MOLECULAR AND CELLULAR BIOLOGY, Nov. 1997, p. 6321–6329 Vol. 17, No. 11
0270-7306/97/$04.00+0

Synergistic Activation of the Fibroblast Growth Factor 4 Enhancer by Sox2 and Oct-3 Depends on Protein-Protein Interactions Facilitated by a Specific Spatial Arrangement of Factor Binding Sites

DAVIDE-CARLO AMBROSETTI, CLAUDIO BASILICO,* AND LISA DAILEY*

Department of Microbiology and Kaplan Cancer Center, New York University School of Medicine, New York, New York 10016

Received 20 June 1997/Returned for modification 18 July 1997/Accepted 1 August 1997

Octamer binding and Sox factors are thought to play important roles in development by potentiating the transcriptional activation of specific gene subsets. The proteins within these factor families are related by the presence of highly conserved DNA binding domains, the octamer binding protein POU domain or the Sox factors HMG domain. We have previously shown that fibroblast growth factor 4 (FGF-4) gene expression in embryonal carcinoma cells requires a synergistic interaction between Oct-3 and Sox2 on the FGF-4 enhancer. Sox2 and Oct-3 bind to adjacent sites within this enhancer to form a ternary protein-DNA complex (Oct-3*) whose assembly correlates with enhancer activity. We now demonstrate that increasing the distance between the octamer and Sox binding sites by base pair insertion results in a loss of enhancer function. Significantly, those enhancer "spacing mutants" which failed to activate transcription were also compromised in their ability to form the Oct* complexes even though they could still bind both Sox2 and the octamer binding proteins, suggesting that a direct interaction between Sox2 and Oct-3 is necessary for enhancer function. Consistent with this hypothesis, Oct-3 and Sox2 can participate in a direct protein-protein interaction in vitro in the absence of DNA, and both this interaction and assembly of the ternary Oct* complexes require only the octamer protein POU and Sox2 HMG domains. Assembly of the ternary complex by these two protein domains occurs in a cooperative manner on FGF-4 enhancer DNA, and the loss of this cooperative interaction contributes to the defect in Oct-3* formation observed for the enhancer spacing mutants. These observations indicate that Oct-3* assembly results from protein-protein interactions between the domains of Sox2 and Oct-3 that mediate their binding to DNA, but it also requires a specific arrangement of the binding sites within the FGF-4 enhancer DNA. Thus, these results define one parameter that is fundamental to synergistic activation by Sox2 and Oct-3 and further emphasize the critical role of enhancer DNA sequences in the proper assembly of functional activation complexes.

The ability of the cell to selectively activate the transcription of specific gene subsets is fundamental to many biological processes such as development and differentiation. While cell-type-specific gene activation is mediated by tissue-specific transcription factors that bind DNA sequences within the promoter or enhancer, it has become clear that transcriptional activation of a given gene is not simply defined by the activity of an individual factor or a single promoter or enhancer DNA binding site but, rather, depends on combinatorial interactions between multiple proteins. Thus, insight into the mechanism of tissue-specific or developmentally specific transcriptional activation requires first the identification of the protein components that make up the multifactor complex and then the definition of both the protein-protein and protein-DNA interactions that determine its assembly and function.

The focus of our work has been to decipher the regulatory mechanisms underlying differential transcription of the fibroblast growth factor 4 (FGF-4) gene. FGF-4 is a secreted peptide-signaling molecule that was originally identified as an oncogene product capable of transforming fibroblasts in tissue culture (6, 35). Subsequently, FGF-4 gene expression was shown to be both temporally and spatially restricted to discrete tissues of the developing embryo (24), and essential roles for FGF-4 in viability of the blastocyst and in outgrowth and patterning of the developing limb have been clearly demonstrated (8, 25). Thus, regulated expression of the FGF-4 gene is essential to proper development.

In tissue culture, FGF-4 gene transcription is restricted to undifferentiated embryonic stem (ES) cells and embryonal carcinoma (EC) cell lines (39). We have previously characterized enhancer DNA sequences that specifically direct transcriptional activation of the murine FGF-4 gene in EC cell lines and presumably also in the early embryo (4, 5, 43). The FGF-4 enhancer is conserved in both the human and murine genes and can promote transcriptional activation from both homologous and heterologous promoters (4). We have shown (5, 43) that FGF-4 enhancer activity depends on a DNA binding site for the HMG domain protein Sox2 (12, 31) and an adjacent octamer motif that can bind either the Oct-1 or Oct-3 POU domain proteins (30) present in EC and ES cells. Upon binding of Sox2 and either Oct-1 or Oct-3 to the enhancer DNA, ternary complexes, designated Oct-1* and Oct-3*, respectively, are observed, and the ability of enhancer mutants to form the Oct* complexes correlates with their ability to activate transcription (5). However, transfection experiments demonstrated that only the specific combination of Sox2 and Oct-3 could elicit enhancer activity whereas neither factor alone nor Oct-1 in combination with Sox2 was functional (43). Thus, EC cell-specific FGF-4 enhancer function is minimally defined by a synergistic interaction between the EC cell-specific proteins Sox2 and Oct-3 which form the Oct-3* complex on the FGF-4 enhancer DNA.

To understand the mechanisms determining synergistic activation of the FGF-4 gene by Oct-3 and Sox2, we have begun

* Corresponding author. Phone for C. Basilico: (212) 263-5341. Phone for L. Dailey: (212) 263-0525. Fax: (212) 263-8714. E-mail: dailel01@mcrcr.med.nyu.edu.

a characterization of the molecular parameters required for assembly of a functional Oct-3* complex. In this report, we show that the Oct-3 POU domain and the Sox2 HMG domain can mediate both direct protein-protein interaction and cooperative binding to the FGF-4 enhancer DNA. Increasing the spacing between the normally juxtaposed octamer- and Sox-binding elements in the FGF-4 enhancer results in a progressive loss in the ability of these altered enhancers to establish cooperative binding between Sox2 and Oct-3, resulting in reduced Oct-3* assembly and impaired transcriptional activity. Together, these results suggest that direct protein-protein contact between Sox2 and Oct-3 is a prerequisite for the formation of a functional Oct-3* complex and that this interaction is facilitated by the specific arrangement of the factor binding sites within the FGF-4 enhancer. This observation underscores the fundamental role played by enhancer DNA in stereospecific assembly of active transcription complexes and illustrates one regulatory mechanism by which common DNA elements, which are widely dispersed throughout the genome and can bind multiple proteins, can nonetheless direct tissue-specific, selective transcriptional activation.

MATERIALS AND METHODS

Plasmid DNAs and transfections. To construct the wild-type reporter, the pM-380DrDr plasmid (4) was used as a template to amplify FGF-4 enhancer DNA from position 12 (DIR primer) to 239 (REV primer) by PCR. Both primers contained the recognition site for *Bam*HI. After *Bam*HI digestion, one copy of the 244-bp PCR product was inserted in the *Bam*HI site of plasmid pKfgfCAT (4), which contains approximately 1 kb of human FGF-4 promoter sequence upstream of the chloramphenicol acetyltransferase (CAT) coding sequence. The mutant enhancers were derived from the wild-type enhancers by insertion of additional bases by using sequential PCR steps. Briefly, for each enhancer mutant, two overlapping fragments (A and B) containing the same insertion were obtained by PCR. Fragment A was obtained with oligonucleotides DIR and A (see below), and fragment B was obtained with oligonucleotides B and REV. Fragments A and B were then annealed to each other and extended by mutually primed synthesis. The resulting fragment was then amplified by a second PCR step with DIR and REV as the primers and inserted in pKfgfCAT after *Bam*HI digestion. Oligonucleotides A and B for each mutation were as follows: +1, (A) TAGCATCCCAAACAAAGAGTTTTC and (B) GTTTGGG ATGCTAATGGGATACTTA; +2, (A) TAGCATTCCCAAACAAAGAGTT TTC and (B) TTTGGGAATGCTAATGGGAATAACTTA; +3, (A) TAGCA TGTCCCAAACAAAGAGTTTTC and (B) GTTTGGGACATTGCTAATG GGATACT; +5, (A) TTAGCATCCATGCCAAACAAAGAGTTTTCT and (B) CATGGATGCTAATGGGATACTTAAAATAC; and +10, (A) CCATGG CGTACCAAACAAAGAG and (B) GGTACGCCATGGATGCTAATGG. F9 cells were transfected with 5 μg of each of the indicated plasmids by the calcium phosphate method as previously described (4). CAT activity was determined as previously described (4).

