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The Oct-1 POU domain activates snRNA gene transcription by contacting a region in the $SNAP_c$ largest subunit that bears sequence similarities to the Oct-1 coactivator OBF-1

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The RNA polymerases II and III snRNA gene promoters contain an octamer sequence as part of the enhancer and a proximal sequence element (PSE) as part of the core promoter. The octamer and the PSE bind the POU domain activator Oct-1 and the basal transcription factor $SNAP_c$, respectively. Oct-1, but not Oct-1 with a single E7R mutation within the POU domain, binds cooperatively with $SNAP_c$ and, in effect, recruits $SNAP_c$ to the PSE. Here, we show that $SNAP_c$ recruitment is mediated by an interaction between the Oct-1 POU domain and a small region of the largest subunit of $SNAP_c$, SNAP190. This SNAP190 region is strikingly similar to a region in the B-cell-specific Oct-1 coactivator, OBF-1, that is required for interaction with octamer-bound Oct-1 POU domain. The Oct-1 POU domain–SNAP190 interaction is a direct protein–protein contact as determined by the isolation of a switched specificity SNAP190 mutant that interacts with Oct-1 POU E7R but not with wild-type Oct-1 POU. We also show that this direct protein–protein contact results in activation of transcription. Thus, we have identified an activation target of a human activator, Oct-1, within its cognate basal transcription complex.

[*Key Words*: Oct-1; SNAP*c;* snRNA gene; transcription; transcription activation; POU domain]

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Promoters can be divided into a core promoter region, which is sufficient to direct basal levels of transcription in vitro, and a regulatory region, which modulates the levels of transcription by recruiting activators and/or repressors. How activators and repressors regulate core promoter function is a fundamental mechanistic question in eukaryotic transcription, and yet it is still poorly understood. Activators generally contain two functional domains, the DNA-binding domain and the activation domain. Typically, the role of the DNA-binding domain is to target the activator to the correct promoter, whereas the role of the activation domain is to activate transcription (Ptashne 1988). Activation domains can perform a large number of functions including (1) recruiting chromatin remodeling complexes (Struhl 1998), (2) inducing conformational changes (and/or covalent changes) in members of the basal machinery (Horikoshi et al. 1988a,b; Lieberman and Berk 1994; Roberts and Green 1994; Chi et al. 1995; Chi and Carey 1996), and (3) interacting with a variety of basal transcription factors (Burley and Roeder 1996). Such interactions are thought to mediate recruitment of an active basal machinery to promoters, a limiting step for transcription initiation.

The human snRNA promoters constitute a family of related promoters, some of which are recognized by RNA polymerase II (Pol II) and others by RNA polymerase III (Pol III) (Lobo and Hernandez 1994). In the RNA Pol II snRNA promoters, basal transcription is directed by a single element, the proximal sequence element (PSE). In the RNA Pol III snRNA promoters, basal transcription is directed by the combination of a PSE and a TATA box. The RNA Pol II and Pol III PSEs are interchangeable and recruit a five-subunit complex known as $SNAP_c$ (Sadowski et al. 1993; Henry et al. 1998) or PTF (Murphy et al. 1992), which is composed of SNAP190, SNAP50/PTFβ, SNAP45/PTFδ, SNAP43/PTFγ, and SNAP19 (Henry et al. 1998, and references therein). The TATA box in the RNA Pol III snRNA promoters recruits TBP. $SNAP_c$ and TBP bind cooperatively to their respective binding sites on an RNA Pol III snRNA promoter, in a

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manner that is dependent on the nonconserved amino-terminal domain of TBP (Mittal and Hernandez 1997).

Activated snRNA gene transcription depends on the distal sequence element (DSE), which, like the PSE, is interchangeable between the RNA Pol II and Pol III snRNA promoters. The DSE is typically composed of several protein-binding sites, one of which is an octamer sequence (Lobo and Hernandez 1994). The octamer sequence recruits Oct-1, a POU domain protein (Herr et al. 1988; Sturm et al. 1988). The POU domain is a bipartite DNA-binding structure consisting of a POU-specific (POU_S) domain linked by a flexible linker to a POU homeodomain (POU_H) (Herr and Cleary 1995). Oct-1 binds cooperatively with $SNAP_c$ through its POU domain. Because the human PSEs are often very low-affinity binding sites for $SNAP_c$, the Oct-1 POU domain in effect recruits $SNAP_c$ to the PSE (Murphy et al. 1992; Mittal et al. 1996). We have shown before that substitution of a glutamic acid at position 7 in the Oct-1 POU_S domain for an arginine (E7R mutation) strongly reduces the ability of Oct-1 to both recruit $SNAP_c$ to the PSE and activate transcription in vitro (Mittal et al. 1996). Recently, we showed that the largest subunit of $SNAP_c$, SNAP190, associates on its own with octamer-bound Oct-1 POU domain (Wong et al. 1998).

Here we show that SNAP190 interacts with the Oct-1 POU domain through a small region which, remarkably, is similar to an Oct-1 POU-interacting region in OBF-1 (Strubin et al. 1995) [also called OCA-B (Luo and Roeder 1995)] and Bob1 (Gstaiger et al. 1995), a B-cell-specific coactivator that associates with octamer-bound Oct-1 and stimulates transcription from immunoglobulin promoters in an octamer site-dependent manner. The interaction is a direct protein–protein contact as determined by the isolation of a switched specificity SNAP190 mutant that interacts with Oct-1 POU E7R but not with wild-type Oct-1 POU. We also show that recombinant SNAP complexes containing a single or double amino acid change in the Oct-1 POU-interacting region of SNAP190 are significantly impaired in their abilities to mediate activated, but not basal, transcription in vitro. The activation defects correlate with defects in the abilities of these complexes to bind cooperatively with Oct-1 POU. Together, these observations show that the DNA-binding domain of Oct-1 is involved in a direct protein–protein interaction with the largest subunit of $SNAP_c$, which results in recruitment of $SNAP_c$ to snRNA promoters and activation of transcription. Thus, these results clearly establish the identity of a target for Oct-1, a human transcriptional activator, within the basal transcription machinery.

Results

In vivo selection of the smallest amino-terminal SNAP190 truncation capable of interacting with octamer-bound Oct-1

A cDNA encoding the carboxy-terminal half of SNAP190 was originally obtained in a yeast one-hybrid screen designed to identify proteins capable of associating with Oct-1 bound to an octamer motif (Wong et al. 1998). In this screen, illustrated in Figure 1A, the parental yeast strain carried, as a selectable marker, an integrated copy of a *HIS3* allele with six octamer sites upstream of the TATA element, as well as a plasmid that directs constitutive expression of full-length Oct-1 (Strubin et al. 1995). Because Oct-1 does not activate transcription in yeast, this strain transcribes the *HIS3* gene at basal levels and does not grow in the presence of aminotriazole (AT), a competitive inhibitor of the *HIS3* gene product. However, in the presence of a cDNA encoding the VP16 activation domain fused to amino acids 800–1469 of SNAP190, cells are able to grow under selective conditions, and this region of SNAP190 can associate with the Oct-1 POU domain in an electrophoretic mobility shift assay (EMSA), suggesting that this region of SNAP190 is capable of interacting with the Oct-1 POU domain bound to an octamer site (Wong et al. 1998).

To determine the amino-terminal border of the region of SNAP190 able to associate with octamer-bound Oct-1, we used the strategy illustrated in Figure 1B. We subjected a cDNA clone encoding a VP16 activation domain fusion to amino acids 800–1137 of SNAP190, which is active in the yeast one-hybrid assay (data not shown), to limited digestion with exonuclease III to create a SNAP190 amino-terminal deletion library. The yeast tester strain described in Figure 1A was then transformed with the entire deletion library and grown under selective conditions in medium containing AT. This step selected for cells with plasmids expressing SNAP190 truncations capable of associating with octamer-bound Oct-1. Surviving cells were collected and plasmid DNA analyzed by PCR with a forward primer hybridizing to the region corresponding to the end of the VP16 activation domain and a reverse primer hybridizing to a region within the SNAP190 800–1137 fragment, as shown in Figure 1B.

