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Mechanisms for Flexibility in DNA Sequence Recognition and VP16-Induced Complex Formation by the Oct-1 POU Domain

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DNA binding by the Oct-1 protein is directed by its POU domain, a bipartite DNA-binding domain made up of a POU-specific (POU_S) domain and a POU-homeo (POU_H) domain, two helix-turn-helix-containing DNA-binding modules that cooperate in DNA recognition. Although the best-characterized DNA target for Oct-1 binding is the octamer sequence ATGCAAAT, Oct-1 also binds a number of different DNA sequence elements. For example, Oct-1 recognizes a form of the herpes simplex virus VP16-responsive TAATGARAT element, called the ($OCTA^-$)TAATGARAT site, that lacks octamer site similarity. Our studies suggest two mechanisms by which Oct-1 achieves flexible DNA sequence recognition. First, an important arginine found in the Oct-1 POU_S domain tolerates substitutions of its base contacts within the octamer site. Second, on the ($OCTA^-$)TAATGARAT site, the POU_S domain is located on the side of the POU_H domain opposite from where it is located on an octamer site. This flexibility of the Oct-1 POU domain in DNA binding also has an impact on its participation in a multiprotein-DNA complex with VP16. We show that Oct-1 POU_S domain residues that contact DNA have different effects on VP16-induced complex formation depending on whether the VP16-responsive element involved has overlapping octamer similarity or not.

Eukaryotic *trans*-acting regulators of transcription often display a remarkable flexibility in promoter recognition that derives, in part, from their ability to bind to a number of different DNA sequences. An excellent example of a DNA-binding protein that recognizes different *cis*-acting elements is the mammalian POU domain factor Oct-1 (reviewed in reference 13). Although the best-characterized *cis*-regulatory target for Oct-1 is the octamer motif ATGCAAAT, Oct-1 also binds to sites that bear little resemblance to the octamer consensus. For example, Oct-1 binds a promoter element of the herpes simplex virus (HSV) immediate-early genes called the TAATGARAT (where R is a purine) element.

The TAATGARAT sequence is the target of activation by the HSV transcriptional activator VP16, which induces formation of a DNA-dependent complex (called the VP16-induced complex) with Oct-1 and another cellular factor, HCF (reviewed in reference 39). Two forms of the TAATGARAT site exist in HSV promoters. The first, called the ($OCTA^+$)TAATGARAT motif, contains an overlapping octamer sequence (e.g., ATGCTAATGATAT from the ICP0 promoter), to which Oct-1 binds with a relatively high affinity (12, 18, 23). The second type of TAATGARAT site, called ($OCTA^-$)TAATGARAT, lacks an overlapping octamer sequence (e.g., GCGG TAATGAGAT from the ICP4 promoter). On these sites, Oct-1 binds with a low affinity across the entire TAATGARAT sequence, which may bear as little as a 4-of-8-bp match to the octamer consensus (1, 5).

The Oct-1 DNA-binding domain consists of a POU domain. This evolutionarily conserved DNA-binding domain exhibits a unique mode of DNA recognition that results from the synergistic effect of two separate, but linked, DNA-binding subunits (2, 4, 6, 10, 15, 17, 19, 37, 41): a POU-specific (POU_S) domain joined by a hypervariable linker to a C-terminal POU-homeo (POU_H) domain (14). Both of these POU domain subunits contain helix-turn-helix (HTH) motifs. The POU_H domain is very similar to other homeo domains (17, 32), whereas the POU_S domain is more related to the DNA-binding domains of the bacteriophage proteins λ repressor, 434 repressor, and 434 Cro (2, 10).

A crystal structure of the Oct-1 POU domain bound to an octamer site (17) has revealed that both of the POU domain subunits bind to the major groove on opposite sides of the DNA. The interactions of the POU_S and POU_H domains with DNA closely resemble those of their bacteriophage or homeo DNA-binding counterparts, respectively. Significantly, the POU_S and POU_H domains do not touch one another when bound to an octamer site, and the linker is apparently flexible.

Here, we have analyzed the contribution of the POU_S domain to flexible DNA sequence recognition by Oct-1 and to assembly of the VP16-induced complex. We assayed the effects of amino acid substitutions in the POU_S domain and of base substitutions in octamer and ($OCTA^-$)TAATGARAT sites on Oct-1 POU domain binding to DNA. In addition, we tested the relative effects of the POU_S domain amino acid substitutions on VP16-induced complex formation on the ($OCTA^+$)- and ($OCTA^-$)TAATGARAT sites. The results suggest that flexible DNA sequence recognition can be achieved by both flexible amino acid-base interactions and flexible arrangements of the Oct-1 POU_S and POU_H domains on DNA. Furthermore, POU_S domain residues that contact DNA have different roles in stabilizing the VP16-induced complex on ($OCTA^+$)- and ($OCTA^-$)TAATGARAT sites.

MATERIALS AND METHODS

POU_S domain mutagenesis. Mutant Oct-1 POU_S domain proteins were generated by oligonucleotide-mediated site-directed mutagenesis (20, 44) of the plasmid pET11c.G.POU-1 (21), which encodes the Oct-1 POU domain fused to

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the glutathione-*S*-transferase (GST) gene product. The following sequences, which contain cosegregating restriction site polymorphisms, were created to generate the mutant proteins: Q27A, ACg **gcc** GGT (*Eag*I); G35P, ATG **ccG** AAg CTt TAT (*Hin*dIII); Q44A, AGC **gcA** ACT (*Hha*I); and R49A, TCg **gcc** TTT (*Hae*III). In this notation scheme, the bases that are altered in the wild-type Oct-1 POU domain sequence are shown in lowercase, the new amino acid codon is in boldface, and the engineered restriction sites for the nucleases indicated in parentheses are underlined. The double mutant POU domain protein (Q27A/Q44A) was made by two sequential rounds of site-directed mutagenesis. The pET11c.G.POU_H-1 Oct-1 POU_H domain expression plasmid was generated by loop-out mutagenesis of the POU_S domain and linker-coding regions from the pET11c.G.POU-1 plasmid with an oligonucleotide that spans the 3′ sequence of the GST-coding region and the 5′ sequence of the homeo domain-coding region, generating the junction sequence CGTGGATCTAGA/CGCCGGAAGAAA. Each mutation was verified by dideoxy DNA sequencing (31).

Preparation of in vitro-translated and *Escherichia coli*-expressed proteins. Wild-type and mutant forms of pET11c.G.POU-1 were used as templates for PCR with the upstream primer SP65′OcPOU, which matches the 5′ coding sequences of the Oct-1 POU_S domain and provides in vitro transcription and translation signals, and the downstream primer OcHBam, which matches the 3′ coding sequences of the Oct-1 POU_H domain (4).

These PCR-amplified products were used to synthesize RNA, which was used to program reticulocyte lysates (Promega, Inc.) in the presence of [^{35}S]methionine. The amount and quality of POU domain protein were determined by use of a Fuji BAS1000 Phosphorimager or by scintillation counting of bands after sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis. Oct-1 POU domain protein concentrations were normalized by dilution in unprogrammed reticulocyte lysate.

Fusion constructs between GST and the full-length POU and POU_H domains were expressed in *E. coli* BL21(DE3) cells by use of the T7 expression system (35). Growth conditions and purification steps were as described previously for the GST–Oct-1 POU domain fusion (21).

Wild-type and mutant DNA-binding sites. Most of the binding sites used were based on single-copy forms of the H2B/OCTA and ICP4/TAAT-1 sites described previously (9). In addition, we used a single-copy form of an ($OCTA^+$)TAATGARAT site taken from the ICP0 gene promoter referred to as ICP0/TAAT-1 (tcgagCATGCTAATGATATTCTTctcga), which was generated in a similar way (9). In addition to the wild-type forms of these sites, we used two previously described double mutant forms: H2B/OCTA*dpm1* (A_1T_2→CG) and ICP4/TAAT-1*dpm1* (A_8T_9→CG) (9). For this study we generated the following new mutant sites by oligonucleotide-mediated site-directed mutagenesis: H2B/OCTA*spm1* (A_1→C), H2B/OCTA*spm2* (T_2→G), ICP4/TAAT-1*spm1* (A_8→C), ICP4/TAAT-1*spm2* (T_9→G), and ICP4/TAAT-1*dpm2* (A_2A_3→CC). The H2B octamer mutation G_3C_4→TT is based on a previously described double-point mutation in an octamer site derived from the simian virus 40 enhancer (B20*dpm7*) (38); for consistency we refer to this site as H2B/OCTA*dpm7*.