Preparation of Sox2 proteins in vitro. Templates for in vitro transcription by SP6 polymerase were generated as described previously (1) by PCR of pCMVSox2 (43) with 5′ oligonucleotide primers composed of promoter sequences for SP6 DNA polymerase (5′ CTATTTAGGTGACACTATAGAAAC AGACACC 3′) and sequences complementary to Sox2 cDNA at nucleotides (nt) 362 to 381 (numbering as in reference 43) (5′ ATGTATAACATGATGGAGAC 3′; primer 1), nt 563 to 579 (5′ AT GCACAACTCGGAGAT 3′; primer 2), or nt 670 to 690 (5′ ATGAAGGAGCACCCCGATTA 3′; primer 3). The 3′ primers used were complementary to DNA sequences downstream of the Sox2 cDNA insert within plasmid pCEP4 (Invitrogen) (5′ AAAGCAATAGCATCACAA 3′; primer 4) or nt 1088 to 1105 within Sox2 cDNA (5′ CCGGGATCCTCACTCG GACTTGACCACAGA 3′; primer 5). Templates were generated by PCR with primers 1 and 4 (wild type), 2 and 4 (mutant F), 3 and 4 (mutant G), and 1 and 5 (mutant B). The template for mutant A was generated by *Bcl*I digestion of the wild-type PCR product (3′ nucleotide at position 1181), while mutants D and E were generated by *Sma*I and *Sac*I digestion, respectively (*Sma*I site at nt 524, and *Sac*II site at nt 703). Template DNA for mutant C was generated by PCR of the truncated Sox2 plasmid variant NP2 (43) with primers 1 and 4. Template DNAs were transcribed in vitro with SP6 DNA polymerase (Promega), and the RNA products were used to program rabbit reticulocyte lysates (Promega) in the presence (for protein binding assays) or absence (for production of proteins to be used in DNA binding assays) of [^{35}S]methionine (40 μCi; New England Nuclear) for 1 h at 30°C.

Protein binding assays. Overnight cultures of bacteria harboring expression plasmids for recombinant glutathione *S*-transferase (GST) octamer binding protein rOct-3 (5), POU-3 (Oct-3 amino acids 118 to 295) (22, 26), or POU-1 (Oct-1 amino acids 269 to 450) (22, 34) were diluted 1:10 (final volume of 100 ml) in Luria broth plus ampicillin and incubated for an additional 1.5 to 2 h at 30°C. After addition of 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG), growth was continued for another 5 to 7 h prior to lysate preparation in 1 ml of BC100N buffer (20 mM HEPES [pH 7.9], 100 mM KCl, 20% glycerol, 0.02% Nonidet P-40, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride) by sonication. Due to the low solubility of rOct-3, rOct-3–GST was purified by resuspending the cell pellet in BC100N buffer containing 6 M urea and the extracted, denatured proteins were renatured by extensive dialysis against BC100N buffer. Glutathione-Sepharose 4B beads (Pharmacia) were equilibrated with BC100N buffer, incubated with bacterial lysates (100 to 600 μl) for 30 min at 4°C, and washed at least three times with 500 μl of BC100N. The final bead pellet was resuspended in a final volume of 100 μl, and the quantity of bound fusion protein was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie blue staining of a 1-μl aliquot. The volume of bead suspension corresponding to approximately 1 to 2 μg of fusion protein was incubated with 1 μl of reticulocyte extract containing in vitro-translated [^{35}S]methionine-labeled Sox2 protein, and the final volume was brought to 100 μl with BC100N buffer plus 0.5 mg of bovine serum albumin per ml. Samples were incubated with rotation for 2 h at 4°C, and the beads were washed four or five times with 300 μl of BC100N buffer. Experiments involving ethidium bromide (EtBr) (18) were performed as above except that 10, 100, or 400 μg of EtBr per ml was included in all binding and wash buffers throughout the procedure. The final pellet was resuspended in Laemmli sample buffer, boiled, and applied to an SDS-polyacrylamide gel (10 to 12% polyacrylamide as indicated in the figure legends).

DNA binding assays and quantification. Protein binding to FGF-4 enhancer DNA probes was assessed by the electrophoretic mobility shift assay (EMSA) essentially as described previously (5). Thrombin was included in the samples that contained the recombinant GST-POU or GST-Oct fusion protein. All samples were incubated for 1 h at room temperature to allow the reaction to come to equilibrium. The mutant and wild-type probes used in the experiments in Fig. 2 and 7 were generated to equivalent specific activities. The experiments in Fig. 6 and 7 were quantitated by PhosphorImager analysis. The amount of POU-3* complex expected to form as a result of noncooperative binding of the POU-3 and MUT C proteins (POU-3* Predicted in Fig. 6 and 7) was determined as the multiple of the amount of probe bound by MUT C in the absence of POU-3 and the amount of probe bound by POU-3 in the absence of MUT C. For example, if 25% of the DNA probe were bound by a given concentration of MUT C and 50% of the probe were bound by a given amount of POU-3, the amount of POU-3* expected to form from noncooperative binding upon combination of these proteins would be equivalent to 25% × 50%, or 12.5%.

RESULTS

Increasing the distance between the Sox and octamer DNA binding motifs abolishes enhancer activity. Our previous studies had indicated that intact DNA binding sites for octamer binding proteins and Sox2, normally juxtaposed within the FGF-4 enhancer, were required for both full enhancer function and Oct* formation (5). The close juxtaposition of the Sox and octamer elements is conserved in both the mouse and human FGF-4 enhancers, suggesting that enhancer function may require a particular spatial organization of these factors on the DNA. To address this possibility, an increasing number of base pairs were inserted between the octamer and Sox DNA binding motifs within the FGF-4 enhancer (Fig. 1A) and the effect of these insertions on enhancer activity was assessed after transfection of the mutant enhancer-CAT constructs into F9 EC cells. Insertion of 1 bp had no discernible effect, while insertion of 2 bp caused a greater than twofold reduction in enhancer activity (Fig. 1B, mutant +1 and mutant +2). Insertion of 3 or 5 bp between the protein binding sites practically eliminated enhancer function, which was not restored by the insertion of 10 bp, i.e. approximately one turn of the DNA double helix (Fig. 1B, mutant +3, mutant +5, and mutant +10, respectively). Thus, increasing the distance between the octamer and Sox DNA binding motifs results in a loss of enhancer function.