The results of this experiment are shown in Figure 1C. PCR analysis of the plasmids present in the yeast population before selection showed a continuous ladder of bands corresponding to fragments differing by one nucleotide in size, suggesting that the deletion library was complete in this region (lane 1; the under-representation of longer fragments may reflect the composition of the library or less efficient PCR amplification of these longer fragments). After one (lane 2) or two (lane 3) rounds of in vivo selection, we obtained a ladder of bands across only a portion of the deletions and the ladder in this region corresponded to fragments differing by three nucleotides, indicating that growth in AT had resulted in the selection of only one of the three possible reading frames, presumably the one that is in frame with the VP16 activation domain (see Fig. 1B). To identify the end points of the most extensive deletions allowing survival in AT, we excised from the gel the two smallest fragments in the ladder, reamplified the DNA by PCR (lanes 4,5), and sequenced it by the Maxam–Gilbert chemical sequencing method. The results showed that, in these fragments, the VP16 activation domain was fused in

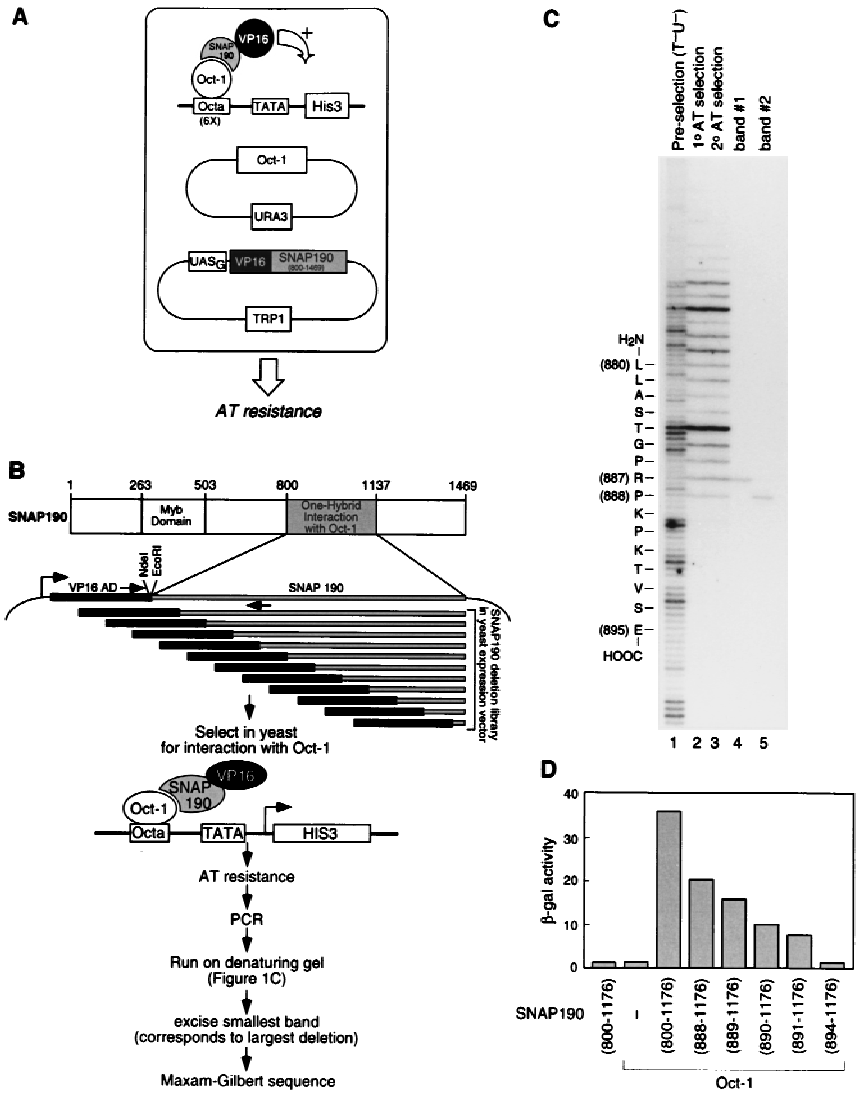

Figure 1. High-resolution mapping of the smallest amino-terminal SNAP190 truncation capable of interacting with Oct-1 in a one-hybrid screen in yeast. (*A*) Schematic diagram of the yeast one-hybrid screen used to isolate originally part of the SNAP190 cDNA (Wong et al. 1998). AT stands for Aminotriazole, a competitive inhibitor of the *HIS3* gene product. (*B*) Schematic diagram of the yeast one-hybrid screen used to map the smallest amino-terminal SNAP190 truncation still capable of interacting with Oct-1. The primers used for the PCR amplification are indicated by small horizontal arrows. (*C*) DNA from yeast cells containing an integrated copy of the *HIS3* reporter and the Oct-1 expression plasmid as diagrammed in *A*, as well as the SNAP190 deletion library diagrammed in *B*, was isolated, amplified in *E. coli*, and used as a template for PCR reactions with the primers depicted in *B*. The DNA template was isolated from yeast cells grown in synthetic medium lacking tryptophan and uracil, which selects for the retention of the two expression plasmids shown in *A* but not the expression of the *HIS3* reporter gene (lane *1*); yeast cells selected for one round (lane *2*) or two rounds (lane *3*) in synthetic medium lacking tryptophan, uracil, and histidine but containing galactose to induce expression of the SNAP190 deletion library and 10 mM AT to select for AT resistance. (Lanes *4*,*5*) Gel purified DNA corresponding to the second to lowest (lane *4*) and the lowest (lane *5*) bands in lane *3* after PCR amplification. The amino-acid sequence corresponding to the DNA ladder is shown at *left* and was determined by: (1) Maxam–Gilbert sequencing of the PCR products in lanes *4* and *5*, and (2) size of the DNA fragments. (*D*) Deletion constructs containing an HA tag between the VP16 activation domain and SNAP190 sequences and the SNAP190 sequences indicated were transformed into a tester yeast strain similar to that shown in *A* except that it contained a *lacZ* reporter gene bearing a TATA box driven by six reiterated octamer motifs. The resulting β-galactosidase activity was that measured in yeast strains expressing VP16-AD–SNAP190 fusion proteins and Oct-1 as indicated.

frame with SNAP190 sequences starting at codon R887 and P888, respectively. Together, these results suggest that the smallest amino-terminal SNAP190 truncation still capable of directing the induced levels of *HIS3* transcription required for survival in AT starts at P888. However, we could not exclude another possibility, namely, that SNAP190 truncations smaller than the P888 truncation were not stably expressed in yeast.

To determine whether SNAP190 truncations were stably expressed in yeast and to confirm the results above, we generated constructs in which we inserted the HA epitope between the VP16 activation domain and SNAP190 sequences, and introduced them into a yeast tester strain similar to the one used above, except that the reporter construct expressed the *lacZ* gene instead of the *HIS3* gene. Immunoblot analysis with monoclonal antibody 12CA5, directed against the HA epitope, confirmed that all SNAP190 truncations were expressed (data not shown). Transcription activation was then measured by a β-galactosidase assay. As shown in Figure 1D, β-galactosidase activity decreased as smaller and smaller SNAP190 truncations, extending from I800, P888, K889, P890, and even K891, were tested. The next truncation, starting at S894, was inactive in this assay. The weak activity of the P890 and K891 truncations in the HA-tagged, but not the untagged construct above, may be the result of the different amino acid sequence at the junction between the VP16 activation domain and SNAP190 sequences. Alternatively, it may result from the use of two very different assays, one scoring for survival in AT and the other for synthesis of the β-galactosidase enzyme. Nevertheless, the two assays point to the

same amino-terminal border with a remarkable accuracy of a few amino acids. This suggests that the approach used here may be generally useful to map protein–protein interaction domains with high resolution.