For all binding assays with in vitro-translated proteins, probes were generated by PCR amplification of the pUC119 polylinker containing the appropriate binding site with ^{32}P-end-labeled primers. For the binding assays with the GST-POU and GST-POU_H domain fusion proteins, probes were also generated by PCR, but the ^{32}P-end-labeled primers used (pUC/HIII [5′-AGCTTGCATGC CTGCA] and pUC/RI [5′-AATTCGAGCTCGGTAC]) allowed amplification of a shorter segment of the pUC119 polylinker region. This shorter fragment lacks alternate POU_H domain-binding sites present in the longer fragment.

Electrophoretic mobility retardation assays. Protein-DNA binding reactions (12.5-μl total reaction mixture volume) were performed as follows. The in vitro-translated Oct-1 POU domain proteins (2 μl) were incubated with 2×10^4 cpm (1 μl) of DNA probe in 8 mM Na-HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.9)–60 mM NaCl–4 mM spermidine–2 mM EDTA–0.2 mM dithiothreitol–0.03% Nonidet P-40–0.1 mg of bovine serum albumin (BSA) per ml–0.13 mg of unsonicated poly(dI-dC) (Pharmacia) per ml for 30 min at 30°C. After incubation, the reaction mixtures were loaded onto a Tris-glycine 5% polyacrylamide gel (acrylamide/bisacrylamide ratio, 29:1), which had been subjected to prior electrophoresis for 30 min at room temperature. Mobility retardation analysis of the VP16-induced complex was performed as described previously (9), except that in vitro-translated wild-type and mutant POU domain proteins were used instead of full-length Oct-1. Levels of complex formation were measured with a Fuji BAS1000 Phosphorimager.

DEPC modification interference analysis. Diethylpyrocarbonate (DEPC) interference analysis was carried out essentially as described previously (36). The ($OCTA^-$)TAATGARAT site-containing probes used were generated by PCR with one 5′-end-labeled primer and one unlabeled primer. Binding reactions with DEPC-modified DNAs (50-μl volumes) were in 10 mM HEPES (pH 7.9)–60 mM KCl–0.25 mM dithiothreitol–1 mM EDTA–0.1 mg of BSA per ml–0.06 mg of poly(dI-dC) per ml–2% Ficoll. The sources of Oct-1 and HCF were two separate fractions of the same HeLa cell nuclear extract passed over heparin-agarose (36), and the source of VP16 was a protein A-VP16 fusion protein (33, 43). The relative intensities of the bands were determined by densitometry.

RESULTS

Figure 1 shows features of the Oct-1 POU domain bound to an octamer site. When bound to the octamer site (Fig. 1A), the POU_S domain makes base contacts with the 5′ ATGC half of the octamer sequence (Fig. 1C), and the POU_H domain contacts the 3′ AAAT half (17). Three amino acid residues within the POU_S domain make base contacts within the octamer site, a glutamine at position 44 (Q-44), a threonine at position 45 (T-45), and an arginine at position 49 (R-49). Of these three, Q-44 and R-49 are invariant among all POU domain proteins. Q-44 contacts the adenine at position 1, and R-49 makes a bifurcated contact with the two GC base pairs at positions 3 and 4 of the octamer sequence (Fig. 1C).

The conservation of Q-44 and another invariant POU_S domain glutamine residue (Q-27) extends beyond POU domains to the λ repressor, 434 repressor, and 434 Cro protein DNA-binding domains. These glutamines participate in one of the key amino acid side chain-DNA interactions used by both the POU_S domain and the bacteriophage proteins. For this interaction (Fig. 1B), the two glutamines, which lie at the beginning of each of the two α helices of the POU_S HTH motif (Fig. 1A), participate in a coordinated interaction with each other and with an adenine base (A_1) and backbone phosphate (between T_{-1} and A_1) in the DNA (17).

An invariant arginine residue makes flexible contacts with consensus and variant octamer sites. To study the contribution of the POU_S domain to DNA binding by the Oct-1 POU domain, we first analyzed binding of the wild-type Oct-1 POU domain to wild-type and mutant octamer sites with mutations in the POU_S domain-binding site. Figure 2B shows an electrophoretic mobility retardation assay testing binding to the wild-type and mutant octamer sites shown in Fig. 2A. We mutated each of the four bases contacted by the Oct-1 POU_S domain, either individually or in combination, as follows: A_1, which is contacted by Q-44, was changed to C; T_2, which is contacted by T-45, was changed to G; and G_3 and C_4, which belong to base pairs contacted by R-49, were both changed to T. We also assayed binding to a previously described mutant (9) that represents a combination of the A_1 and T_2 mutations (A_1T_2→CG).

Consistent with previous DNase I footprinting results (9), the A_1T_2→CG mutation has a severe effect on the binding of the Oct-1 POU domain to the histone H2B octamer sequence in an electrophoretic mobility retardation assay (Fig. 2B; compare lane 4 with lane 1). Each of the corresponding individual mutations also impairs Oct-1 POU domain binding (Fig. 2B, lanes 2 and 3). Thus, changes in the sites of Q-44 or T-45 contact affect DNA binding. In contrast, the G_3C_4→TT mutation, which affects the bifurcated contact made by the invariant R-49 residue, had relatively little impact (a threefold decrease) on binding of Oct-1 to the octamer site (Fig. 2B; compare lanes 1 and 5).

We envisioned two possibilities to explain the flexibility in recognition of the variant G_3C_4→TT octamer site. One possibility was that although R-49 is invariably conserved in all POU domains and is seen in the crystal structure to contact the GC base pairs at positions 3 and 4 of the octamer site, these contacts are not necessary for Oct-1 POU domain binding to DNA. Alternatively, R-49 might adapt to the new base pairs at positions 3 and 4 of the G_3C_4→TT mutation and still stabilize binding. To discriminate between these two possibilities and to compare the relative contributions to DNA binding of individual POU_S domain amino acid side chains, we tested the effects of mutagenesis of those residues.