Each of the enhancer spacing mutant DNAs was then analyzed by EMSA for its ability to form individual octamer binding and Sox complexes as well as ternary Oct* complexes. DNA oligonucleotide probes corresponding to the sequences within each of the enhancer variants were generated to equivalent specific activities and incubated with F9 nuclear extracts. As expected, all five complexes (Oct-3, Sox2, Oct-3*, Oct-1,

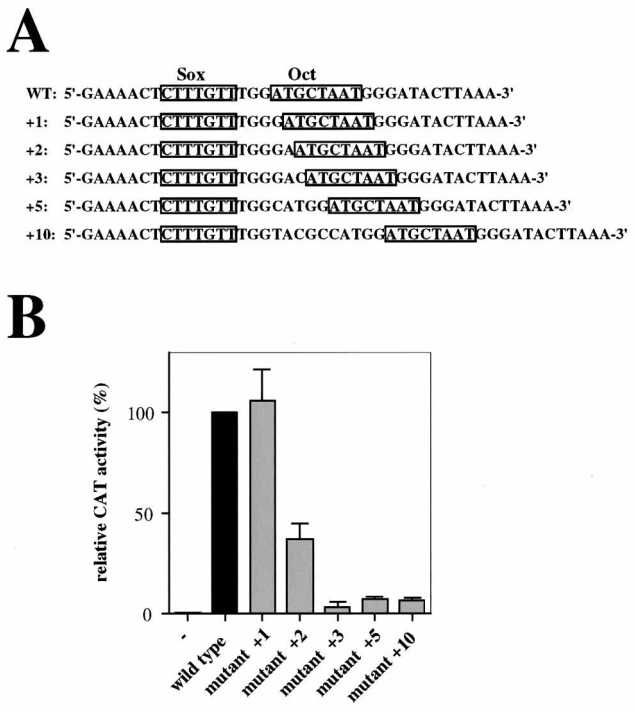

FIG. 1. Increasing the distance between the octamer and Sox binding motifs impairs FGF-4 enhancer function. (A) Base pair composition of the wild-type (WT) and mutant (+1 to +10) FGF-4 enhancers in the region spanning the octamer and Sox protein binding sites. The wild-type (nt 12 to 239 [43]) or mutant enhancers containing the base pair insertions shown between the octamer and Sox protein binding elements were cloned downstream of FGF-4 promoter-CAT sequences as described in Materials and Methods. The locations of the Sox and octamer binding sites are indicated in the boxes. (B) Relative enhancer activity of the wild-type and spacing mutant FGF-4 enhancers. Reporter CAT plasmids containing wild-type or spacing mutant enhancers +1 to +10 were transfected into F9 cells and assessed for transcriptional activation by CAT assays as described in Materials and Methods. The results shown are the averages of four independent experiments; −, activity of the pKfgfCAT plasmid (lacking the enhancer sequences).

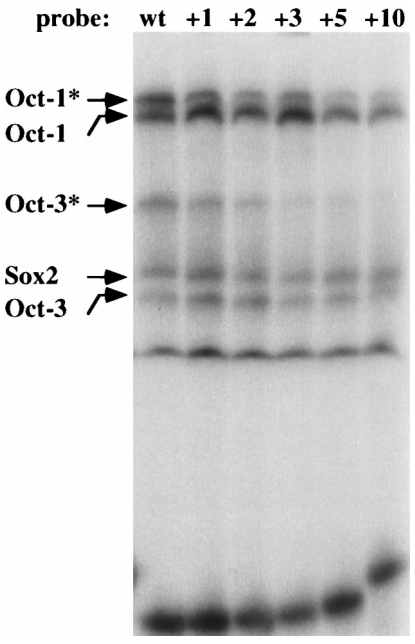

FIG. 2. EMSAs comparing Oct* assembly on wild-type (wt) and spacing mutant FGF-4 enhancer DNA. DNA oligonucleotides comprising the sequences shown in Fig. 1A were incubated with F9 nuclear extracts and analyzed by EMSA. The probe used in each sample (shown in Fig. 1) is indicated above the lanes, and the protein-DNA complexes are identified on the left.

and Oct-1*) were formed with the wild-type probe (Fig. 2). However, the use of probes derived from the mutant enhancers demonstrated that increasing the distance between the octamer and Sox sites caused a progressive decrease in the ability to form the Oct-1* and Oct-3* complexes (Fig. 2). The impaired formation of the Oct* complexes on these probes occurred even though these mutant DNAs were unaffected in their overall ability to bind each of the individual octamer binding and Sox2 proteins (Fig. 2). Thus, while binding of Sox2 and Oct-3 to the enhancer is necessary for the formation of the Oct-3* complex and enhancer function, it is not in itself sufficient, since transcriptional activation is observed only when the DNA binding sites for these factors exist in a specific spatial orientation with respect to one another.

Direct protein-protein interaction between Oct-3 and Sox2 in vitro. A likely interpretation of the results presented above was that they reflect a role for the FGF-4 enhancer DNA to facilitate proper protein-protein interactions between domains within Oct-3 and Sox2. We therefore examined the ability of Sox2 and Oct-3 to interact in solution in the absence of DNA by using in vitro binding assays. Bacterially expressed recombinant Oct-3–GST fusion protein (rOct-3) was immobilized on glutathione-Sepharose resin and incubated with in vitro-translated, [^{35}S]methionine-labeled Sox2 protein. After extensive washing of the rOct-3 beads and analysis by SDS-PAGE, a significant portion of the input [^{35}S]methionine-labeled Sox2 protein was found to have copelleted with the rOct-3 beads (Fig. 3B, lane 5). The interaction of Sox2 with Oct-3 was not affected by inclusion of up to 400 μg of EtBr per ml, indicating that we were detecting a true protein-protein interaction (18) (Fig. 3C). In contrast, less than 2% of the input Sox2 protein was detected when using beads to which the GST protein alone had been coupled (Fig. 3B, lane 9). These results suggest that Sox2 and Oct-3 can participate in a direct protein-protein interaction in solution.

To determine the region(s) within Sox2 mediating the interaction with Oct-3, [^{35}S]methionine-labeled Sox2 variants (mutants A to G, Fig. 3A) containing different deletions from the N or C terminus were generated by in vitro translation and similarly tested. The deletions in Sox2 variants A to D, which progressively remove amino acids from the C-terminal portion of the protein but do not impinge on the DNA binding HMG domain (amino acids 41 to 120 [42]), had no effect on the binding of these proteins to Oct-3 (Fig. 3B, mutants A to D, lanes 6 to 8 and 14). However, further extension of the deletion to sequences within the HMG domain abolished detectable Sox2 binding to Oct-3 (mutant E, lane 15). These results indicate that the direct protein-protein interaction between Sox2 and Oct-3 is mediated by the Sox2 HMG domain. While we had previously shown that a putative transactivation domain is contained within the C-terminal half of Sox2 downstream of amino acid 178 (43), this region clearly does not appear to be involved in the efficient binding of Sox2 to Oct-3 in these assays (Fig. 4B, lane 8).