A small SNAP190 region is sufficient for association with Oct-1 bound to an octamer motif in vitro

We also mapped the SNAP190 region required for interaction with Oct-1 by taking advantage of the EMSA described previously, in which SNAP190 is capable of retarding the mobility of the Oct-1 POU domain bound to an octamer sequence (Wong et al. 1998). The cDNA clone encoding amino acids 800–1137 of SNAP190 was subjected to further amino- and carboxy-terminal deletions, and the corresponding protein truncations were synthesized by translation in a reticulocyte lysate and normalized to equimolar amounts by SDS-PAGE and autoradiography. As shown in Figure 2, none of the SNAP190 truncations were able to bind directly to the octamer-containing DNA probe, as expected (lanes 4–10). In contrast, several of the SNAP190 truncations could associate with a complex containing the Oct-1 POU domain bound to the octamer sequence (cf. lanes 14–20 with lane 11). In particular, carboxy-terminal truncations extending to amino acids 912 and 903 were still active (lanes 16,17). However, combination of these carboxy-terminal deletions with amino-terminal deletions showed that a 869–912 truncation was active (although less so than the 800–912 truncation), whereas both the 869–903 and 874–912 truncations were not (lanes 18–20). Thus, additional sequences at either end of the protein can compensate for deletions at the other end. Note, however, that because the signal intensities obtained in the EMSA are influenced by the varying sizes of the complexes (data not shown), these data are not strictly quantitative. Nevertheless, these results show that in the context of the 869 amino-terminal truncation, the carboxy-terminal border of the Oct-1 POU interaction domain lies between SNAP190 amino acids 903 and 912; they also suggest that amino acid 903 is close to or at the carboxy-terminal boundary of the Oct-1-interacting domain even in the presence of additional amino-terminal sequences, although this was not tested directly. Most importantly, they identify a small, 44-amino-acid region of SNAP190, encompassing residues 869 to 912, that is sufficient to interact with octamer-bound Oct-1 POU in an EMSA.

The inability of the full-length and truncated SNAP190 proteins to associate with the Oct-1 POU domain mutant POU_S E7R (lanes 21–30), which does not bind cooperatively with $SNAP_c$ (Mittal et al. 1996), indicates that the SNAP190–Oct-1 association described above reflects an interaction mediating cooperative binding of Oct-1 POU and $SNAP_c$ on snRNA promoters.

The SNAP190 and OBF-1 regions required for association with the Oct-1 POU/octamer complex are similar

Figure 3 shows an alignment of the region of SNAP190 and OBF-1 involved in Oct-1 POU domain association. The smallest SNAP190 fragment tested still active in an EMSA (amino acids 869–912) is indicated, as well as the largest amino-terminal deletion allowing survival of yeast cells in AT (deletion library boundary, amino acids 888). The largest amino-terminal deletion (to amino acid 891) still scoring in the β-galactosidase assay is indicated by the black arrow above the sequence. Carboxy-terminal deletions were tested only in the EMSA. The most

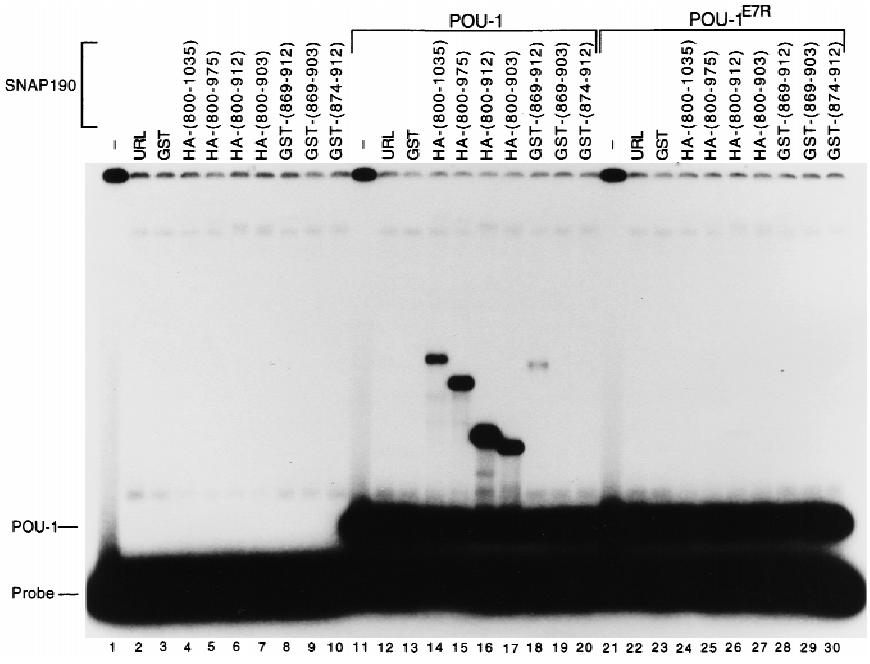

Figure 2. A 44 amino acid SNAP190 region is sufficient for association with the Oct-1 POU domain bound to an octamer sequence in vitro. An EMSA was performed with a DNA probe containing the IgH octamer and the proteins indicated above each lane. Equimolar amounts of each SNAP190 protein were added to each lane as determined by SDS-PAGE and autoradiography (data not shown). The positions of the free probe and the Oct-1 POU–DNA complex are indicated at *left*. (URL) Unprogrammed reticulocyte lysate; (GST) GST protein alone translated in vitro.

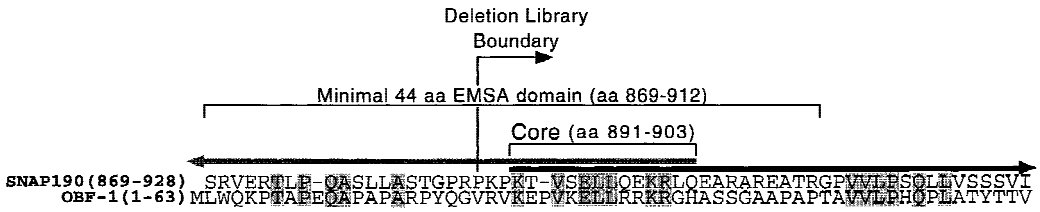

Figure 3. The regions of SNAP190 and OBF-1 that interact with the Oct-1 POU domain contain sequence similarity. The Oct-1 POU interacting regions of SNAP190 and OBF-1 are shown. Identical amino acids are shaded. The arrow marks amino acid 888, the amino-terminal boundary of the most extensively deleted fragment still capable of conferring growth in AT (deletion library boundary) (Fig. 1C). The smallest SNAP190 fragment (amino acids 869–912) that retained the ability to interact with the Oct-1 POU domain by EMSA (Fig. 2) is shown. The black and gray arrows indicate sequences still present in the most extensive amino-terminal (Fig. 1D) and carboxy-terminal (Fig. 2) truncations, respectively, that retained the ability to interact with the Oct-1 POU domain. The core region contains residues absolutely required for interaction with Oct-1 POU.

extensive carboxy-terminal deletion tested that was still active (to amino acid 903) is indicated by the gray arrow above the sequence. The most extensive amino-terminal and carboxy-terminal deletions tested thus define a core extending from amino acid 891 to amino acid 903, which contains residues absolutely required for interaction with octamer-bound Oct-1 POU.

OBF-1 (Strubin et al. 1995), a B-cell-specific coactivator that associates with both Oct-1 or Oct-2 bound to octamer motifs and increases transcription from immunoglobulin promoters, was originally isolated in the same yeast one-hybrid screen used to isolate the SNAP190 cDNA (Strubin et al. 1995). Remarkably, although a comparison with the BLAST program (Altschul et al. 1997) of full-length SNAP190 with sequences in the databases failed to identify any related sequence in OBF-1, a direct comparison of SNAP190 amino acids 869–928 with full-length OBF-1 revealed a striking region of similarity, as shown in Figure 3: SNAP190 amino acids 869–922 and OBF-1 amino acids 2–57 share 17 identical (31%) residues. These are the regions of these two proteins responsible for interaction with DNA-bound Oct-1 POU (Gstaiger et al. 1995). Moreover, OBF-1 residues E30 and L32, which align with the SNAP190 core in the shared sequence ELL, are essential for Oct-1 interaction (Gstaiger et al. 1995). These observations suggest that there are similarities in how OBF-1, a cell-specific transcriptional coactivator, and SNAP190, a basal transcription factor, interact with the transcriptional activator Oct-1.