Figure 2D shows the results of such an experiment. The

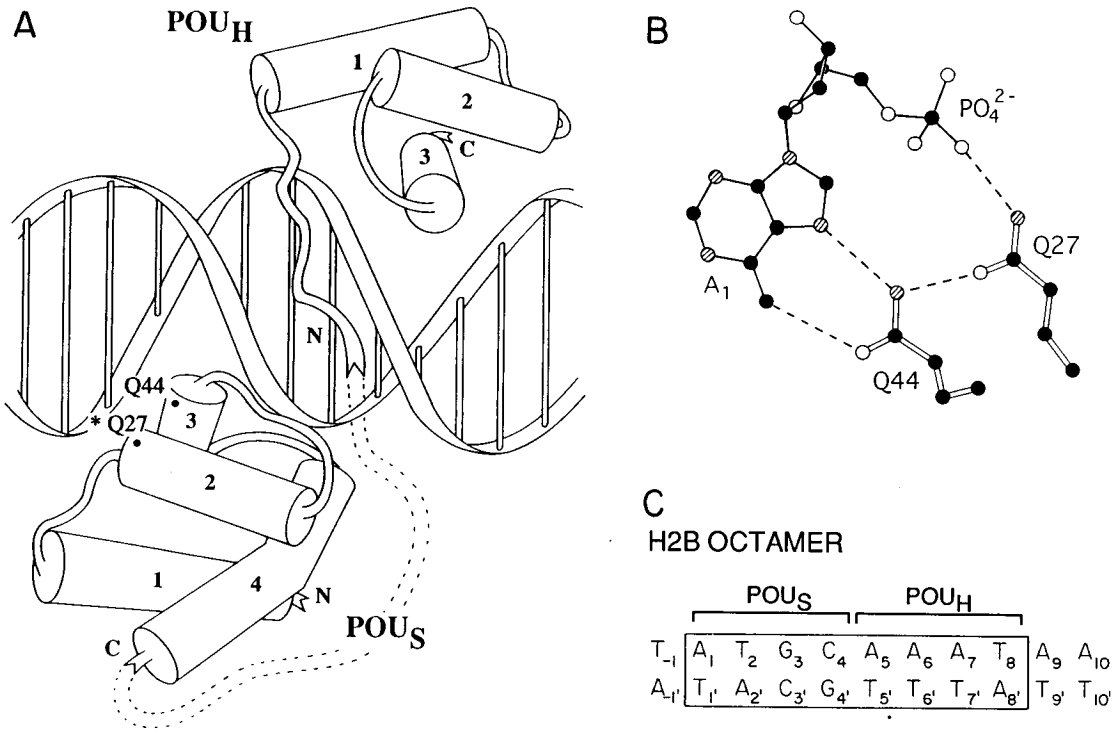

FIG. 1. Structural properties of the bipartite Oct-1 POU domain bound to the histone H2B octamer site. (A) Illustration of the Oct-1 POU domain bound to the histone H2B octamer sequence (adapted from reference 17). The two POU domain subunits, POU_S and POU_H, do not contact one another, and the linker joining the two subunits is likely to be unstructured, as indicated by broken lines. Cylinders indicate α helices. The positions of glutamines Q-27 and Q-44, which make coordinated DNA contacts, are indicated by dots. The asterisk identifies the position of the phosphate contacted by residue Q-27. (B) Coordinated glutamine-DNA interaction shared by the POU_S domain, λ repressor, 434 repressor, and 434 Cro protein DNA-binding domains. This interaction involves glutamine residues Q-27 and Q-44 in the POU_S domain HTH motif. Q-44 forms two hydrogen bonds (dashed lines) with the first adenine (A_1) in the octamer sequence, Q-27 forms a hydrogen bond with the phosphate 5′ of A_1 (PO_4^{2-}), and the two glutamines hydrogen bond to each other. (C) Sequence of the H2B octamer site used for determination of the X-ray crystal structure of the octamer-bound Oct-1 POU domain (17) and described in this work. The POU_S and POU_H domain-binding sites are indicated by brackets.

sequence of the HTH structure and the positions and identities of mutations that we tested are shown in Fig. 2C superimposed on an illustration of the HTH structure. We created alanine substitutions of three residues, the two invariant glutamines Q-27 and Q-44 (Q27A and Q44A) and the invariant arginine R-49 (R49A). In addition, we mutated a residue that does not contact DNA, replacing a nonconserved glycine residue, G-35, with a proline residue (G35P). This substitution mimics a naturally occurring human dwarfism mutation in the pituitary POU factor Pit-1, which affects activation of transcription but has a minimal effect on DNA binding (26).

Figure 2D shows the binding of these POU_S HTH mutants to the wild-type and G_3C_4→TT mutant H2B octamer probes. Consistent with the ability of the analogous Pit-1 mutation to bind DNA like wild-type Pit-1, the G35P substitution has a minimal effect on binding to the wild-type or G_3C_4→TT mutant octamer sites (Fig. 2D, lane 3). In contrast, alanine substitutions of the invariant glutamine and arginine residues show large reductions in DNA binding to both the wild-type and mutant octamer sites (Fig. 2D, lanes 2, 4, and 5), underscoring the importance of these residues in DNA binding. Of the glutamine-for-alanine substitutions, the Q27A mutation is more deleterious than the Q44A mutation (Fig. 2D; compare lanes 2 and 4). The effect of the Q27A mutation may be greater because this residue, in addition to contributing to DNA binding, participates in a further network of POU_S domain side chain interactions (17).

The loss of binding with the Q44A mutation correlates with the loss of binding when the glutamine contact site is mutated (i.e., A_1→C) (Fig. 2B). In studies not shown here, we found that on the A_1→C mutant binding site (Fig. 2A), where the Q-44 base contact has been disrupted, the wild-type Oct-1 POU domain binds no better than the Q44A mutant, which is missing the glutamine that contacts A_1. In contrast, the Q44A mutant still binds less effectively than wild-type Oct-1 on the T_2→G mutant octamer site, where the Q-44 contact with DNA has not been disrupted (8). These results are typical of a "loss-of-contact" analysis (reviewed in reference 11) and are similar to the results of Weiss and colleagues (7, 16), who studied the same amino acid-base interactions between the POU_S domain of the Oct-2 protein and an octamer site. In a loss-of-contact analysis, an amino acid-base interaction is established if the loss of a particular amino acid results in loss of sensitivity to particular substitutions in DNA. The results of such an analysis of Oct-1 and Oct-2 are consistent with the known structure of Oct-1 on an octamer site.

Like the Q27A and Q44A glutamine substitutions, the R49A arginine substitution dramatically reduced binding to both the wild-type and G_3C_4→TT mutant sites (Fig. 2D, lane 5); thus, even when the bases contacted by R-49 are mutated as in the G_3C_4→TT site, R-49 still contributes considerably to DNA binding (Fig. 2D, lower panel; compare lanes 1 and 5). The effect of the R-49 substitution is unlikely to result from disruption of the overall POU_S domain structure because the R-49 side chain does not interact with other residues in the POU_S domain (2, 10, 17). Therefore, this result argues that R-49

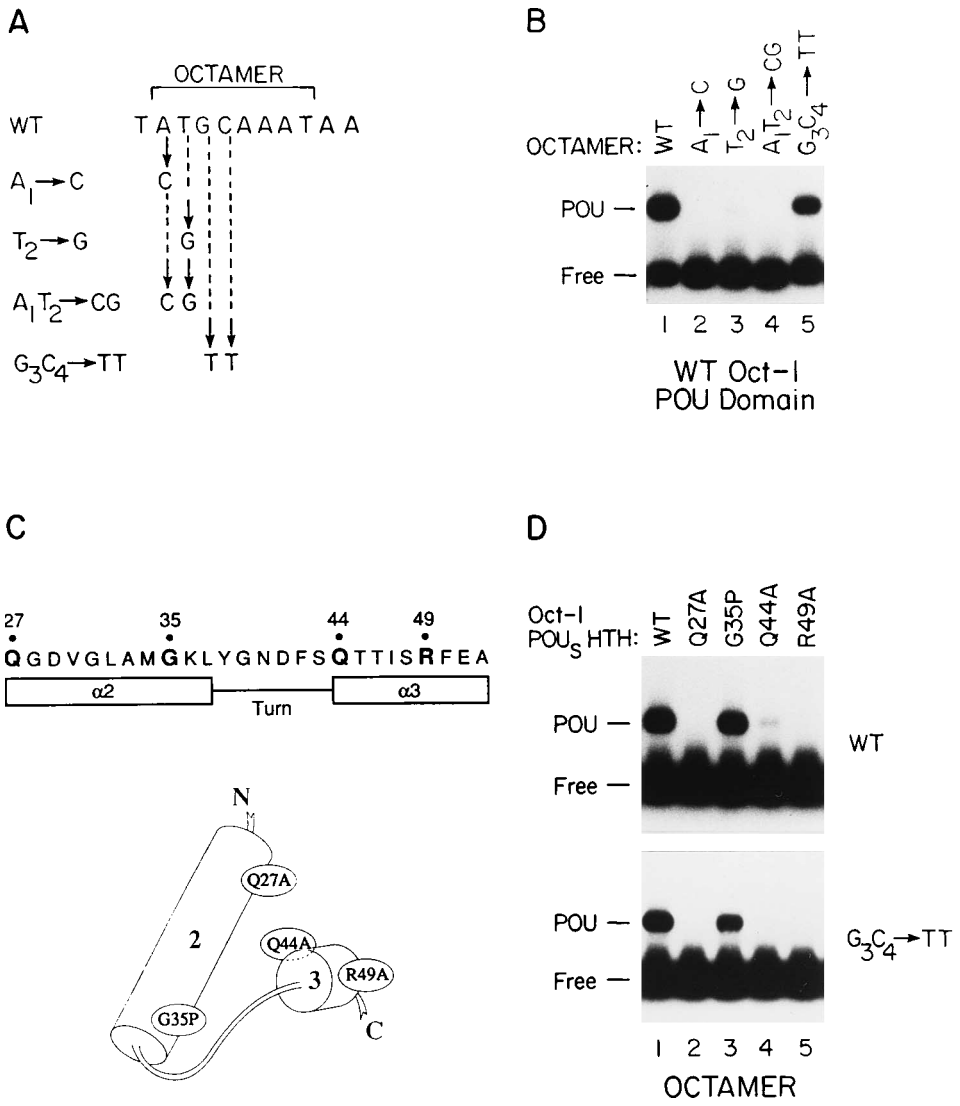

FIG. 2. Binding of wild-type (WT) and POU_S domain mutant forms of the Oct-1 POU domain on wild-type and mutant sites. (A) Wild-type and mutant octamer sites. The mutations are named according to the numerical designations shown in Fig. 1C, with the identities of the base substitutions indicated after the arrows. (B) Electrophoretic mobility retardation analysis of wild-type Oct-1 POU domain binding to the wild-type and mutant sequences shown in panel A. Lanes show POU domain binding to a wild-type probe and to probes mutated as indicated above each lane. POU, POU domain-DNA complex; Free, free probe. (C) Oct-1 HTH region and positions and identities of the residues mutated to study the DNA-binding properties of the POU_S domain. (Top) Primary sequence of the HTH structure of the Oct-1 POU_S domain, with the residues targeted for mutagenesis shown in boldface. Two residues lie in helix 2 (α2) (Q-27 and G-35), and two lie in helix 3 (α3) (Q-44 and R-49), the DNA recognition helix. The turn between the two helices (Turn) is also indicated. (Bottom) Illustration of the secondary structure of the HTH and the amino acid substitutions. Residues were changed to either alanines (Q27A, Q44A, and R49A) or a proline (G35P). (D) Electrophoretic mobility retardation analyses comparing the binding of the wild-type Oct-1 POU domain (lanes 1) with that of Oct-1 POU_S domain variants described in panel C on the wild-type (top) and G_3C_4→TT mutant (bottom) octamer sites. The HTH variants tested are indicated above the lanes.