Based on several reports demonstrating the involvement of the POU domain in a number of protein-protein interactions (10, 21, 22, 32, 33, 40, 44), we next tested whether this domain within Oct-3 was sufficient for binding Sox2. To this end, bacterially expressed, recombinant Oct-3 POU domain-GST fusion protein (POU-3) (Fig. 4A, Oct-3 amino acids 118 to 295)

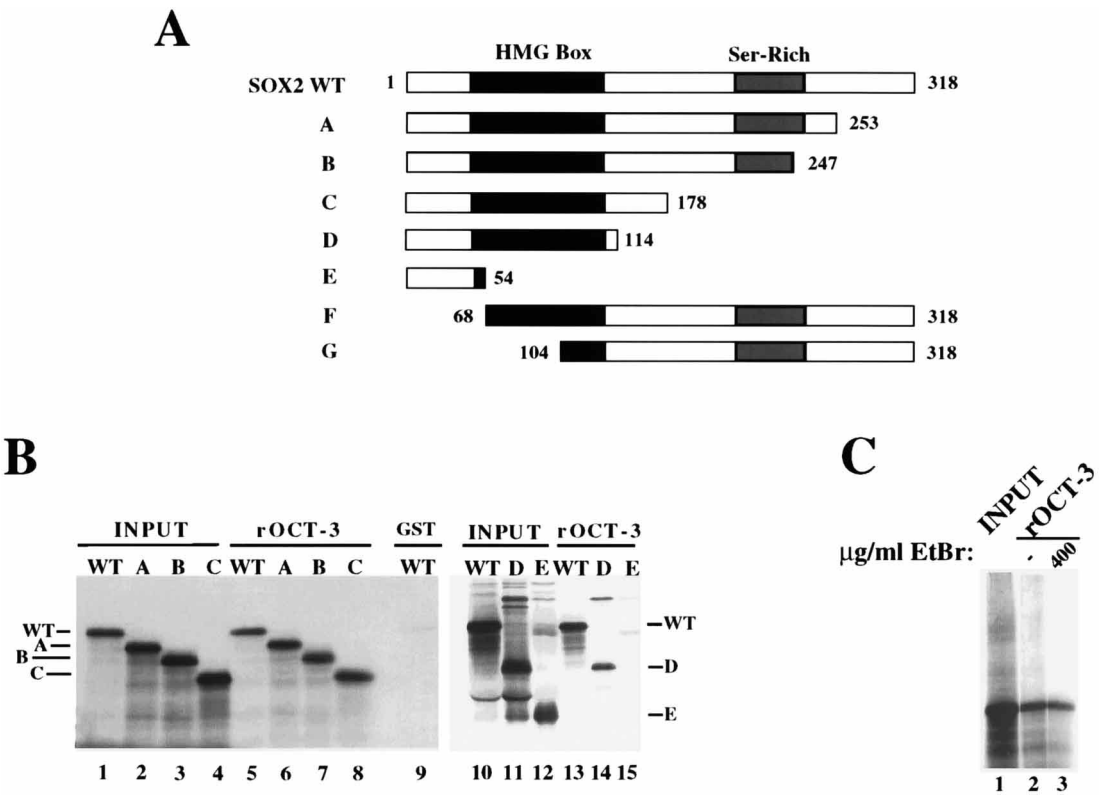

FIG. 3. Sox2 and Oct-3 can interact in the absence of DNA. (A) Schematic representation of wild-type (WT) and truncated Sox2 proteins A to G. [^{35}S]methionine-labeled Sox2 proteins were produced by in vitro translation as described in Materials and Methods. The amino acid sequences included in each protein are indicated by the numbers at the N and C termini. The HMG domain (amino acids 41 to 120) is depicted by the black box, and the serine-rich domain (amino acids 210 to 262) is denoted by the grey box. (B) In vitro binding assays. Lysate (1 μl) containing [^{35}S]methionine-labeled wild-type or mutant A to E Sox2 protein was incubated with rOct-3–GST fusion protein (lanes 5 to 8 and 12 to 14) or GST protein (lane 9) that had previously been coupled to glutathione-Sepharose beads and processed as described in Materials and Methods. The samples were resolved by SDS-PAGE with 10% (lanes 1 to 9) or 12% (lanes 10 to 15) polyacrylamide gels. (C) Challenge of the protein complexes with EtBr. The samples were treated as above except that all binding and wash buffers of that depicted in lane 3 contained 400 μg of EtBr per ml. INPUT, 1 μl of reticulocyte lysate that had been programmed with the indicated Sox2 templates. rOCT-3, material recovered from the OCT-3 GST-bound fraction.

(26) was coupled to GST-Sepharose beads, incubated with [^{35}S]methionine-labeled Sox2 proteins, and analyzed as above. As shown in Fig. 4B (lane 5), the POU domain of Oct-3 was sufficient to retain Sox2. Further analyses with Sox2 proteins containing truncations extending from either the C terminus (Fig. 4B, lane 6) or the N terminus (lanes 7 and 8) showed that, just as was observed with full-length Oct-3 (Fig. 3B and data not shown), the interaction of Sox2 with the Oct-3 POU domain occurs via amino acids within the Sox2 HMG domain.

Binding of Sox2 to the Oct-1 POU domain. We have previously shown that activation of the FGF-4 enhancer by Sox2 can occur only in conjunction with Oct-3 but not in conjunction with the closely related Oct-1 (43). Based on the observations described above, we considered the possibility that the differential activities of these two octamer binding proteins reflect a difference in their abilities to interact with the HMG domain of Sox2. To test this, the in vitro binding of [^{35}S]methionine-labeled Sox2 proteins to immobilized POU-1-GST (POU-1) (Fig. 4A, Oct-1 amino acids 269 to 450) (34) was compared with the ability of these proteins to bind POU-3–GST (POU-3). This analysis showed that Sox2 bound in a comparable manner, via its HMG domain, to both POU-1 and POU-3 (Fig. 4C). Thus, the observation that the Sox2 HMG domain does not discriminate between the POU domains of Oct-1 and Oct-3 in our assays suggests that the differential activation properties of these two octamer binding proteins on the FGF-4 enhancer does not result solely from selective interactions between these domains.

Assembly of the Oct* complexes involves cooperative interactions between the HMG and POU domains. Previous analysis of FGF-4 enhancer base substitution mutants (5), as well as analysis of the enhancer spacing mutants in Fig. 1 and 2, demonstrated that enhancer function correlates with the ability to form ternary Oct* protein-DNA complexes composed of Sox2 and octamer binding protein (5, 43). The additional finding that the octamer binding protein POU domain and the Sox2 HMG domain can directly bind each other suggests that proper juxtaposition of these two factors by the enhancer DNA may foster interactions between these domains and actually underlie assembly of the Oct* complexes. To determine whether these protein domains are sufficient for Oct* complex formation, we combined Sox2 mutant C (MUT C), which contains the HMG domain, with the purified, recombinant POU domains of either Oct-1 or Oct-3 and analyzed the resulting complexes by EMSA with an FGF-4 enhancer DNA probe. As shown in Fig. 5, these protein subregions were by themselves sufficient to generate the POU* complexes and did not require the presence of any of the known transactivating domains of Sox2 or octamer binding proteins (2, 15, 36, 43). This result indicates that the POU and HMG domains are sufficient to mediate assembly of the Oct* complexes on the FGF-4 enhancer.