Single and double amino acid changes in the core Oct-1-interacting region of SNAP190 affect activated but not basal transcription from RNA Pol II and Pol III snRNA promoters

To evaluate the importance of the SNAP190 domain that interacts with octamer-bound Oct-1 POU domain for both basal and activated snRNA gene transcription, we introduced a number of amino acid changes within the 870–920 region of SNAP190. We were, ultimately, interested in obtaining a switched specificity mutant $SNAP_c$ capable of binding cooperatively with the E7R mutant Oct-1 POU domain (see below). We reasoned that the Oct-1 POU_S E7 might be involved in a side chain–side chain interaction with a basic residue in SNAP190, and that the effect of the E7R mutation might be reversed by mutation of an interacting basic residue in SNAP190 to glutamic acid. Therefore, we mutated the seven basic amino acids in the SNAP190 region extending from amino acid 869 to 903 (see Fig. 3) to glutamic acid, and tested the effects for association with both wild-type and E7R Oct-1 POU in the EMSA described above (data not shown). We then introduced two of these mutations, a single K900E mutation and a double K900E/R101E mutation, into recombinant SNAP complexes by coinfecting insect cells with four baculoviruses expressing the small $SNAP_c$ subunits (SNAP19, SNAP43, SNAP45, and SNAP50) as well as with a baculovirus expressing either wild-type or mutant full-length SNAP190. The resulting wild-type and mutant SNAP complexes were immunopurified as described previously (Henry et al. 1998), and their transcription potential was tested in an in vitro transcription assay with constructs containing the basal RNA Pol III U6 snRNA promoter, the related basal RNA Pol III 7SK promoter (Lucito and Hernandez 1988), or the basal RNA Pol II U2 snRNA promoter, downstream of either a high-affinity H2B or a mutant octamer sequence.

As shown in Fig. 4A, B, and C, the templates containing a wild-type H2B octamer were, in each case, more active than those containing a mutant octamer sequence (cf. lanes 1 with lanes 9), indicating that as in vivo (see Lobo and Hernandez 1994), and as observed before (Murphy et al. 1992; Mittal et al. 1996), the octamer motif activates transcription in this in vitro transcription assay. We have shown previously that this activation of transcription is dependent on the Oct-1 POU domain, and is suppressed when the POU domain is replaced by the POU_S E7R POU domain mutant (Mittal et al. 1996). When the extract was depleted of endogenous SNAP complex with antibody beads directed against SNAP190, both activated and basal transcription were strongly reduced (lanes 2,10). We then determined the abilities of the various recombinant SNAP complexes to reconstitute basal transcription by testing the templates with mutated octamer motifs. Basal transcription from all

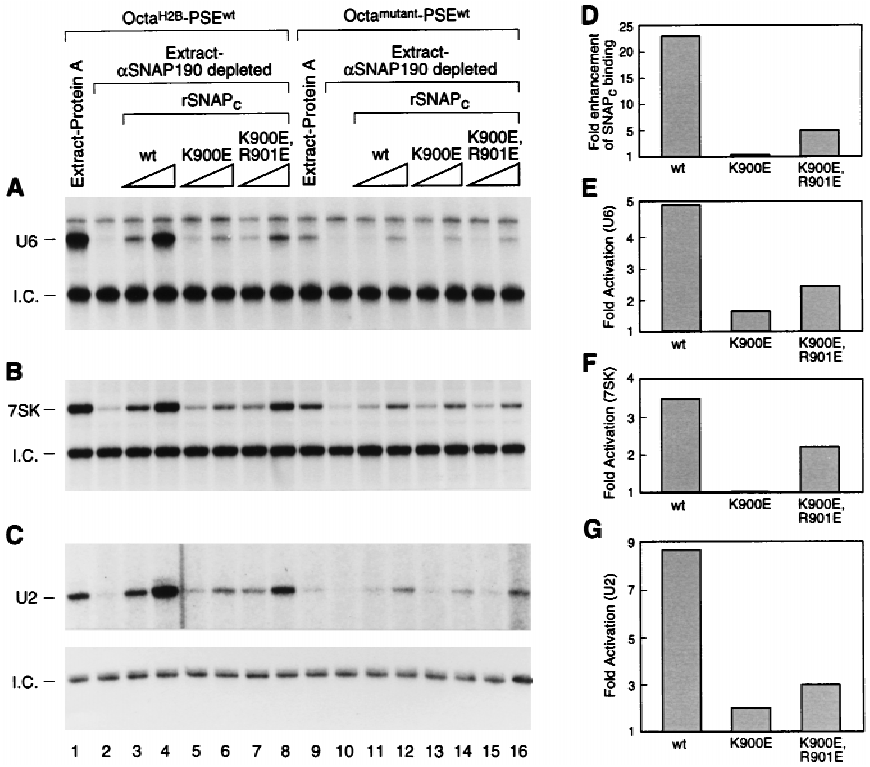

Figure 4. SNAP190 mutants K900E and K900E/E901E specifically disrupt activated but not basal transcription. (*A–C*). In vitro transcription assays were performed for the U6 (*A*; RNase T_1 protection), 7SK (*B*; RNase T_1 protection), and U2 (*C*; Gless cassette) templates containing an H2B octamer motif and a wild-type PSE (lanes *1–8*) or a mutant octamer and a wild-type PSE (lanes *9–16*). HeLa cell extracts (which contain Oct-1 but not Oct-2) were either mock depleted with protein A agarose beads (lanes *1*,*9*) or depleted of $SNAP_c$ with anti-SNAP190–protein A–agarose beads (lanes *2–8* and *10–16*; antibody 402). A total of 0.2 and 0.8 units (U6), 0.2 and 0.8 units (7SK), and 0.5 and 2 units (U2) of wild-type or mutant $SNAP_c$ was added to each reaction as indicated. Transcriptions were stopped and a nonspecific radiolabeled fragment of DNA (U6 and 7SK) or RNA (U2) was added to each reaction to normalize for nucleic acid recovery. Reactions were processed and fractionated on a denaturing polyacrylamide gel (data not shown). The internal control (I.C.) was quantitated and a second normalized gel was run. The U6, 7SK, and U2 transcription experiments were performed five, two, and four times, respectively, with similar results. (*D–G*). The ability of wild-type and mutant $SNAP_c$ complexes to support activated transcription correlates with their ability to be recruited to the PSE by the Oct-1 POU domain. (*D*) The fold enhancement of $SNAP_c$ binding in the presence of Oct-1 POU was determined by quantitating the EMSA in Fig. 5 and dividing the amount of $SNAP_c$ bound to DNA when Oct-1 POU was present in the reaction by the amount of $SNAP_c$ bound to DNA when Oct-1 POU was absent, i.e., (1) the signal in lane *3* divided by that in lane *2* (wt), (2) the signal in lane *6* divided by that in lane *5* (K900E), or (3) the signal in lane *9* divided by that lane *8* (K900E/R901E). (*E–G*). The fold activation of transcription was calculated by dividing the levels of transcription obtained with the H2B octamer-wild-type PSE templates by the corresponding levels obtained with the mutant octamer-wild-type PSE templates. For the U6 and U2 templates, only the fold activation obtained with the higher amount of $SNAP_c$ was calculated, because the levels of basal transcription obtained with the lower amount of $SNAP_c$ were not sufficiently above background. For the 7SK template, the fold activation obtained with both amounts of $SNAP_c$ was calculated and averaged.

three templates, U6, 7SK, and U2, could be rescued by addition of increasing amounts of recombinant wild-type and mutant SNAP complexes (cf. lanes 11–16 with lanes 9 and 10). Significantly, wild-type and mutant SNAP complexes restored transcription to the same extent (for example, cf. lanes 12, 14, and 16) indicating that neither the K900E nor the K900E/R901E mutation in SNAP190 affected the ability of $SNAP_c$ to mediate basal transcription.