adapts to the base changes in the G_3C_4→TT octamer site. This adaptation by R-49 provides one mechanism for flexible sequence recognition by the Oct-1 POU domain, wherein an amino acid side chain is able to adapt and contribute to binding to sites of different nucleotide sequence.

The relative positions of the POU_S and POU_H domains differ on an (OCTA$^-$)TAATGARAT site. Although the above-described results provide one mechanism for flexible DNA sequence recognition by the Oct-1 POU domain, they do not explain how the Oct-1 POU domain can recognize an (OCTA$^-$)TAATGARAT site. Here, flexible DNA sequence recognition is likely to arise from flexibility in the arrangement of the POU_S and POU_H domains on the DNA.

Figure 3 shows where, from previous results, we envision the Oct-1 POU_S and POU_H domains to bind an (OCTA$^+$)- and an (OCTA$^-$)TAATGARAT site. On the (OCTA$^+$)TAATGARAT site the POU domain probably recognizes the overlapping octamer sequence much like it recognizes the histone H2B octamer sequence (Fig. 1), with the POU_S domain contacting the 5′ ATGC octamer half site (Fig. 3A). On the (OCTA$^-$)TAATGARAT site, however, the POU_S domain is likely to contact the 3′ GARAT sequence, while the POU_H domain likely contacts the 5′ TAAT sequence (Fig. 3B). We base this model on two criteria. First, because the crystal structure of the Oct-1 POU domain bound to the octamer site shows that the POU_H domain binds DNA like other known homeo domains (17) and because the optimal POU_H domain-binding site is the sequence TAATGA (40), the Oct-1 POU_H

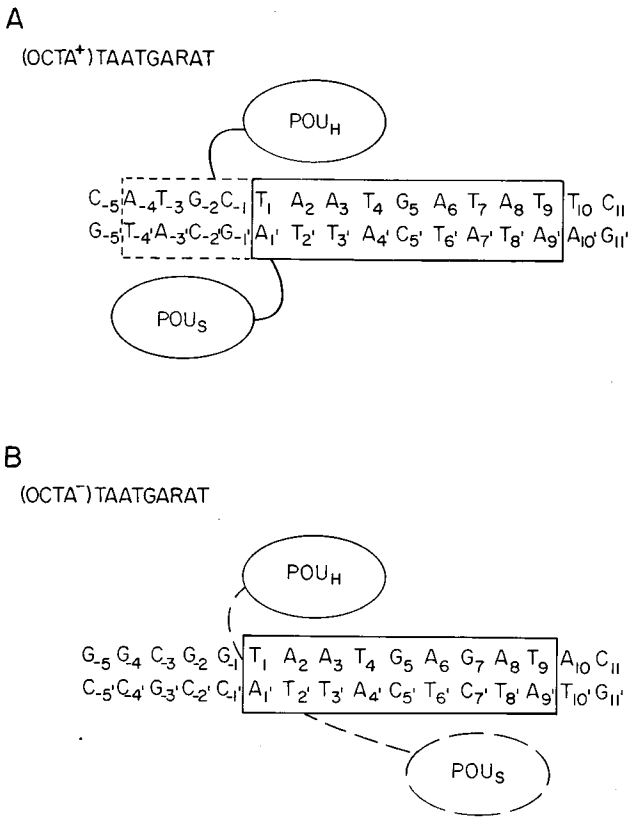

FIG. 3. Models for binding of the Oct-1 POU domain to the (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites. (A) The Oct-1 POU domain probably recognizes an (OCTA$^-$)TAATGARAT site in a manner similar to its recognition of the octamer site (see Fig. 1A and C), with the POU_S domain contacting the 5′ ATGC sequence and the POU_H domain contacting the 3′ TAAT sequence. (B) When the Oct-1 POU domain is bound to the (OCTA$^-$)TAATGARAT site, the POU_S domain is located on the opposite side of the POU_H domain contacting the 3′ GARAT sequence, whereas the POU_H domain contacts the 5′ TAAT sequence. For this site, the POU_S domain is shown with dashed lines because its mode of interaction with the GARAT region is not known.

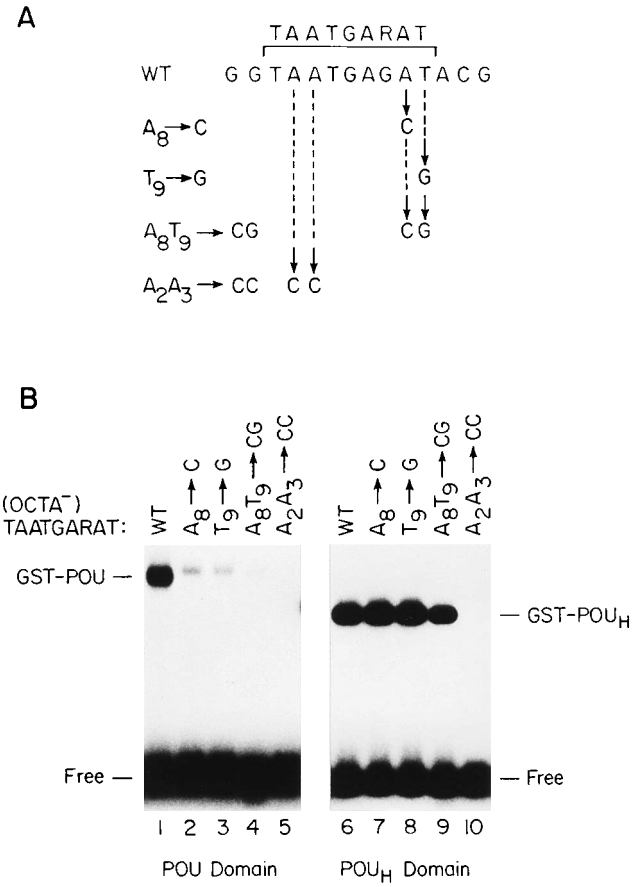

FIG. 4. Binding of the Oct-1 POU domain and POU_H domain to wild-type (WT) and mutant (OCTA$^-$)TAATGARAT sites. (A) Mutations within the (OCTA$^-$)TAATGARAT sequence used in these studies. (B) Electrophoretic mobility retardation analyses comparing the effects of the sites described in panel A on binding by the Oct-1 GST-POU (lanes 1 to 5) and GST-POU_H (lanes 6 to 10) domain fusion proteins. The identities of the (OCTA$^-$)TAATGARAT site probes tested are indicated above each lane. GST-POU, GST-POU domain–DNA complex; GST-POU_H, GST-POU_H domain–DNA complex; Free, unbound probe.

domain probably recognizes the 5′ TAAT sequence of the (OCTA$^-$)TAATGARAT site as it would recognize the 3′ AAAT sequence of an octamer site. Second, although the (OCTA$^-$)TAATGARAT site contains an optimal POU_H domain-binding site, there is no clear 5′ POU_S domain-binding site (GCGG instead of ATGC). Chemical modification interference analysis of Oct-1 binding to this site, however, has shown that besides contacts within the 5′ TAAT sequence, the only other contacts made by Oct-1 lie in the 3′ GARAT sequence (5), suggesting that the POU_S domain contacts this region.