We next examined whether cooperative binding of the HMG

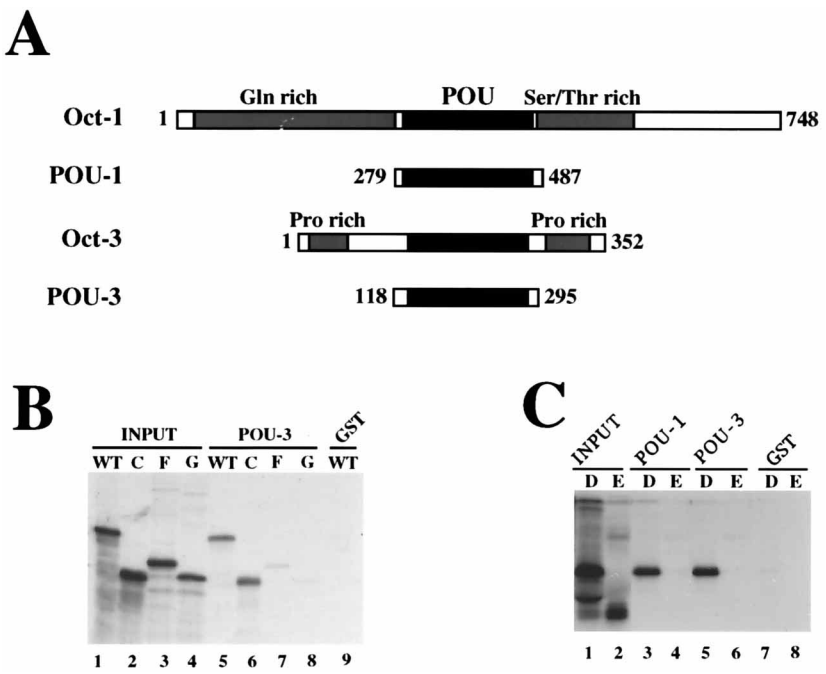

FIG. 4. The Sox2 HMG domain interacts with the POU domains of both Oct-1 and Oct-3. (A) Schematic representation of wild-type Oct-1 and Oct-3 proteins used to derive the truncated proteins POU-1 and POU-3. The location of each POU domain is depicted by the black boxes, and regions containing a high degree of proline (Pro), seronine (Ser), or threonine (Thr) residues and possibly acting as transcriptional activation domains (2, 15, 36) are depicted by the grey boxes. (B) Wild-type (WT) or truncated Sox2 proteins C, F, and G (Fig. 3) were incubated with POU-3–GST (lane 9) or GST protein (lanes 9 to 12) that had previously been coupled to glutathione-Sepharose beads and processed as above. (C) [^{35}S]methionine-labeled Sox2 mutant proteins D and E (Fig. 3) were produced by in vitro translation and incubated with equivalent amounts of either POU-3–GST, POU-1–GST, or GST (as indicated above the lanes) and processed as described in Materials and Methods and the legend to Fig. 3.

and POU domains to the FGF-4 enhancer DNA might contribute to the efficient assembly of the Oct-3* complex. Formation of the POU-3* complex on the FGF-4 enhancer DNA probe was assessed as a function of increasing concentrations of MUT C in the presence of a fixed amount of POU-3. As shown in Fig. 6A (lanes 7 to 11) and B, the vast majority, if not all, of the added MUT C protein was found in the ternary POU-3* complex. The multiple of the fraction of probe bound by POU-3 alone (18%, lane 6) and the fraction of probe bound independently for each concentration of MUT C (0.5, 0.5, 0.7, 1.6, and 4.2%, lanes 1 to 5) allowed us to determine the amount of POU-3* that ought to be observed if its assembly occurred by independent binding by POU-3 and MUT C (Fig. 6B, POU-3* predicted). Significantly, the actual amount of POU-3* that was detected experimentally for each point was four- to sixfold greater than the amount of POU-3* predicted, suggesting that MUT C and POU-3 bind cooperatively to FGF-4 enhancer DNA to form the POU-3* complex.

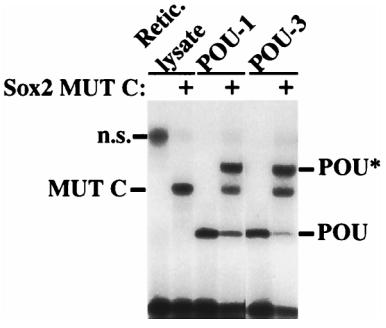

FIG. 5. The Sox2 HMG and the octamer binding protein POU domains are sufficient to assemble Oct* complexes on the FGF-4 enhancer. Wild-type FGF-4 enhancer DNA oligonucleotide probe was incubated with unlabeled, in vitro-translated truncated Sox2 mutant C (MUT C [Fig. 3]) and thrombin-cleaved (to remove the GST portion of the fusion protein) POU-3 or POU-1 as indicated above the lanes. The samples were analyzed by EMSA as described previously (5). Retic. Lysate indicates the sample in which the DNA probe was incubated with control unprogrammed rabbit reticulocyte; n.s. denotes the position of a nonspecific protein-DNA complex observed after incubation of the DNA probe with some rabbit reticulocyte extracts.

The complementary experiment, in which POU-3* formation was examined as a function of increasing concentrations of POU-3 protein in the presence of a fixed amount of MUT C, was also performed (Fig. 6C and D). Again, as long as MUT C was in excess over POU-3, the majority of the added POU-3 was found in the ternary POU* complex (Fig. 6C, lanes 7 to 11, and D). Comparison of the amount of POU-3* observed experimentally with the value of POU-3* predicted for each concentration of POU-3 showed that the actual value was approximately 8- to 10-fold greater than that predicted (Fig. 6D). Together, these results support the notion that efficient Oct-3* assembly involves cooperative interactions between the Sox2 HMG domain and the Oct-3 POU domain and that this phenomenon facilitates formation of the Oct-3* complex and may contribute to the transcriptional synergism of these two proteins on the FGF-4 enhancer.

Increasing the distance between the octamer and Sox DNA binding motifs results in a loss of cooperativity. To examine the defect in Oct-3* formation exhibited by the enhancer spacing mutants (Fig. 2) in more detail, we compared the ability of each of the mutant enhancer DNAs to allow cooperative binding between MUT C and POU-3. The amount of POU-3* formed with fixed amounts of MUT C and POU-3 (Fig. 7B, showing the quantitation of Fig. 7A, lanes 3, 6, 9, 12, and 15) was compared to the amount of POU-3*