To determine the abilities of the recombinant SNAP complexes to respond to Oct-1, we tested the wild-type and mutant SNAP complexes with the templates containing the H2B octamer sequence. As in the absence of the octamer motif, transcription from the U6, 7SK, and U2 templates could be rescued by addition of increasing amounts of recombinant $SNAP_c$, but the levels obtained with wild-type and mutant $SNAP_c$s were very different. Whereas wild-type recombinant $SNAP_c$ restored transcription to levels much higher than those achieved with the templates containing the mutant octamer sequence (cf. lanes 3 and 4 with lanes 11 and 12), $SNAP_c$ K900E restored transcription only to basal levels (cf. lanes 5 and 6 with lanes 13 and 14), and $SNAP_c$ K900E/R901E had intermediate activity (cf. lanes 7 and 8 with lanes 15 and 16). A quantitation of the fold activation mediated by recombinant wild-type and mutant $SNAP_c$s on the U6, 7SK, and U2 promoters is shown in Figure 4E, F, and G, respectively. Together, these results show that SNAP complexes carrying single or double point mutations in their largest subunit are specifically impaired in their ability to mediate Oct-1 activated transcription. In particular, a SNAP complex with the single amino acid change K900E is essentially unresponsive to the presence of an octamer motif, even though it directs normal levels of basal transcription.

The abilities of wild-type and mutant SNAP complexes to mediate Oct-1 activated transcription correlate with their abilities to bind cooperatively with the Oct-1 POU domain

Above, we have defined a small region in SNAP190 that is required for association of SNAP190 with octamer-bound Oct-1 in the absence of a PSE. We have also shown

that recombinant SNAP complexes carrying mutations within this SNAP190 core region are specifically defective for activation of transcription. Next, we wanted to determine whether these mutations might also affect the ability of the reconstituted $SNAP_c$ to bind cooperatively with the Oct-1 POU domain to probes mimicking snRNA promoters and thus containing both an octamer motif and a PSE. As shown in Figure 5, wild-type and mutant SNAP complexes formed weak $SNAP_c$/PSE complexes of similar sizes (complex labeled $SNAP_c$, lanes 2,5,8) and bound specifically to the PSE (lanes 22,25,28), indicating that the mutations introduced into SNAP190 did not interfere with assembly of $SNAP_c$ nor its binding to DNA. This result is consistent with the observation that these three SNAP complexes are equally active for basal transcription (see Fig. 4). On probes containing both a PSE and an octamer, addition of the Oct-1 POU domain resulted in a large increase in the binding of recombinant wild-type $SNAP_c$, and the complex migrated slightly slower than in the absence of the Oct-1 POU domain (cf. lanes 3 and 2). This effect was dependent on the presence of a wild-type octamer motif (cf. lanes 12 and 13). Thus, as with endogenous $SNAP_c$ purified from HeLa cells (Murphy et al. 1992; Mittal et al. 1996), the Oct-1 POU domain is capable of recruiting wild-type recombinant $SNAP_c$ to the PSE. Remarkably, however, addition of the Oct-1 POU domain did not enhance the binding of $SNAP_c$ K900E, even though the Oct-1 POU domain was bound to the probe, as evidenced by the slightly retarded mobility of the complex (cf. lanes 5 and 6). Consistent with its partial activity in the in vitro transcription assay, the $SNAP_c$ mutant K900E/R901E had an intermediate ability to bind cooperatively with the Oct-1 POU domain (lanes 8,9).

Quantitation of the fold enhancement of $SNAP_c$ binding in the presence of the Oct-1 POU domain for recombinant wild-type $SNAP_c$ and mutant $SNAP_c$s is shown in Figure 4D. The correlation of cooperative binding with enhancement of transcription (Fig. 4E–G) is striking. Thus, the abilities of the various recombinant SNAP complexes to bind cooperatively with the Oct-1 POU domain correlated with their abilities to mediate activated transcription. These data suggest that the association of SNAP190 with octamer-bound Oct-1 POU reflects a protein–protein interaction that mediates cooperative binding of the two factors. They also suggest that the ability of Oct-1 POU to recruit $SNAP_c$ to the PSE by cooperative binding is key to transcription activation in vitro.

The K900E mutation results in an altered specificity SNAP complex capable of binding cooperatively with the Oct-1 POU_S E7R mutant but not wild-type Oct-1 POU domain

Our previous observation that mutating the glutamic acid at position seven within the Oct-1 POU_S domain to an arginine (mutation E7R) strongly reduces cooperative binding between the Oct-1 POU domain and $SNAP_c$ (Mittal et al. 1996), as well as the results described above, suggest that the Oct-1 POU_S domain, and in particular residue E7, is engaged in a direct protein–protein contact with the SNAP complex. A convincing demonstration of such a direct protein–protein contact would be the isolation of a compensatory mutation in SNAP190 that enables cooperative binding with Oct-1 POU E7R, hence, the design (basic residue to glutamic acid) of the SNAP190 mutations.

We tested the ability of recombinant wild-type, K900E, and K900E/R901E $SNAP_c$s to bind cooperatively with the Oct-1 POU E7R mutant. As shown in Figure 5, wild-type $SNAP_c$ was recruited much less efficiently to the PSE by Oct-1 POU E7R than by wild-type Oct-1 POU (cf. lanes 4 and 3). Strikingly, however, the reverse was true for K900E $SNAP_c$: This complex was recruited more efficiently by Oct-1 POU E7R than by wild-type Oct-1

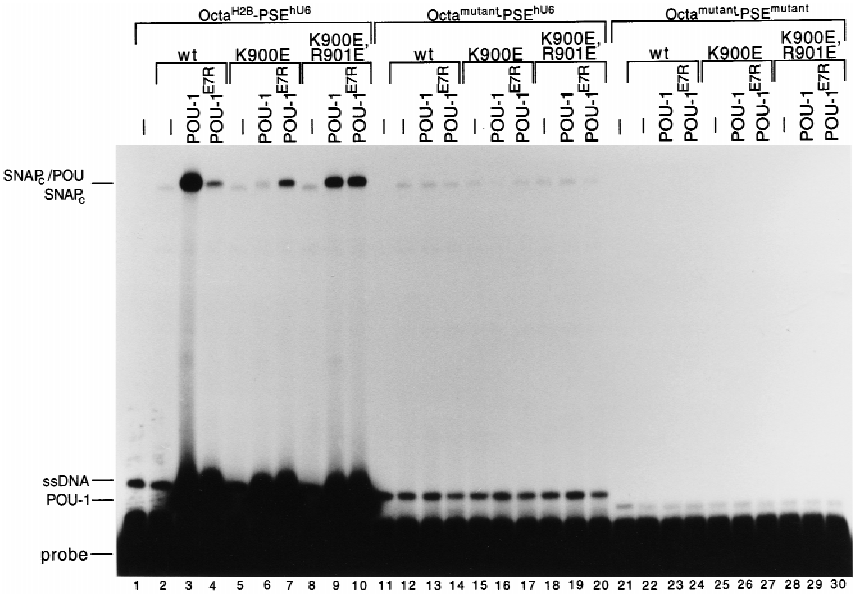

Figure 5. SNAP190 mutants K900E and K900E/R901E form SNAP complexes that bind DNA and interact with switched or relaxed specificity with the wild-type Oct-1 POU domain and the Oct-1 POU domain containing the E7R mutation. An EMSA was performed with DNA probes containing the H2B octamer and the human U6 (hU6) PSE (lanes *1–10*), a mutant octamer and the hU6 PSE (lanes *11–20*), or a mutant octamer and a mutant PSE (lanes *21–30*). Proteins were added as indicated above the gel. Wt, K900E, and K900E/R901E indicate the recombinant $SNAP_c$ complexes used. The positions of the free probe, single stranded probe (ss probe), and complexes containing Oct-1 POU (POU), $SNAP_c$, and $SNAP_c$ together with Oct-1 POU ($SNAP_c$/POU) are indicated at *left*.

POU domain (cf. lanes 6 and 7) demonstrating a switched specificity interaction. In contrast, K900E/R901E $SNAP_c$ was recruited equally by both wild-type and mutated Oct-1 POU domains (cf. lanes 9 and 10) demonstrating a relaxed specificity interaction. These results provide compelling evidence that cooperative binding of the Oct-1 POU domain and $SNAP_c$, and the resulting activation of transcription, derive from a direct protein–protein contact between the two factors involving E7 in Oct-1 POU and K900E in the largest subunit of $SNAP_c$.