To test this hypothesis, we compared the binding affinity of the full-length Oct-1 POU domain with that of the Oct-1 POU_H domain on a wild-type (OCTA$^-$)TAATGARAT site and on (OCTA$^-$)TAATGARAT sites mutated in the putative POU_H-binding (TAAT) and POU_S-binding (GARAT) sites (Fig. 4). We were unable to detect binding of the in vitro-translated POU_H domain alone to the (OCTA$^-$)TAATGARAT site (data not shown), probably because the binding affinity of the POU_H domain alone for the (OCTA$^-$)TAATGARAT site is lower than that of the entire POU domain (19, 37, 41). Therefore, to increase the concentration of the POU_H domain in the (OCTA$^-$)TAATGARAT site binding assay, we expressed the POU_H domain at high levels as a GST fusion protein in *E. coli*.

Figure 4B shows a mobility retardation assay comparing the binding of low levels of purified *E. coli* GST-POU and high levels of GST-POU_H domain fusion proteins on the wild-type and mutant (OCTA$^-$)TAATGARAT sites. Consistent with the POU_H domain recognizing the homeo domain-binding TAAT sequence, both the GST-POU and GST-POU_H domain proteins bound much better to the wild-type site than to the mutated TAAT site A_2A_3→CC (Fig. 4B; compare lanes 1 and 5 and lanes 6 and 10). Although binding of the full-length POU domain was significantly affected by mutations in the GARAT sequence (Fig. 4B; compare lane 1 with lanes 2 to 4), the GST-POU_H protein did not distinguish among the wild-type and mutant GARAT sites (compare lane 6 with lanes 7 to 9). Thus, the presence of the POU_S domain (and linker) confers specificity for the GARAT sequence to the GST-POU protein. These results are consistent with the POU_S domain interacting with the GARAT sequence and the POU_H domain interacting with the TAAT sequence of the (OCTA$^-$)TAATGARAT site, as shown in Fig. 3B.

To characterize the interaction of the POU_S domain with the GARAT sequence further, we used the loss-of-contact approach described above to compare the bindings of the Oct-1 wild-type and POU_S HTH mutants described in Fig. 2 to

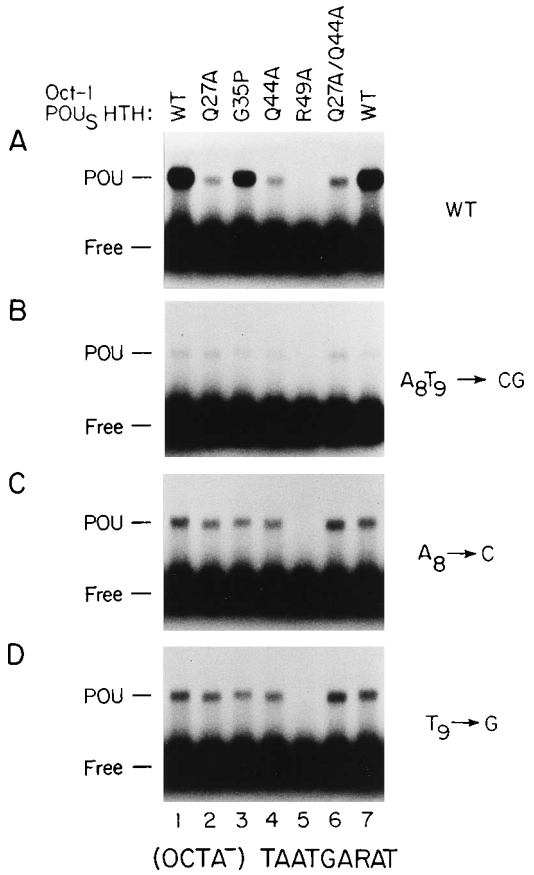

FIG. 5. Binding of the wild-type and mutant Oct-1 POU domains to the wild-type and mutant (OCTA$^-$)TAATGARAT sites. A series of electrophoretic mobility retardation analyses with the HTH mutants described in Fig. 2 and indicated above each lane is shown. In addition, a double glutamine mutant, Q27A/Q44A (lane 6), was assayed. The identities of the probes used are indicated on the right and are as described in Fig. 4. Abbreviations are as in Fig. 2.

the wild-type and GARAT mutant (OCTA$^-$)TAATGARAT sites described in Fig. 4.

Figure 5 shows the results of such an experiment. As with binding to the wild-type and mutant octamer sites (Fig. 2D), the G35P substitution had little effect on Oct-1 POU domain binding to all four (OCTA$^-$)TAATGARAT-related sites, whereas the R49A substitution disrupted binding to all four sites (Fig. 5; compare lanes 1, 3, and 5). The latter result shows that the Oct-1 POU$_S$ domain contributes to binding to all four of these sites, but because G35P has little effect on binding and binding by R49A is undetectable, these mutations cannot be used for a loss-of-contact analysis. We therefore used the glutamine substitution mutants and first compared binding to the wild-type (OCTA$^-$)TAATGARAT site and a previously described double mutant ($A_8T_9 \rightarrow CG$), as shown in Fig. 5A and B, respectively.

On the wild-type (OCTA$^-$)TAATGARAT site, both the Q27A and Q44A mutations disrupted DNA binding (Fig. 5A; compare lanes 1, 2, and 4), indicating that these two elements of the coordinated glutamine interaction with the octamer sequence also contribute to binding to an (OCTA$^-$)TAATGARAT site. The relative level of disruption, however, was not as strong as that on the octamer site (compare Fig. 2D with Fig. 5A), suggesting that the contribution of these residues to (OCTA$^-$)TAATGARAT site binding relative to other interactions is less than that to octamer site binding.

Binding by both the Q27A mutant and the Q44A mutant to the (OCTA$^-$)TAATGARAT site, although reduced, is clearly evident (Fig. 5A). This residual binding allowed us to ask whether combining these mutations had any further deleterious effect. We found, as shown in Fig. 5A (lane 6), that the double glutamine substitution mutant (Q27A/Q44A) binds as well as the two individual mutants. This result suggests that a likely coordinated interaction on the (OCTA$^-$)TAATGARAT site by the two glutamines is already fully disrupted by the individual Q27A and Q44A alanine substitutions.

Figure 5B shows the binding of the Oct-1 POU$_S$ HTH mutants to the (OCTA$^-$)TAATGARAT site mutated in the putative GARAT POU$_S$ domain target sequence ($A_8T_9 \rightarrow CG$). On this site, the wild-type Oct-1 POU domain binds no better than the single and double glutamine Oct-1 POU$_S$ domain substitution mutants (Fig. 5B; compare lanes 2, 4, and 6 with lanes 1 and 7). This result suggests that the $A_8T_9 \rightarrow CG$ GARAT mutation disrupts the coordinated interaction of glutamines Q-27 and Q-44 with the (OCTA$^-$)TAATGARAT site, further establishing the interaction of the Oct-1 POU$_S$ domain with the GARAT portion of the (OCTA$^-$)TAATGARAT site.

The GARAT and 3′ neighboring sequence of the ICP4 (OCTA$^-$)TAATGARAT site used here contains two candidate Oct-1 POU$_S$ domain-binding sites. The optimal Oct-1 POU$_S$ domain-binding site is TATGC (40). The upper strand of the (OCTA$^-$)TAATGARAT sequence contains the related POU$_S$ domain-binding site sequence 5′-G**AT**A**C**, and the complementary strand contains a second related sequence, 5′-**TAT**CT, which overlaps the first sequence (Fig. 3B). If one or both of these sequences serve as the recognition site of the POU$_S$ domain, then the 5′ adenine residues in each sequence (A_8 and $A_{9'}$ [Fig. 3B]) are prime candidates for contact by Q-44. We therefore tested the binding of the Oct-1 wild-type and POU$_S$ HTH mutant proteins to the individually mutated $A_8 \rightarrow C$ and $T_9 \rightarrow G$ (OCTA$^-$)TAATGARAT sites.

Figure 5C and D show the results of this experiment. Curiously, the Q27A and Q44A single mutants and the Q27A/Q44A double mutant display the same relative binding patterns on each of the individually mutated (OCTA$^-$)TAATGARAT sites as they do on the combined doubly mutated site (compare Fig. 5B to D); the binding of the glutamine substitution mutants is similar to that of the wild-type protein, suggesting that both sites can serve as contacts for the coordinated glutamine interaction. Perhaps the POU$_S$ domain can adopt two different positions on the GARAT sequence, utilizing either A_8 of the upper-strand GATAC sequence or $A_{9'}$ of the lower-strand TATCT sequence as the target of a coordinated interaction by the two HTH glutamines.

