$_S$-SNAP$_c$ interaction, the critical Oct-1 residue is a glutamic acid (26, 36, 41). The recent observation that a hybrid protein consisting of a zinc finger domain linked to the Oct-1 POU$_H$ domain can promote formation of the VP16-induced complex confirms that the Oct-1 POU$_S$ domain provides no essential protein contacts for this interaction (37). Similarly, the Oct-1 POU domain stimulates adenovirus DNA replication by enhancing the formation of the preinitiation complex containing the viral precursor terminal protein and DNA polymerase by direct protein-protein interactions between the Oct-1 POU$_H$ domain and the preinitiation complex (6). The POU$_H$ domains of Oct-1 and Oct-2 have also been shown to interact with TBP and high-mobility group 2 protein (53). In contrast, the Oct-1 POU$_S$ domain has, so far, been implicated only in homo- or heterodimerization with other POU-domain proteins (17, 28, 47). It is now clear that it can also participate in protein-protein contacts with heterologous proteins.

The recruitment of the SNAP$_c$-related PTF to the PSE by the Oct-1 POU domain is independent of the distance separating the octamer motif and the PSE (34). In addition, we find that the Oct-1 POU domain can facilitate the binding of SNAP$_c$ to the PSE regardless of the orientation of the octamer motif. In both octamer orientations, the Oct-1 E7R mutation disrupts and the Pit-1 R7E mutation generates the ability to recruit SNAP$_c$ to the PSE, suggesting that the protein-protein contacts are similar in both cases. Yet, if the Oct-1 POU domain recognizes the octamer motif similarly in both cases, the amino terminus of the POU$_S$ domain faces away from SNAP$_c$ when the octamer motif is in the reverse orientation. This raises several possibilities: (i) the SNAP$_c$ region contacted by the Oct-1 POU$_S$ domain may be different depending on the orientation of the octamer site relative to the PSE; (ii) the Oct-1 POU domain may bind differently to the octamer motif in each case; (iii) the POU$_S$ domain may come off the DNA when contacting SNAP$_c$; and (iv) the conformation of SNAP$_c$ itself may be flexible.

Although it is possible that the Oct-1 POU domain contacts different regions of SNAP$_c$ depending on the orientation of the octamer motif, the observation that the Oct-1 E7R and Pit-1 R7E mutations have identical effects in both cases strongly suggests that the protein-protein contacts are the same. Similarly, there is no indication that the Oct-1 POU domain can recognize the H2B octamer site in more than one conformation. It is conceivable, however, that in the Oct-1 POU-SNAP$_c$-DNA complex, the POU$_S$ domain does not actually contact the DNA but instead contacts SNAP$_c$ while off the DNA. Alter-

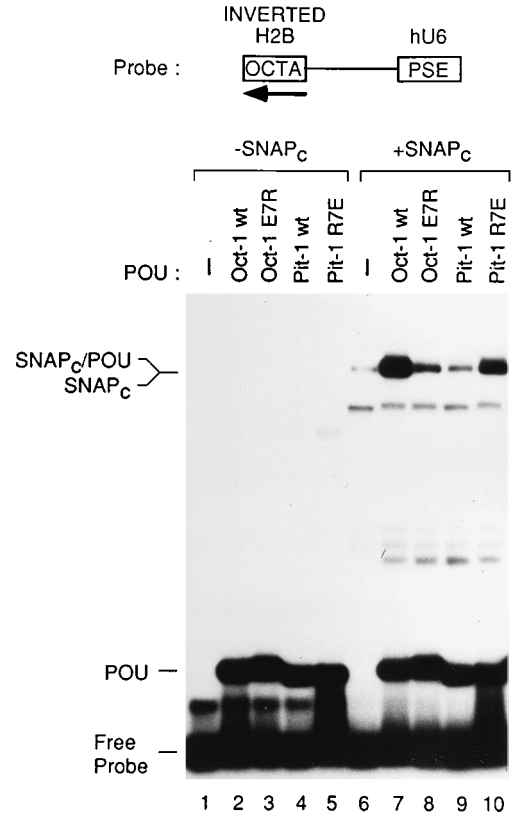

FIG. 8. The orientation of the octamer motif does not affect the ability of the Oct-1 POU domain to recruit SNAP$_c$ to PSE. EMSA was performed with a probe containing an inverted H2B octamer sequence and the human U6 PSE and with the proteins indicated above the lanes. The POU domain derivatives were produced by in vitro translation. wt, wild type.