Discussion

Although it is clear that, at least in yeast, transcriptional activators can mediate transcription activation by recruiting the basal machinery to promoters, it has been difficult to identify the natural targets of activators with certainty. Here, we have described in molecular details the interaction of a human activator with the basal transcription machinery. We have shown that the Oct-1 POU domain makes a direct protein–protein contact with a small region of the SNAP190 subunit of $SNAP_c$, which, remarkably, is similar in sequence to the region of OBF-1 required for interaction with octamer-bound Oct-1 POU. This contact is direct as determined by the isolation of altered specificity mutants, and mediates both cooperative binding of Oct-1 POU and $SNAP_c$ to the DNA and transcriptional activation.

A similar Oct-1 POU interaction domain in two unrelated factors

An unexpected finding of this study was the striking sequence similarity between the Oct-1 POU-interacting regions of OBF-1 and SNAP190. OBF-1 is a B-cell-specific coactivator, in which the Oct-1 POU interacting domain is fused to a strong activation domain of mRNA gene transcription. SNAP190 is a subunit of a ubiquitously expressed basal transcription complex. Apart from the Oct-1 POU interacting regions, these two proteins do not share any obvious sequence similarity, nor are they known to share any functional role. Thus, two proteins that differ in their tissue distribution, their function, and their primary structure nevertheless share a common Oct-1 POU-binding domain.

The similarity between the SNAP190 and OBF-1 regions required for interaction with octamer-bound Oct-1 POU suggests that these two proteins may contact overlapping surfaces within Oct-1 POU. The region of the Oct-1 POU domain contacted by OBF-1 has been mapped in detail by a mutagenesis of all the surface residues in the Oct-1 POU domain (Babb et al. 1997), and encompasses residues in both the POU_H and the POU_S domains. In particular, this interaction, like that of the Oct-1 POU domain and $SNAP_c$, is sensitive to the Oct-1 POU E7R mutation. This suggests that the interactions of OBF-1 and $SNAP_c$ with the Oct-1 POU domain are mutually exclusive, which would ensure that in B cells, transcription of snRNA genes and immunoglobulin genes do not interfere with each other. It will be very interesting to map the Oct-1 surface contacted by $SNAP_c$.

Cooperative binding of Oct-1 POU and $SNAP_c$ is mediated by a direct protein–protein contact

Perhaps one of the most convincing ways to demonstrate a direct protein–protein contact is through the isolation of switched specificity mutants, in which a mutation in one of the partners affects the interaction, and can be compensated for by a second mutation in the other partner. In the case of TBP and TFIIB, such a switched specificity protein–protein interaction could be engineered by changing the TBP E284–TFIIB R169 polar side chain–side chain interaction revealed by the crystal structure (Nikolov 1995) to the reverse TBP E284R–TFIIB R169E polar side chain–side chain interaction (Tansey and Herr 1997). In our case, in a screen specifically designed to identify altered specificity mutations that might enable interaction with the Oct-1 POU domain mutant E7R, we isolated one SNAP190 switched specificity mutant, K900E, which interacts with Oct-1 POU E7R but not with wild-type Oct-1 POU, and a relaxed specificity mutant, K900E/R901E, which interacts similarly with both. These results show that it is possible to obtain, out of a small set of mutants, altered specificity mutations without the information provided by a crystal structure. Both the switched specificity TBP–TFIIB interaction described previously (Tansey and Herr 1997) and the switched specificity $SNAP_c$–Oct-1 POU interaction described here involve substitution of an acidic residue for a basic residue in one partner and substitution of a basic residue for an acidic residue in the other partner. Thus, this type of mutation may be an easy method for the generation of altered specificity interactions.

Remarkably, the altered specificity mutations we isolated fall precisely within the SNAP190 core region (amino acids 891–903) that is required for interaction with Oct-1 POU, and that is highly conserved in OBF-1 (see Fig. 3). In fact, both K900 and R901 are conserved in OBF-1. Thus, although we do not know the exact details of the side chain–side chain interactions, our results suggest that K900 in SNAP190 and the corresponding K in OBF-1 (K35) may contact E7 in Oct-1 POU. At any rate, our results clearly demonstrate a direct protein–protein contact between SNAP190 and Oct-1 POU involving the regions around K900 of SNAP190 and E7 of the POU domain.

Recruitment of $SNAP_c$ results in transcription activation

The precise correlation between the relative abilities of the $SNAP_c$ mutants to be recruited to the PSE by Oct-1 POU and their relative activation potential strongly suggests that the Oct-1 POU domain mediates transcription activation by recruitment of the core promoter-binding factor $SNAP_c$ to snRNA promoters. The discovery of the

holoenzyme, in which most of the transcription initiation factors are assembled in a complex with RNA Pol II, has led to the proposal that it is the holoenzyme, rather than individual initiation factors, that constitutes the physiological target for recruitment by activators (Carey 1995; Koleske and Young 1995; Orphanides et al. 1996). In yeast, artificial recruitment of the holoenzyme results in transcription activation in vivo (Ptashne and Gann 1997), and, recently, the GAL4-activation domain was shown to activate transcription in yeast through a protein–protein contact with SRB4, a subunit of the yeast holoenzyme (Koh et al. 1998).

The holoenzyme, however, does not contain all of the general transcription factors. Most notably, most preparations of holoenzyme lack (or only contain limiting amounts of) the core promoter-binding factors TFIID and TFIIA (Kim et al. 1994; Koleske and Young 1994; Maldonado et al. 1996; Cho et al. 1997). It seems highly likely, therefore, that activation domains can also recruit core promoter-binding factors. Such a dual role for activation domains is supported by the observation that in yeast, transcription activation can be mediated by artificial recruitment to a core promoter of either a TFIIB-containing complex, which may correspond to the holoenzyme, or TFIID (Gonzalez-Couto et al. 1997; see also references therein). In the mammalian system, studies with the VP16 and ZEBRA activation domains have shown that both enhance binding of TFIID and TFIIA to the core promoter (Lieberman and Berk 1994; Chi et al. 1995; Kobayashi et al. 1995; Chi and Carey 1996). Further, the abilities of partial TFIID complexes to mediate transcription activation by various activation domains correlate with the presence in the partial TFIID complexes of the specific TAFs known to interact with the activation domains in vitro (Chen et al. 1994; Klemm et al. 1995; Sauer et al. 1995; Thut et al. 1995), consistent with transcription activation in these cases resulting from recruitment of TFIID by the various TAF-activation domain interactions.

Together, these considerations lead to the model shown in Figure 6A, in which activation domains can recruit either the core promoter-binding factor TFIID (perhaps with TFIIA), or the holoenzyme, through direct protein–protein contacts. Different activation domains may recruit either or both of these complexes, and the importance of each recruitment event for activation of transcription may vary for different core promoters and in different organisms. In particular, recruitment of core promoter-binding factors may be much more important in mammalian cells than in yeast cells, reflecting the much more complicated transcriptional regulatory network in higher eukaryotes.

Our identification of $SNAP_c$ as a target for the activator Oct-1 provides us with a clear case of transcription activation by recruitment of a core promoter-binding factor, as shown in Figure 6B. In this case, the activator–core promoter-binding factor interaction is known in molecular detail and consists of a direct protein–protein contact involving well-defined regions in both $SNAP_c$ and Oct-1 POU. Interestingly, the Oct-1 activator recruits $SNAP_c$ through its DNA-binding domain. Thus, the Oct-1 DNA-binding domain performs one of the functions generally ascribed, in eukaryotes, to transcription-activation domains, namely recruitment of a core binding factor. How the Oct-1 activation domains activate snRNA gene transcription is not known, but they contribute little to recruitment of $SNAP_c$ to the PSE in vitro (Ford and Hernandez 1997), suggesting that they perform another function. The observation that transcription from snRNA promoters is efficiently activated by Oct-1 activation domains and a glutamine-rich activation domain derived from Sp1 (Das et al. 1995), but much less efficiently by good activators of mRNA transcription derived from VP16 or Oct-2 (Tanaka and Herr 1990; Tanaka et al. 1992; Das et al. 1995), suggests that they accelerate a step that is specifically limiting for snRNA gene transcription.