The precise nature of the interaction of the Oct-1 POU$_S$ domain with the GARAT sequence remains to be elucidated. If there are indeed two overlapping Oct-1 POU$_S$ domain recognition sites, then a DNA mutation at the position of one amino acid-base contact in one site could affect a different amino acid-base contact in the other site, making it difficult to analyze further the interaction of the POU$_S$ domain with the GARAT sequence by a loss-of-contact approach. Nevertheless, these studies, together with the GST-POU and GST-POU$_H$ domain comparison shown in Fig. 4, argue that the relative positionings of the Oct-1 POU$_S$ and POU$_H$ domains on octamer and (OCTA$^-$)TAATGARAT sites are different, thus providing a second mechanism for flexible DNA sequence recognition by the Oct-1 POU domain.

VP16 does not extend DNA contacts made by the Oct-1 POU domain on an (OCTA$^-$)TAATGARAT site. Oct-1 participates in formation of the VP16-induced complex on the two types of TAATGARAT site: (OCTA$^+$) (12, 18, 23, 34) and (OCTA$^-$)

(1, 9). This VP16-induced complex results from association of Oct-1 with a preformed binary complex (the VP16-HCF complex) of VP16 and the other cellular factor in the VP16-induced complex, HCF (reviewed in reference 39). For VP16-induced complex formation on an octamer-containing (OCTA$^+$)TAATGARAT site, it is probable that Oct-1 recognizes the octamer sequence, with the POU_S domain contacting the high-affinity Oct-1 POU_S domain-binding site ATGC and the POU_H domain contacting the high-affinity POU_H domain-binding site TAAT (18, 19, 40); the VP16-HCF complex probably contacts the GARAT sequence (12, 18, 19, 24, 33). On the (OCTA$^-$)TAATGARAT site, however, because the Oct-1 POU_S domain apparently already contacts the GARAT sequence (Fig. 3B), the question arises as to where the VP16-HCF complex interacts with the DNA during VP16-induced complex formation on this site.

To address this question, we did a DEPC chemical modification interference analysis of Oct-1 and VP16-induced complex binding to the (OCTA$^-$)TAATGARAT site. End-labeled DNA probes containing the (OCTA$^-$)TAATGARAT site were randomly modified at adenines and guanines with DEPC and subsequently used in an electrophoretic mobility retardation assay with a HeLa cell nuclear extract in the presence or absence of the VP16-HCF complex. Both the free and bound probes were analyzed.

Figure 6 shows such a comparison for both the lower (A) and upper (B) strands of the DNA sequence shown in the summary in panel C. For both strands, the cleavage patterns resulting from the free and bound probes with Oct-1 alone (Fig. 6A and B, lanes 2 and 3, respectively) and with the VP16-induced complex (lanes 5 and 4, respectively) reactions are shown, flanked by the cleavage pattern of the initially modified DNA (lanes 1 and 6).

As described previously for a different (OCTA$^-$)TAATGARAT site (5), DEPC modifications over the entire TAATGARAT sequence interfered with Oct-1 binding (Fig. 6A and B; compare lanes 2 and 3; see panel C). The extent of the DEPC interference pattern for VP16-induced complex formation was similar to that of the dimethyl sulfate interference pattern described previously (28). Importantly, DNAs isolated from the VP16-induced complex displayed neither an extension of nor new bases within the interference pattern of Oct-1 alone. The cleavage pattern of the lower strand is identical in the Oct-1 complex and the VP16-induced complex (Fig. 6A; compare lanes 3 and 4). On the upper strand, the only difference in the cleavage patterns of the DNA isolated from the two complexes is that three modifications that interfere only partially with Oct-1 binding alone interfere strongly with VP16-induced complex formation (Fig. 6B, lanes 3 and 4 [dots]). These three residues lie within or immediately 3′ of the GARAT sequence. Thus, on an (OCTA$^-$)TAATGARAT site, the interactions of the POU_S domain and the VP16-HCF complex with the GARAT sequence appear to be superimposed.

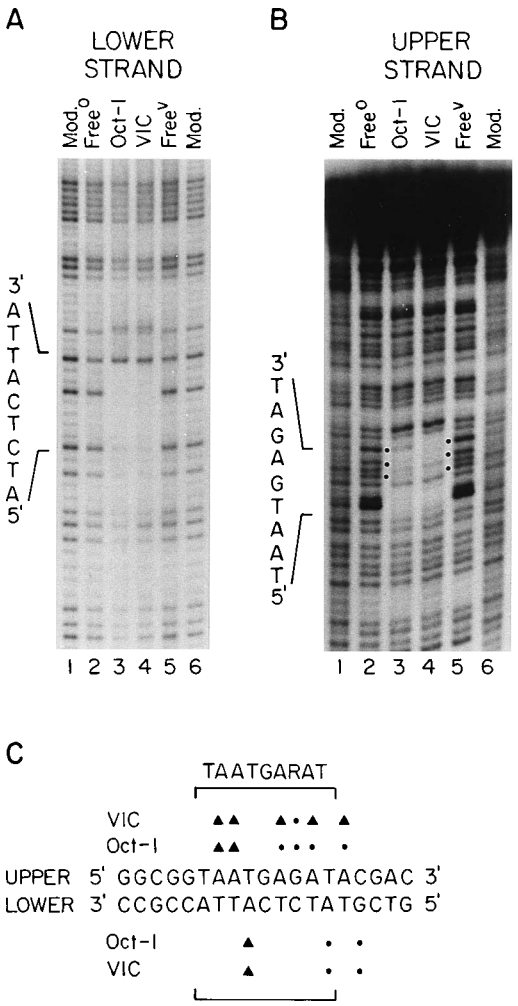

FIG. 6. Chemical modification interference analysis of binding of Oct-1 in the absence and presence of VP16-HCF. Adenine and guanine bases were tested for importance in the formation of a complex on the (OCTA$^-$)TAATGARAT site by Oct-1 alone or Oct-1 in the presence of VP16 and HCF by DEPC modification interference analysis. (A and B) Cleavage ladders generated after piperidine treatment of modified DNA probes eluted from gel slices containing bound and unbound species. The lower strand (A) contains a sequence complementary to the actual TAATGARAT motif, whereas the upper strand (B) contains the actual TAATGARAT nucleotides. The individual bound species from which the DNAs shown were eluted are indicated above each lane. Mod., (OCTA$^-$)TAATGARAT element-containing probe that was treated with DEPC but not subjected to binding conditions before cleavage; Free^0, unbound modified DNA that was incubated in the presence of Oct-1 alone; Oct-1, Oct-1-bound modified DNA in the absence of VP16; VIC, VP16-induced complex; Free^V, unbound modified DNA that was incubated in the presence of components of the VP16-induced complex. The locations of modified bases corresponding to the TAATGARAT sequence are indicated on the left of each panel. The dots identify residues that interfere partially with Oct-1 binding alone but more completely with VP16-induced complex formation. (C) Summary of the results shown in panels A and B. Dots represent weakly interfering bases, and closed triangles represent strongly interfering bases.

The influence of the POU_S domain on VP16-induced complex formation differs on (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites. Oct-1 serves two functions in promoting VP16-induced complex formation: it binds to DNA, and it recruits VP16 and HCF to form the complex. Thus, mutations in the Oct-1 POU domain that affect DNA binding also affect VP16-induced complex formation simply by virtue of not allowing Oct-1 to reach its target site. Some mutations, however, affect the formation of the VP16-induced complex without affecting DNA binding by Oct-1. For example, changes on the solvent-exposed surface of the Oct-1 POU_H domain, which is critical for recruiting VP16 and HCF into the complex, can specifically disrupt VP16-induced complex formation without affecting DNA binding by Oct-1 (21, 27, 34).