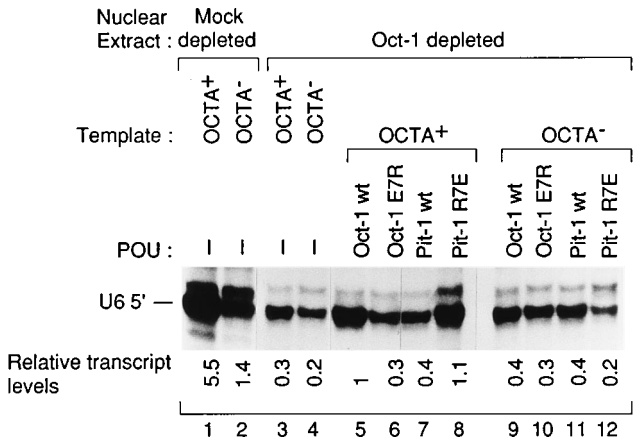

FIG. 9. The abilities of POU-domain derivatives to enhance the binding of $SNAP_c$ to the PSE correlate with their abilities to enhance snRNA gene transcription in vitro. Mock-depleted (lanes 1 and 2) or Oct-1-depleted (with MAbs YL15 and YL8) (lanes 3 to 12) HeLa nuclear extracts were programmed with the U6 templates $OCTA^+$ and $OCTA^-$, containing wild-type (wt) and mutant octamer, respectively (see Materials and Methods for details). Where indicated, the reactions were supplemented with wild-type (wt) Oct-1 POU domain (lanes 5 and 9), Oct-1 POU domain containing the E7R mutation (lanes 6 and 10), wild-type Pit-1 POU domain (lanes 7 and 11), and Pit-1 POU domain containing the R7E mutation (lanes 8 and 12) expressed in *E. coli*. The resulting RNAs were analyzed by RNase T_1 protection with the U6/RA.2/143 probe (29). The signal corresponding to RNA correctly initiated from the U6 promoter is indicated (U6 5′).

natively, the SNAP complex itself may be flexible, and its conformation on the DNA may be affected by protein-protein contacts with the Oct-1 POU domain.

The SNAP complex contains at least four polypeptides in addition to TBP (13); the $SNAP_c$ subunit that contacts the Oct-1 POU domain in the Oct-1 POU-$SNAP_c$-DNA complex remains to be determined. Both the Oct-1 and Oct-2 POU domains have been reported to interact with TBP in the absence of DNA, but this interaction involves the POU_H domain (52). Thus, although a POU_H-TBP interaction may well play a role in the recruitment of $SNAP_c$ to the PSE, the E7R mutation probably disrupts another protein-protein contact involving the POU_S domain and another member of $SNAP_c$. As cDNAs corresponding to all the $SNAP_c$ subunits become available, we will be able to address this question.

Recruitment of basal transcription factors is usually enhanced by the activation domains of *trans* activators. The recruitment of $SNAP_c$ to the PSE is, however, achieved by the DNA-binding domain of Oct-1. That this event actually plays a role in transcription in vivo is suggested by the direct correlation we observe between the abilities of different wild-type and mutant POU domains to recruit $SNAP_c$ to the PSE and their abilities to stimulate snRNA gene transcription in vitro. However, because Oct-1 contains activation domains that lie outside the POU domain and that activate snRNA gene transcription in vivo better than the Oct-2 activation domains do (7, 45, 46), activation by the natural Oct-1 protein in vivo probably results from the concerted effects of its DNA-binding and activation domains. Thus, as with mRNA promoters, for which transcription activation may involve two steps (recruitment of TFIID and subsequent recruitment of other basal transcription factors, either separately or as a holoenzyme complex), activation of snRNA promoters may involve recruitment of $SNAP_c$ to the PSE followed by recruitment of the rest of the initiation complex. Here, however, the first step may be accomplished in large part by the DNA-binding domain rather than by the activation domain.

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