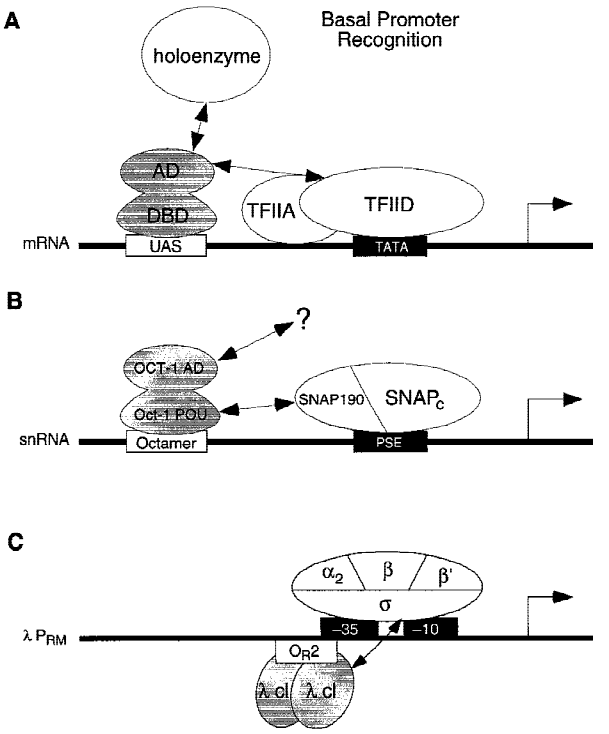

Figure 6. Various examples of transcription activation by recruitment. (*A*) A typical activator on a mRNA promoter contains a DNA-binding domain (DBD) and an activation domain (AD). The activation domain may perform a number of functions, among them recruitment of core promoter-binding factors, such as TFIID and TFIIA, and recruitment of the holoenzyme. (*B*) The Oct-1 activator contains a DNA-binding domain consisting of a POU domain and an activation domain. On snRNA promoters, the Oct-1 POU domain recruits the core promoter-binding factor $SNAP_c$ through a direct protein–protein interaction with a small region of SNAP190. How the Oct-1 AD activates snRNA gene transcription is not known. (*C*). The λ *c*I repressor activates transcription from the λ P_{RM} promoter by binding as a dimer close to the binding site for RNA polymerase and recruiting, through a direct protein–protein contact, the core promoter–recognizing subunit of the RNA polymerase, σ factor.

The Oct-1 POU domain and the bacteriophage λ cI protein activate transcription by a similar mechanism

The mechanism of transcription activation in eukaryotes by the Oct-1 POU domain is reminiscent of transcription activation in prokaryotes by the λ *c*I repressor. As shown in Figure 6C, in the λ P_{RM} promoter, the cI repressor binds as a dimer upstream of the RNA polymerase-binding site, and in this position, activates transcription by recruiting RNA polymerase to the DNA through direct protein–protein contacts with its DNA-binding domain (Guarente et al. 1982; Hochschild et al. 1983; Bushman et al. 1989; Li et al. 1994). Bearing similarity to the interactions of the Oct-1 POU domain with $SNAP_c$, the RNA polymerase subunit contacted by the cI DNA-binding domain is the σ subunit, which, like $SNAP_c$, is a core promoter-binding factor. Moreover, the three-dimensional structure of the cI DNA-binding domain (Jordan and Pabo 1988; Beamer and Pabo 1992) is very similar to the Oct-1 POU_S domain structure (Assa-Munt et al. 1993; Dekker et al. 1993; Klemm et al. 1994), which, as shown here, is involved in protein–protein contact with $SNAP_c$ (Mittal et al. 1996). Thus, despite the enormous apparent complexity of the transcription apparatus of higher eukaryotes as compared with prokaryotes, there are striking similarities in how a common transcription activation process is achieved: The DNA-binding domain of an activator recruits the basal transcriptional machinery through analogous protein–protein contacts.

Materials and methods

Plasmid constructs

Yeast expression vectors The yeast expression plasmid pK35#2 contains the following features in the following order: (1) the G_{UAS} promoter, (2) a nuclear localization signal, (3) the VP16 activation domain, (4) unique *Nde*I and *Eco*RI sites, (5) the coding sequence for amino acids 800–1137 of SNAP190, (6) a unique *Not*I site, and (7) stop codons in all three frames (Wong et al. 1998). A *Bam*HI site present in the vector backbone of pK35#2 was destroyed by cutting with *Bam*HI, filling in with Klenow, and religated to create pK35#2^{Bam-}. The plasmid pK35#2^{Bam-} was then cut with *Eco*RI and *Not*I to release the coding sequence of SNAP190, which was replaced by an insert generated by PCR. The insert contained the following features: (1) an *Eco*RI site, (2) a HA tag, (3) a *Nhe*I site, (4) the coding sequence for amino acids 800–1137 of SNAP190, (5) a *Bam*HI site, (6) a stop codon, and (7) a *Not*I site. The resulting plasmid is called pKHA–SNAP190(800–1137) with the numbers in the parenthesis referring to the SNAP190 amino acid sequence expressed by the construct. The inserts for the SNAP190 deletion constructs pKHA–SNAP190(888–1137), pKHA–SNAP190(889–1137), pKHA–SNAP190(890–1137), pKHA–SNAP190(891–1137), and pKHA–SNAP190(894–1137) were generated by PCR using primers with amino-terminal overhanging *Xba*I sites and carboxy-terminal overhanging *Bam*HI sites. Because *Nhe*I and *Xba*I have compatible overhangs, these inserts were then inserted into the pKHA35 plasmid that had been linearized with *Nhe*I and *Bam*HI.

Rabbit reticulocyte expression constructs All deletion mutants of SNAP190 were generated by PCR with amino-terminal *Xba*I sites and carboxy-terminal *Bam*H1 sites. The deletions were either inserted into a modified version of pCite2a+, which contains an amino-terminal HA tag and unique *Xba*I and *Bam*HI cloning sites (a kind gift of Angus Wilson, New York University, New York, NY), or into a modified version of pET11c containing an amino-terminal GST tag and unique *Xba*I and *Bam*HI sites (Aurora and Herr 1992; Wong et al. 1998).

Glutamic acid substitution mutants Glutamic acid substitutions were introduced into the SNAP190 coding sequence as described previously (Kunkel 1985).

Baculovirus expression constructs The coding sequences of SNAP190, SNAP190 K900E, SNAP190 K900E/R901E, SNAP50, SNAP45, SNAP43, and SNAP19 were cloned into a modified version of the baculovirus transfer vector pAcUW51 (a kind gift of Paul Kaufman, University of California at Berkeley).

Reporter construct The U6 reporter constructs were described previously (Mittal et al. 1996). The 7SK reporter constructs were generated with oligonucleotides containing (1) the human H2B octamer and the human 7SK PSE (5′-GCAGATCTATACGTACACCTTATTTGCATAAGCGAATCTCGAGCGTCCAACTTGACCTAAGTGTAAAGTTGAGACTTCCTTCAG-3′ and 5′-CGGGGTACCGAGGTACCCAAGCGGCGCACAAGCTATATAAACCTGAAGGAAGTCTCAAC-3′), or (2) a mutant octamer and the human 7SK PSE (5′-GCAGATCTATACGTACACCTTAggTGCcgAAGCGAATCTCGAGCGTCCAACTTGACCTAAGTGTAAAGTTGAGACTTCCTTCAG-3′ and 5′-CGGGGTACCGAGGTACCCAAGCGGCGCACAAGCTATATAAACCTGAAGGAAGTCTCAAC-3′). These oligonucleotides were annealed, filled in with Klenow, and cut with *Sna*BI and *Kpn*I before they were inserted into the vector pU6/Hae/RA.2/Asp2 (a gift of P. Reinagel, Cold Spring Harbor Laboratory) that had also been cut with *Sna*BI and *Kpn*I. The resulting plasmids are called p7SK/Kpn/O^+P^+ and p7SK/Kpn/O^-P^+. The U2 reporter constructs were generated with oligonucleotides containing the human H2B octamer and the human U2 PSE (5′-CCCGGAATTCGCTTATGCAAATAAGGGGTACCGAGGCTGGGGCTTCACCGCGACTTGA-3′ and 5′-CTTTCCGCTCGAGGCCGTCACCGTCCCACTCTCATCCACATTCAAGTCGCGGTGAGAGC-3′). The oligonucleotides were annealed, filled in with Klenow, and cut with *Eco*RI and *Xho*I before they were inserted into the plasmid pU2*G^- (Sadowski et al. 1993) that had also been cut with *Eco*RI and *Xho*I. This created plasmid pU2VMGless–H2B/hU2PSE. The U2 reporter construct with the mutant octamer site was generated with oligonucleotides containing a mutant octamer site (5′-CCGGAATTCGCTTcgGCAccTAAGGGGTACCCCG-3′ and 5′-CGGGGTACCCCTTAGGTGCCGAAGCGAATTCCGG-3′). These oligonucleotides were annealed, cut with *Eco*RI and *Kpn*I, and inserted into the plasmid pU2VMGless–H2B/hU2PSE that had also been cut with *Eco*RI and *Kpn*I. This created the plasmid pU2VMGless–mutOctamer/hU2PSE.