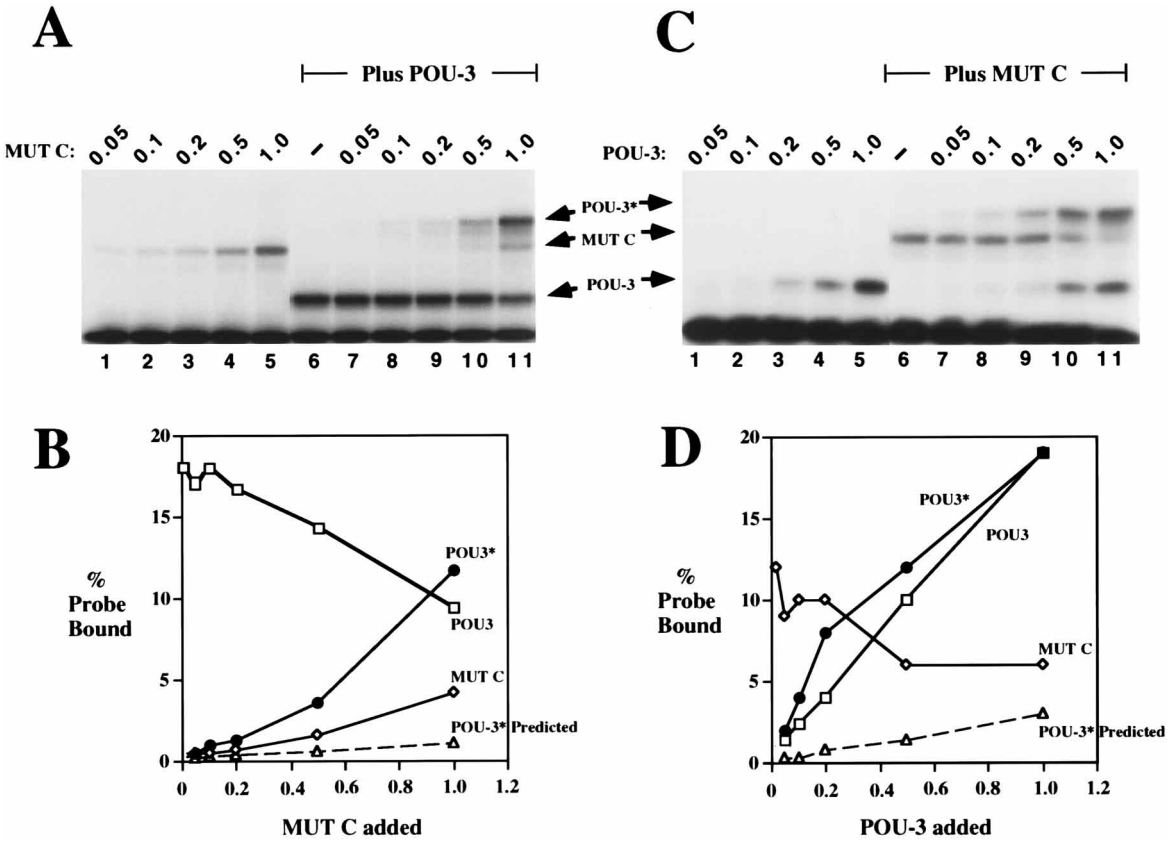

FIG. 6. Formation of the ternary POU-3* complex involves cooperative interactions between the Sox2 HMG domain and the Oct-3 POU domain. (A) DNA binding assay. Increasing quantities of the truncated Sox2 protein MUT C (Fig. 3) were incubated with wild-type FGF-4 enhancer DNA probe in the absence (lanes 1 to 5) or presence (lanes 7 to 11) of a fixed amount (shown in lane 6) of purified recombinant POU-3 protein. The resulting POU-3, MUT C, and ternary POU-3* protein-DNA complexes were resolved by electrophoresis in a polyacrylamide gel. The position of each complex is indicated to the right of the panel (POU-3*, MUT C, and POU-3). Numbers above the lanes represent the amounts (in microliter equivalents) of in vitro-translated MUT C protein analyzed in each sample, where 1 = 1 μl of MUT C-containing reticulocyte lysate. (B) Quantitative representation of the DNA binding data presented in panel A. The amount of probe present in each of the POU-3, MUT C, and POU-3* protein-DNA complexes of lanes 6 to 11 was determined by PhosphorImager analysis and expressed as the percentage of total probe for each sample (*y* axis, % Probe Bound). The *x* axis represents the different amounts of MUT C-containing reticulocyte lysate used in the experiment in panel A. The theoretical level of POU-3* complexes that ought to be generated by noncooperative interactions was calculated for each concentration of MUT C, as described in Materials and Methods, to give POU-3 Predicted. (C) DNA binding assay. The results are presented as in panel A, except that the assays were performed with increasing quantities of POU-3 protein in the absence (lanes 1 to 5) or presence (lanes 7 to 11) of a fixed amount (shown in lane 6) of MUT C protein-containing reticulocyte lysate. The position of each complex after electrophoresis is indicated to the left of the panel (POU-3*, MUT C, and POU-3). Numbers above the lanes represent the amounts of POU-3 protein analyzed in each sample in microliter equivalents, where 1 = 1 μl of a preparation of purified recombinant POU-3. (D) Quantitative representation of the DNA binding data presented in panel C. The amount of probe present in each of the POU-3, MUT C, and POU-3* protein-DNA complexes of lanes 6 to 11 was determined by PhosphorImager analysis and expressed as the percentage of total probe for each sample (*y* axis, % Probe Bound). The *x* axis represents the different amounts of purified POU-3 preparation used in the experiment in panel C. POU-3* Predicted was calculated as described in Materials and Methods. Overexposure of the autoradiograms, as well as some loss of contrast resulting from image scanning, is responsible for an apparent overrepresentation of the intensity of some bands relative to the free probe in panels A and C.

predicted to form by noncooperative binding for some of the mutant DNAs. The results of this comparison show that as the distance between the octamer binding protein and Sox binding sites was increased, cooperativity of factor binding was lost, such that the amount of POU-3* formed with the +3, +5, or +10 mutants approached or equaled the value predicted for noncooperative, independent complex formation (Fig. 7B). These results are consistent with the notion that the specific spatial orientation of factor binding sites within the FGF-4 enhancer plays a directive role in the assembly of a functional Oct-3* activation complex by facilitating proper protein-protein interactions and cooperative binding between the POU and HMG domains of Oct-3 and Sox2.

DISCUSSION

The events that ultimately result in gene activation or silencing represent a complex interplay of multiple protein-protein and protein-DNA interactions. In an attempt to better understand the nature and requirements for these interactions, we have focused our efforts on deciphering the parameters that govern the activity of the FGF-4 enhancer.

The FGF-4 gene is expressed in specific developmental stages in the mouse and plays essential roles in postimplantation viability and embryonic limb formation (8, 24, 25). The previously identified FGF-4 enhancer is active only in EC and ES cells in tissue culture (4) and probably represents the main element responsible for the specific expression of FGF-4 in the inner cell mass of the blastocyst and possibly other developmental stages. We had previously shown that FGF-4 enhancer function depends on a synergistic interaction between two transcriptional regulators, Sox2 and Oct-3, which are specifically expressed in EC and ES cells, and bind to adjacent sites in the FGF-4 enhancer to generate a ternary complex that we had termed Oct-3* (5, 43). Oct-3* is necessary for enhancer

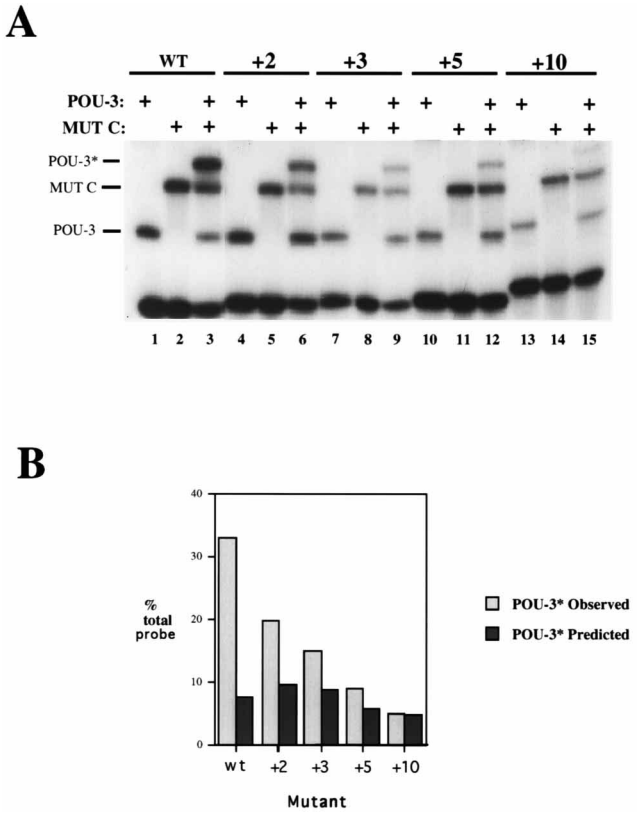

FIG. 7. Increasing the distance between the octamer and Sox DNA binding motifs results in a loss of cooperative binding between MUT C and POU-3. (A) EMSA. DNA probes of each of the FGF-4 enhancer mutants described in the legend to Fig. 1 were incubated for 1 h in the presence of POU-3 alone (lanes 1, 4, 7, 10, and 13), MUT C alone (lanes 2, 5, 8, 11, to 14), or both proteins together (lanes 3, 6, 9, 12, and 15). The protein-DNA complexes were resolved by electrophoresis. The relative positions of the POU-3*–, MUT C-, and POU-3–DNA complexes are indicated to the left of the panel. (B) Quantitation of the binding data of panel A. The percentage of each of the DNA probes in panel A that was present in the POU-3, MUT C, and POU-3* complexes was determined by PhosphorImager analysis. POU-3* Predicted was determined for each mutant enhancer DNA as described in Materials and Methods. Overexposure of the autoradiograms, as well as some loss of contrast resulting from image scanning, is responsible for an apparent overrepresentation of the intensity of some bands relative to the free probe in panel A.