Such VP16-induced complex-specific effects have not been observed with the POU_S domain. Indeed, on either an (OCTA$^+$)- or an (OCTA$^-$)TAATGARAT site, an Oct-1 POU domain carrying the Pit-1 POU_S domain, which differs from Oct-1 over much of the solvent-exposed surface of the DNA-bound POU_S domain, can effectively promote VP16-induced

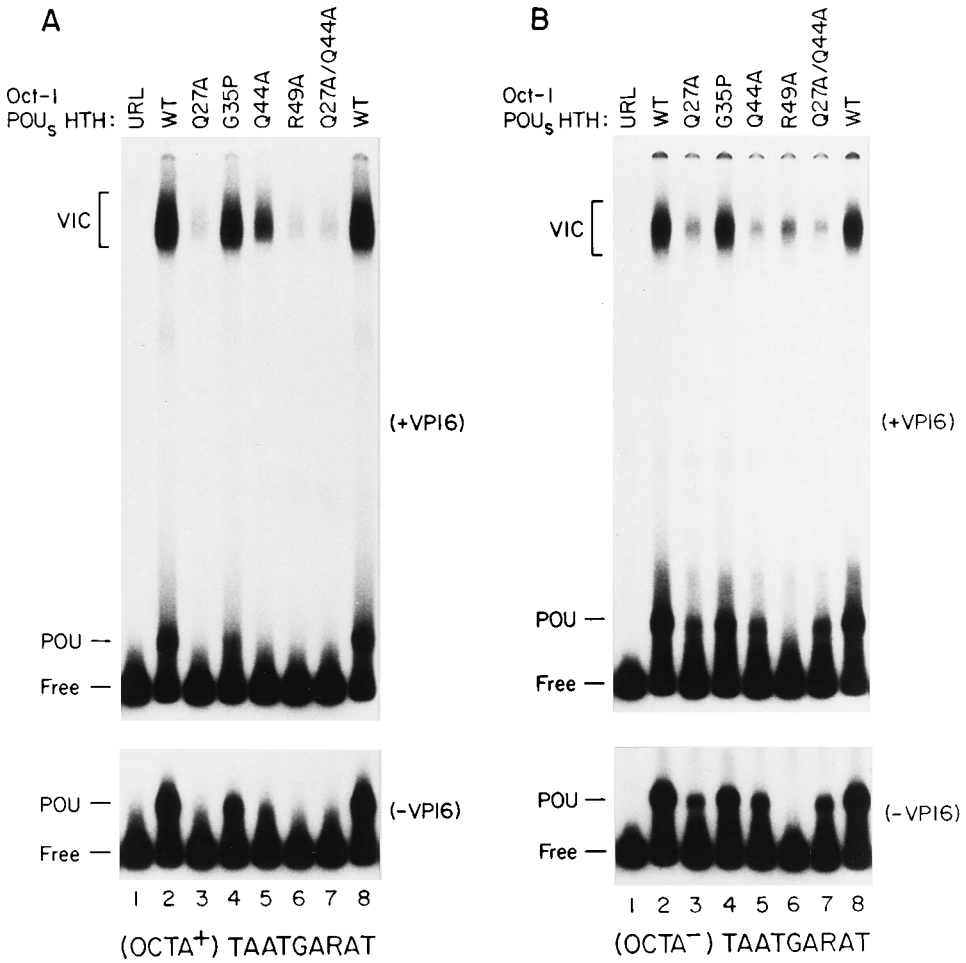

FIG. 7. Comparison of the effects of residues on the DNA-binding surface of the POU_S domain on VP16-induced complex formation on the ($OCTA^+$)-TAATGARAT (A) and ($OCTA^-$)TAATGARAT (B) sites. Electrophoretic mobility retardation analyses of wild-type and mutant Oct-1 POU domains in VP16-induced complex formation on the two sites are shown. In each case, the lower and upper panels show Oct-1 POU domain binding in the absence and presence, respectively, of the VP16-HCF complex. The POU domain species used are indicated above the lanes. Names of HTH variants and most abbreviations are as in Fig. 2. VIC, VP16-induced complex; URL, unprogrammed reticulocyte lysate.

complex formation (8, 34). Thus, many changes on the solvent-exposed surface of the POU_S domain do not necessarily result in a specific defect in VP16-induced complex formation. And yet, the chemical modification interference results described above show that there are superimposed interactions of the Oct-1 POU_S domain and the VP16-HCF complex over the GARAT region of the ($OCTA^-$)TAATGARAT site, suggesting that these two entities could easily influence one another. We therefore wondered whether the DNA-binding surface of the POU_S domain would have a specific effect on VP16-induced complex formation on this site.

To test the influence of POU_S domain residues that contact DNA on VP16-induced complex formation, we compared complex formation with the four single and one double HTH POU_S domain substitutions described in Fig. 2 and 5 on the ICP0 ($OCTA^+$)TAATGARAT site and the ICP4 ($OCTA^-$)-TAATGARAT site, as shown in Fig. 7 and Table 1. We included the ($OCTA^+$)TAATGARAT site, because on this site the likely POU_S domain-binding site is positioned away from the GARAT sequence necessary for VP16-induced complex formation (Fig. 3A), such that POU_S mutations are likely to affect DNA binding but not specifically VP16-induced complex formation. Thus, we might observe contrasting effects of these

TABLE 1. Relative binding and VP16-induced complex formation of Oct-1 wild-type and POU_S HTH mutant POU domains on ($OCTA^+$)- and ($OCTA^-$)TAATGARAT sites[a]

Site and POU domain	Binding	VIC formation
($OCTA^+$)TAATGARAT		
Wild type	1	1
Q27A	—[b]	0.09
G35P	0.3	0.7
Q44A	0.08	0.3
R49A	—	0.08
Q27A/Q44A	—	0.09
($OCTA^-$)TAATGARAT		
Wild type	1	1
Q27A	0.2	0.3
G35P	0.8	1.1
Q44A	0.2	0.3
R49A	—	0.4
Q27A/Q44A	0.2	0.3

[a] Levels of POU domain binding and VP16-induced complex (VIC) formation are relative to those of the wild-type POU domain sample. The ($OCTA^+$)-TAATGARAT site results are from the experiment whose results are shown in Fig. 7A, and the ($OCTA^-$)TAATGARAT site results are from a duplicate of the experiment whose results are shown in Fig. 7B. For each site, three separate experiments gave similar results.

[b] —, less than 0.01.

mutations on VP16-induced complex formation on the two types of TAATGARAT site.

Figure 7A shows the results of VP16-induced complex formation with the Oct-1 POU_S HTH mutants on the ($OCTA^+$)-TAATGARAT site. On this octamer-containing site, the relative affinity of the Oct-1 POU_S HTH mutants for DNA (Fig. 7A, lower panel) parallels that observed on the histone H2B octamer site (Fig. 2D). Furthermore, the relative abilities of these HTH mutants to promote VP16-induced complex formation parallel their relative abilities to bind the ($OCTA^+$)-TAATGARAT site (Fig. 7A; compare POU bands with VIC bands). In particular, the Q44A mutant both binds the ($OCTA^+$)TAATGARAT site on its own and forms a VP16-induced complex better than either the Q27A or R49A mutant on this site (Fig. 7A; compare lane 5 with lanes 3 and 6). Thus, on the ($OCTA^+$)TAATGARAT site, the influence of the POU_S domain on VP16-induced complex formation correlates directly with the affinity of the POU_S domain for this site.

On the ($OCTA^-$)TAATGARAT site, however, we see a different effect. Here, where the POU_S domain and the VP16-HCF complex both interact with the GARAT region of the TAATGARAT sequence, the ability of an HTH mutant to form a VP16-induced complex does not necessarily correlate with its affinity for the site. As in Fig. 5A, all three glutamine substitution mutants (Q27A, Q44A, and Q27A/Q44A) bind the ($OCTA^-$)TAATGARAT site on their own better than the arginine substitution mutant R49A (Fig. 7B, bottom; compare lanes 3, 5, and 7 with lane 6). Unexpectedly, however, the abilities of the single or double glutamine substitution mutants to participate in VP16-induced complex formation are weaker than that of the otherwise inactive R49A mutant (Fig. 7B; compare VIC in lanes 3, 5, and 7 with that in lane 6). Thus, on the ($OCTA^-$)TAATGARAT site the influence of the POU_S domain on VP16-induced complex formation does not correlate directly with the affinity of the POU_S domain for this site.

These results suggest that the coordinated glutamine-DNA interaction specifically stabilizes VP16-induced complex formation on an ($OCTA^-$)TAATGARAT site or that the R-49 arginine specifically interferes with VP16-induced complex formation on this site. Whichever is the case, the different effects of the Oct-1 POU_S domain on the two types of TAATGARAT site demonstrate that, through its ability to bind DNA flexibly, Oct-1 can differentially influence VP16-induced complex formation on different forms of VP16-responsive elements.