Mapping of the SNAP190 domain that mediates interaction with Oct-1 by use of a yeast one-hybrid assay

SNAP190 amino-terminal deletion library The library was generated with the double-stranded Nested Deletion Kit (Pharmacia). A total of 32 μg of the plasmid K35#2 was cut with *Nde*I, filled in with αS-dNTPs using Klenow, and cut with *Eco*RI. One-half of the αS-blocked linearized plasmid was then digested with 100 units of Exonuclease III nuclease at 30°C. Aliquots were removed from the reaction every 0.5–2 min for 42 min. The reactions were stopped by addition of S1 nuclease buffer,

and the single-stranded DNA was removed by digestion with S1 nuclease. The reactions were pooled and treated with Klenow to obtain blunt ends, and the linear DNAs were recircularized with T4 Ligase. To amplify the library, we transformed it into *Escherichia coli* with the *E. coli* Gene Pulsar (BioRad) according to the manufacturer's instructions and plated the cells onto 20 10-cm plates. The cells were grown for 18 hr and DNA was purified with a Qiagen 500 tip.

Selection in yeast The deletion library was introduced into a yeast strain carrying as a selectable marker gene an integrated copy of a *HIS3* allele bearing six octamer sites upstream of the TATA element, and expressing constitutively Oct-1 from a centromeric plasmid marked with the *URA3* gene (Strubin et al. 1995). The library (10 µg) was introduced into the reporter strain according to Schiestl and Gietz (1989) except that human poly(A^-) RNA (130 µg) was used as carrier in place of salmon sperm DNA. An estimated 5×10^5 double transformants were grown for 24 hr at 30°C in 250 ml of glucose medium lacking uracil and tryptophan to maintain selection for both plasmids, at which time the culture consisted of ~80% Trp^+/Ura^+ cells. After centrifugation and wash in TE/LiAc, 1×10^8 cells were resuspended in 50 ml of galactose selective medium and incubated for 24 hr at 30°C to induce expression of the truncated protein library. Transformants (starting with an OD_{600} of 0.1) were then grown in 50 ml of galactose synthetic medium lacking histidine and containing 10 mM AT to an optical density of 1.0, at which time the culture was diluted 15-fold before a second round of selection in AT medium. As Oct-1 cannot activate transcription in yeast, cells that grow under these selective conditions are likely to express truncated SNAP190 proteins that still interact with Oct-1 on the *HIS3* promoter. The SNAP190-containing plasmids were recovered from large pools of transformants (2×10^8 cells) either before, or after one or two rounds of selection in AT. Plasmid rescue was performed according to Robzyk and Kassir (1992), except that the DNA was further purified on a Sepharose CL-4B column to remove impurities that inhibit *E. coli* transformation. The library was amplified in *E. coli* after transformation by electroporation (2×10^5 colonies).

The PCR reaction was performed with 10 ng of the library DNA linearized at a unique *Kpn*I site as template, and 50 ng of forward ^{32}P-labeled oligonucleotide MS70 corresponding to the region encoding the carboxyl terminus of VP16 (5′-GGAATTGACGAGTACGGTG-3′) and oligonucleotide L20 (5′-CTGCTCCTCTGGTCGTCGA-3′) complementary to a region within SNAP190 as primers. Amplification was carried out in standard reaction buffer (2 mM $MgCl_2$); annealing was for 30 sec (65°C–55°C touch down followed by 10 cycles at 55°C) and primer extension at 72°C for 45 sec. After phenol extraction and ethanol precipitation, the PCR products were separated on a 6% acrylamide/urea denaturing gel. The two lower bands were eluted by diffusion in TE (10 mM Tris-HCl at pH7.5, 0.1 mM EDTA) containing 0.3 M of NaAc and 10 mg of tRNA, amplified by PCR, and subjected to Maxam–Gilbert sequencing.

LacZ *assays* Transformed yeast cells were grown in selective medium and assayed for β-galactosidase activity as described (Gonzalez-Couto et al. 1997).

Sources of proteins

Oct-1 POU domain The Oct-1 POU domain and the mutant Oct-1 POU E7R were produced in *E. coli* as GST fusion proteins. The GST moiety was cleaved off with thrombin prior to use as described in Aurora and Herr (1992).

SNAP190 truncations All SNAP190 truncations were produced in rabbit reticulocyte lysate with the TNT T7 Expression System (Promega) and ^{3}H-labeled leucine according to the manufacturer's instructions. Amounts of protein were normalized by SDS–PAGE and autoradiography.

Assembly of recombinant $SNAP_c$ in baculovirus Recombinant baculoviruses expressing the coding sequences for SNAP190, SNAP190 K900E, SNAP190 K900E/R901E, SNAP50, SNAP45, SNAP43, and SNAP19 were generated with the BacVactor 3000 DNA kit (Novagen) according to the manufacturer's instructions. The viruses were purified by two rounds of plaque purification and amplified to a titer of $1–5 \times 10^8$ plaque-forming units/ml. Sf9 cells (ATCC) were coinfected with all five baculoviruses at a multiplicity of infection (MOI) of 10 for each virus. Cells were harvested 48 hr postinfection, washed with PBS, and incubated in 1 ml of Sf9 lysis buffer/2×10^7 cells (10 mM Tris-HCl at pH 7.5, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaP_i, 10 mM $NaPP_i$) on ice for 30 min, and the lysate was spun in a microfuge at 14,000 rpm for 15 min. The supernatants were recovered and recombinant SNAP complexes were purified by immunoaffinity chromatography and peptide elution as described previously (Henry et al. 1998), except that the anti-SNAP190 antibody 402 (Wong et al. 1998) and its corresponding peptide were used, and the extract to bead ratio was 4:1. Purified $SNAP_c$ complexes all contained the same amount of binding activity as determined by binding to the high-affinity mouse U6 PSE in an EMSA. One unit of activity was defined as the amount of $SNAP_c$ needed to shift 25% of 50–100 pg of probe when incubated for 20 min with a mouse U6 PSE DNA probe and assayed by EMSA.

EMSA

SNAP190 supershift assay The EMSA in Figure 2 was performed essentially as described (Strubin et al. 1995) except that 0.5–1 ng of Oct-1 POU proteins expressed in *E. coli* and 0.5 µl of SNAP190 proteins expressed in rabbit reticulocyte lysate were used.

Oct-1/$SNAP_c$ cooperativity assay The EMSA in Figure 5 was performed as described (Mittal et al. 1996), except that 1 unit of recombinant $SNAP_c$ was used.

Immunodepletions and transcription reactions

Whole cell extracts (7SK and U2) or nuclear extracts (U6) from HeLa cells were mixed with an equal volume of anti-SNAP190 (antibody 402)–protein A–agarose beads or protein A–agarose beads equilibrated in Buffer D (Dignam et al. 1983). The extract–bead slurry was incubated for 1 hr at 4°C and for 30 min at room temperature. The transcription reactions were performed with 8 µl (U6 and 7SK) or 16 µl (U2) of extract and 0.5 µg (U6 and 7SK) or 1 µg (U2) of template as described previously (Sadowski et al. 1993). The amount of recombinant $SNAP_c$ ($rSNAP_c$) used is indicated in the legend to Figure 4.

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