function, and its activity depends on at least two conditions: correct assembly of Sox2 and Oct-3 on the enhancer DNA and the presence of transcriptional activation domains contributed by each of these proteins. In this report, we present evidence suggesting that the first step in this process, i.e., assembly of Oct-3*, minimally depends on directive signals from domains both within Sox2 and Oct-3 and within the enhancer DNA itself. We have shown that Sox2 and Oct-3 can interact directly, in the absence of DNA, through amino acid sequences located within their DNA-binding domains. In addition, we show that these domains are sufficient to drive formation of the Oct-3* complex on the enhancer DNA and that they do so in a cooperative manner. Thus, even if additional, secondary interactions between amino acids within the transactivating regions of Sox2 or Oct-3 may contribute to the overall stability or conformation of the Oct-3* complex, they are not strictly required for its assembly.

Previous studies had indicated that formation of a stable ternary Oct-3* complex requires the presence of both binding sites for these proteins on the DNA since probes containing a mutation of either the octamer or Sox binding motifs did not form Oct-3* (5). This suggested that the affinity of the protein-protein interaction between Sox2 and Oct-3 may be too low to allow the detection of Oct-3* in the absence of one of these DNA binding sites. Consistent with this notion, attempts to coimmunoprecipitate a free Oct-3* complex from extracts of transfected HeLa cells or F9 cells proved unsuccessful (data not shown). However, as we show here, the mere presence of both Sox and octamer binding sites on a given segment of DNA does not necessarily lead to effective Oct-3* assembly or enhancer function. FGF-4 enhancer probes in which the distance between these two sites was progressively increased showed a concomitant decrease in their ability to activate reporter gene transcription and to form Oct-3*. The defect in Oct-3* assembly appears to result from the inability of these mutants to facilitate cooperative binding of Sox2 and Oct-3. It thus appears that cooperative rather than independent binding of Sox2 and Oct-3 is a main determinant of FGF-4 enhancer function.

The loss of both enhancer activation and Oct-3* formation is a function of the distance between the binding sites and not simply due to altered helical phasing, since neither of these activities was restored by the insertion of 10 bp, which is assumed to represent approximately one turn of the DNA helix. Furthermore, 3-bp insertions introduced either upstream or downstream of the octamer or Sox binding sites did not effect enhancer activity (data not shown), indicating that the effects we observed are not due to changes in the spacing between these sites and those for other factors bound elsewhere on the enhancer DNA. These observations suggest that Oct-3* assembly results from an interplay of protein-protein interactions between the HMG domain of Sox2 and the POU domain of Oct-3 that leads to their cooperative binding to the DNA and that this interaction is facilitated by the specific spatial arrangement of the binding sites of these factors provided within the FGF-4 enhancer. Only when the factor domains are brought into close proximity by the DNA does a transcriptionally competent Oct-3* complex form.

Investigations into the effect of distance between factor DNA binding sites on ternary complex formation and transcriptional regulation in other systems have revealed that there is a spectrum of tolerance for these variations. At one extreme, the spatial arrangement of binding sites must be strictly maintained. In addition to the results we have presented in this report, this class is represented by the a1-α2 complex that determines yeast cell type by binding cooperatively to two factor half-sites upstream of the haploid-specific genes and repressing their transcription (reviewed in reference 13). Likewise, ternary-complex formation and activation of the human immunodeficiency virus type 1 enhancer by NF-κB and Sp1 is abolished by base pair insertions between the binding sites for these factors (27). In contrast, ternary-complex formation by serum response factor (SRF) and Ets family members Elk-1 or SAP-1 is relatively unaffected by either the distance between or the orientation of the factor binding sites, and comparable complex formation is observed whether the sites are less than 5 bp or as much as 21 bp apart (38). Consistent with these observations, the arrangement of factor binding sites in the target genes of the first class is highly conserved while that of the target genes of the SRF-Ets complexes exhibits a high degree of variability. The apparent difference between these two classes most probably reflects differences in the properties (e.g., length and flexibility) of the domains that mediate the protein-protein interactions. Thus, in the case of the SRF-Ets complexes, greater variations in spacing may be tolerated since the tethering region of the Ets proteins lies within a flexible

domain located approximately 50 amino acids C-terminal to the DNA binding domain. In contrast, the more stringent requirements for binding-site spacing exhibited by the Sox2–Oct-3 complex may exist because the domains mediating this interaction lie within the DNA binding domains themselves. In the case of a1-α2, the spacing constraints in the DNA seem to be governed by the restricted length of an amino acid linker region of α2 that lies between the homeodomain and a short helix mediating the interaction with a1 (16). Thus, it is possible that even for SRF-Ets proteins, further separation of the DNA binding sites, which exceeds the "span" of the tether, will ultimately result in the loss of ternary-complex formation.

The second prerequisite for Oct-3* function, i.e., the presence of specific transactivation domains, is suggested by several observations. First, a region of Sox2 located C-terminal to the HMG domain was shown to be required for transcriptional activation of FGF-4 in conjunction with Oct-3 (43). Second, Sox2 can activate the FGF-4 enhancer only with Oct-3 but not with the closely related Oct-1. While the DNA binding POU domains of Oct-1 and Oct-3 display a high degree of amino acid sequence homology, Oct-1 shows no sequence similarity to amino acids within either the N- or C-terminally located transactivation domains of Oct-3 (2, 15, 34). In fact, binding of the Sox2 HMG domain is not restricted to the Oct-3 POU domain, since we could observe both in vitro protein-protein binding of the Sox2 HMG domain to POU-1 and formation of POU-1*. Separate experiments in which the Sox2–POU-1 or Sox2–POU-3 interaction was challenged with increasing concentrations of salt did not reveal a significant difference in the affinity of these interactions (data not shown). Furthermore, EMSAs with the spacing mutant variant probes of the FGF-4 enhancer demonstrated a loss of Oct-1* formation parallel to that observed for Oct-3* (Fig. 2). These observations are consistent with the hypothesis that the specificity of Oct-3 activation with Sox2 is determined not by the POU domain but by unique features within the Oct-3 transactivation domain(s). Thus, both transcriptional activation per se and specificity most probably derive from additional interactions that may be mediated by the combined transactivation domains within Sox2 and Oct-3. It is expected, then, that the DNA must recruit Sox2 and Oct-3 in a stereospecific manner not only to allow interaction between the HMG and POU domains but also to arrange the transactivation domains in an optimal relative spatial orientation for these subsequent, additional factor interactions.