DISCUSSION

In this study, we have uncovered two mechanisms for how the Oct-1 POU domain achieves flexible DNA sequence recognition. The first mechanism involves a single amino acid residue that makes important contacts for DNA binding by the POU_S domain but allows effective DNA binding to be maintained when its DNA contacts are changed. The second mechanism involves flexibility in the orientation of the two POU domain subunits on DNA, which provides flexibility in how DNA half sites are positioned. Thus, the DNA half sites can be transposed from their positions in the high-affinity octamer site to form a sequence, the TAATGARAT element, that is still functional for DNA binding. This second mechanism has an impact on the interaction of Oct-1 with the HSV *trans* activator VP16, because on one of two different types of VP16-responsive sites, residues within the POU_S domain that contact the DNA appear to influence specifically VP16 association with Oct-1.

An amino acid residue within the POU_S domain, which is important for DNA binding, can adapt to changes in its DNA targets. Our experiments indicate that DNA binding by the Oct-1 POU_S domain involves both fixed and flexible contacts. The fixed contact involves the coordinated glutamine interaction with adenine A_1 in the octamer sequence. This fixed contact has been established in greater detail in studies of the Oct-2 POU_S domain (7, 16). Flexible contacts are exemplified by base substitutions that disrupt contacts made by the invariant and important POU domain arginine R-49 residue but that have only a minor effect on Oct-1 POU domain binding to the histone H2B octamer site (Fig. 2).

Flexibility in DNA sequence recognition by an amino acid side chain is not unprecedented; a glutamine residue in the bacteriophage 434 repressor, the bacteriophage DNA-binding domain most similar to the POU_S domain (17), displays similar DNA-binding properties (30). Like the POU_S domain, the binding of the 434 repressor to its DNA recognition sites involves a similar coordinated glutamine side chain-DNA interaction (17, 25). In a position analogous to that of the conserved POU_S domain residue R-49, the 434 repressor contains a glutamine residue, Q-33, which, like R-49, is not involved in any intramolecular protein interactions. When the 434 repressor binds to sites at which the base contacted by Q-33 differs, the side chain adopts different conformations (30). Perhaps, like the 434 repressor Q-33 residue, the POU_S domain R-49 residue adopts different conformations to recognize different bases in the Oct-1-binding sites.

We observed flexible R-49–DNA contacts with a mutated octamer site, but examples of flexibility in DNA recognition by this residue also exist in naturally occurring POU domain-binding sites. In the adenovirus 2 origin of replication, Oct-1 binds to the octamer site variant ATGATAAT, where the sequence at the positions recognized by R-49 is changed from GC to GA (29). The brain-specific POU domain protein Brn-2 also binds to sites that probably contain changes in the bases contacted by R-49 (22). Given these flexible interactions, it is unclear why R-49 is universally conserved. Perhaps it is precisely the structural flexibility of an arginine side chain that leads to its conservation at this position of POU domains.

If, however, there is flexibility in R-49–DNA contacts, then why is the octamer sequence so highly conserved in both histone H2B and immunoglobulin promoters? Perhaps in these promoters the octamer motif is highly conserved for reasons other than simply affinity for Oct-1, such as for interaction with other proteins. Indeed, when the 434 repressor binds to different sequences, in addition to changes in the conformation of the R-49 counterpart Q-33, the structure of the DNA and the position of the entire DNA-binding domain differ. Analogous changes in the Oct-1 POU_S domain might affect the ability of other regulatory factors to interact with Oct-1 or the related octamer-binding protein Oct-2.

The Oct-1 POU domain can adopt different conformations on different DNA-binding sites. The X-ray crystal structure of the Oct-1 POU domain bound to DNA showed that the two structurally independent POU domain subunits do not contact one another and are connected by a structurally flexible linker (17). Such a structure suggested that there might be flexibility in the positioning of the Oct-1 POU_S- and POU_H-binding sites, although contrary to this suggestion, insertion of 1 to 3 bp between the POU_S- and POU_H-binding sites in the histone H2B octamer had increasingly disruptive effects on Oct-1 binding (17). Our analysis of Oct-1 binding to the ($OCTA^-$)TAATGARAT site suggests, however, that the positions of the Oct-1 POU_S and POU_H domains on DNA can be very flexible (albeit on a lower-affinity site); indeed, even the 5′-to-3′ order of the

two POU domain subunits need not be fixed. Li et al. (22) showed that for the Brn-2 protein POU domain, but not for the Brn-3 protein POU domain, the spacing between POU_S- and POU_H-binding sites is flexible, and they suggested that the Brn-2 POU_S domain can adopt two different orientations on the DNA while maintaining the 5′-to-3′ order of the POU_S and POU_H domains. These studies together with our studies suggest that different POU domains display different modes of flexible sequence recognition.

The abilities of the two Oct-1 POU domain DNA-binding subunits to position themselves differently on different sites affects the interpretation of the relationship of a series of Oct-1-binding sites described before the POU domain was discovered (5). In that study, the Oct-1-binding sites, including octamer and (OCTA$^-$)TAATGARAT sites, were aligned according to a linear comparison of the sequences without the knowledge that they contain two half-sites whose positions vary considerably and indeed can even be transposed. Thus, some of the sequences in that series, particularly the (OCTA$^-$)-TAATGARAT site and the octamer site, are certainly misaligned. These results emphasize the potential difficulties in establishing the sequence relationship among transcription factor-binding sites when only linear DNA sequences are considered.

The POU_S domain has different effects on VP16-induced complex formation on the (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites. The studies described here indicate that Oct-1 recognizes the (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites with the POU_S domain positioned differently on the DNA. On the (OCTA$^+$)TAATGARAT site, the POU_S domain probably favors the 5′ ATGC half site of the overlapping octamer motif, and on the (OCTA$^-$)TAATGARAT site, the POU_S domain recognizes the GARAT sequence. The (OCTA$^+$)TAATGARAT site may have unusual characteristics itself, however, because it has all the functional information of an (OCTA$^-$)-TAATGARAT site. Therefore, it may be able to bind the POU_S domain over either the 5′ ATGC site or the 3′ GARAT site. Consistent with this hypothesis, the affinity of Oct-1 for (OCTA$^+$)TAATGARAT sites with individual sets of mutations in either the 5′ ATGC or the 3′ GARAT POU_S-binding site sequences is higher than that for an (OCTA$^+$)TAATGARAT site with both the 5′ ATGC and the 3′ GARAT sequences mutated simultaneously (3). Thus, an (OCTA$^+$)-TAATGARAT site may be able to bind Oct-1 in both octamer-like and (OCTA$^-$)TAATGARAT-like conformations.

This dual nature of the (OCTA$^+$)TAATGARAT site may explain why, in an $(FeEDTA)^{2-}$ protection assay (42), Oct-1 fails to protect the ATGC segment of an (OCTA$^+$)TAATGARAT site unless the GARAT sequence is mutated. If the POU_S domain can translocate readily between the ATGC and GARAT POU_S domain-binding sites of an (OCTA$^+$)TAATGARAT site, neither site will be fully occupied, and thus neither site may be effectively protected. Mutation of one of the two sites, however (e.g., the GARAT site), should induce greater occupancy of the unmutated site (e.g., the ATGC site), such that the unmutated site is now effectively protected.

The differential effects of substitutions of the HTH residues that contact DNA on VP16-induced complex formation on the (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites demonstrate that residues that recognize DNA can do more than stabilize binding to the DNA. They can also specifically affect assembly of a multiprotein-DNA complex on one of two types of sites. We speculate that these effects are not related to direct protein-protein interaction but rather are mediated through the DNA. For example, perhaps the coordinated glutamine residue interaction with the GARAT sequence stabilizes the conformation of the DNA such that VP16-HCF complex binding is facilitated. Whichever is the case, unlike with the Oct-1 POU_H domain, where two different surfaces contact DNA and specifically promote VP16-induced complex formation, here a single surface of the Oct-1 POU_S domain is used for both purposes.

The differential effects seen on the (OCTA$^+$)- and (OCTA$^-$)TAATGARAT sites may reflect different ways in which these two sites participate in regulation of HSV gene expression. For example, we have shown in earlier studies (9) that the (OCTA$^-$)TAATGARAT site is nonresponsive in vivo for transcriptional activation by either Oct-1 or the related B-cell factor Oct-2 in the absence of VP16, whereas an (OCTA$^+$)TAATGARAT-like site responds to both. Consistent with different activities, these two different types of TAATGARAT site are not equally distributed among HSV immediate-early promoters. Perhaps HSV evolved to employ Oct-1 for regulation of its immediate-early genes because the flexibility of the Oct-1 POU domain in DNA sequence recognition allows the formation of two different types of VP16-induced complex that may have different regulatory properties.

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