Other secondary factor-POU interactions have been described that do demonstrate discrimination among different POU domains. For example, the lymphoid cell-specific OCA-B protein, which participates in the activation of immunoglobulin genes in conjunction with either Oct-1 or Oct-2, can bind specifically to the POU-specific region within Oct-1 or Oct-2 but not to that within Oct-3 or Oct-6 (10, 22, 33). In addition, the herpes simplex virus VP16 protein recognizes a specific amino acid residue within the context of the Oct-1 POU homeodomain that is not present in Oct-2 (19, 29). Thus, in contrast to the results presented here, the specificities of the activation properties of the various octamer binding proteins and secondary-factor combinations can be explained by differential protein-protein interactions mediated by the POU domains.

The previous demonstration of the general interaction of the HMG domain of HMG2 with the POU domain within Oct-1 or Oct-2 (44) suggests that the interaction of Sox2 with POU-1 and POU-3 that we observed reflects a basic feature of this class of proteins that has been evolutionarily conserved. However, unlike that by Sox2, transcriptional activation by HMG2 with the POU proteins does not require DNA binding sites for the HMG factor (44), suggesting that this property has become more refined and restricted in the course of evolution, consistent with the more specialized role of the Sox proteins in the transcriptional activation of specific genes. Studies of the closely related HMG-domain proteins Sox5 (7) and LEF-1 (37, 42) have indicated that these factors also do not function as autonomous transcriptional activators and that, at least for LEF-1, transcriptional activation is dependent on the promoter context (3, 9). In addition, Sox2 has been shown to activate the δ1 crystalline gene enhancer in conjunction with a second factor whose binding site is also located immediately adjacent to that of Sox2 (17). While the identity of this second activator is unknown, the sequence of its putative DNA binding site suggests that it is probably not a POU domain protein. Nonetheless, it is possible that similar enhancer-dependent protein-protein interactions such as those described here for Sox2 and Oct-3 on the FGF-4 enhancer will also occur between Sox2 and this unidentified factor and that this reflects a general mechanism of activation by Sox2 if not by most DNA sequence-specific HMG domain proteins.

The conclusions presented in this report provide one illustration of how selective gene activation can be achieved. While both Sox and octamer binding proteins recognize DNA in a sequence-specific manner, both protein classes are fairly promiscuous in their DNA recognition properties in vitro. As such, potential binding sites for these factors are widely distributed throughout the genome and even within the enhancers or promoters of genes that are expressed in very distinct cell-specific patterns. However, the cell can invoke several mechanisms such that only a fraction of these recognition sites are actually used in transcriptional activation. For example, studies probing transcriptional activation by the Oct-1–VP16 or Oct–OCA-B complexes have shown that subtle differences among the DNA sequences within or flanking an octamer DNA binding motif can determine not only whether a productive octamer binding protein-coactivator complex will form at a given site but also the specificity of the coactivator that is able to interact with the octamer binding protein at that site (11, 14, 41). Since neither VP16 nor OCA-B demonstrates high-affinity DNA binding by itself, one possibility is that, as has been postulated for the interaction of glucocorticoid receptor with its target DNA sequence (20), the DNA acts as an allosteric effector capable of inducing a conformational change in the octamer binding protein bound to it (41). According to this model, the conformation of bound factor may or may not be compatible for subsequent protein interactions and may depend on the DNA sequence bound. An alternate interpretation (14) is that different octamer binding protein-coactivator complexes may possess unique DNA sequence recognition properties. In other examples, complexes composed of different combinations of molecular partners within homo- or heteromeric transcriptional activators may exhibit slightly different DNA sequence recognition properties such that binding to unique subsets of related sites will occur preferentially (23, 28). These considerations, plus the additional imposition of a requirement for a strict, specific relative spatial orientation for the assembly of active complexes, thus severely limit the actual number of factor binding sites that will lead to gene activation in a given cellular environment. In the present example, we suggest that similarly juxtaposed Sox and octamer binding sites within the enhancers of other genes may mark them for expression in EC cells and thus may define a specific code for the activation of a subset of genes in the early embryo.

ACKNOWLEDGMENTS

We thank Yan Luo and Bob Roeder for the gift of the POU-1 and POU-3-GST expression plasmids, Karen Mangasarian for technical advice, and Alessandra Pierani for comments on the manuscript.

This investigation was supported by PHS grant CA42568.

REFERENCES

1. **Aurora, R., and W. Herr.** 1992. Segments of the POU domain influence one another's DNA-binding specificity. Mol. Cell. Biol. **12:**455–467.
2. **Brehm, A., K. Ohbo, and H. Scholer.** 1997. The carboxy-terminal transactivation domain of Oct-4 acquires cell specificity through the POU domain. Mol. Cell. Biol. **17:**154–162.
3. **Carlsson, P., M. L. Waterman, and K. A. Jones.** 1993. The hLEF/TCF-1a HMG protein contains a context-dependent transcriptional activation domain that induces the TCRa enhancer in T cells. Genes Dev. **7:**2418–2430.
4. **Curatola, A. M., and C. Basilico.** 1990. Expression of the K-fgf protooncogene is controlled by 3′ regulatory elements which are specific for embryonal carcinoma cells. Mol. Cell. Biol. **10:**2475–2484.
5. **Dailey, L., H. Yuan, and C. Basilico.** 1994. Interaction between a novel F9-specific factor and octamer-binding proteins is required for cell-type-restricted activity of the fibroblast growth factor 4 enhancer. Mol. Cell. Biol. **14:**7758–7769.
6. **Delli Bovi, P., A. M. Curatola, F. G. Kern, A. Greco, M. Ittmann, and C. Basilico.** 1987. An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell **50:**729–737.
7. **Denny, P., S. Swift, F. Connor, and A. Ashworth.** 1992. An SRY-related gene expressed during spermatogenesis in the mouse encodes a sequence-specific DNA binding protein. EMBO J. **11:**3705–3712.
8. **Feldman, B., W. Poueymirou, V. E. Papaioannou, T. M. DeChiara, and M. Goldfarb.** 1995. Requirement of FGF-4 for post-implantation mouse development. Science **267:**246–250.
9. **Giese, K., and R. Grosschedl.** 1993. LEF-1 contains an activation domain that stimulates transcription only in a specific context of factor-binding sites. EMBO J. **12:**4667–4676.
10. **Gstaiger, M., L. Knoepfel, O. Georgiev, W. Schaffner, and C. M. Hovens.** 1995. A B-cell coactivator of octamer-binding transcription factors. Nature **373:**360–362.
11. **Gstaiger, M., O. Geoegiev, H. van Leeuwen, P. van der Vliet, and W. Schaffner.** 1996. The B cell coactivator Bob1 shows DNA sequence-dependent complex formation with Oct-1/Oct-2 factors, leading to differential promoter activation. EMBO J. **15:**2781–2790.
12. **Gubbay, J., J. Collignon, P. Koopman, B. Capel, A. Economou, A. Munsterberg, N. Vivian, P. N. Goodfellow, and R. Lovell-Badge.** 1990. A gene mapping to the sex-determining region of the mouse Y-chromosome is a member of a novel family of embryonically expressed genes. Nature **346:**245–250.
13. **Herskowitz, I.** 1989. A regulatory hierarchy for cell specialization in yeast. Nature **342:**749–757.
14. **Huang, C. C., and W. Herr.** 1996. Differential control of transcription by homologous homeodomain coregulators. Mol. Cell. Biol. **16:**2967–2976.
15. **Imagawa, M., A. Miyamoto, M. Shirakawa, H. Hamada, and M. Muramatsu.** 1991. Stringent integrity requirements for both trans-activation and DNA-binding in a transactivator, Oct3. Nucleic Acids Res. **19:**4503–4508.
16. **Jin, Y., J. Mead, T. Li, C. Wolberger, and A. K. Vershon.** 1995. Altered DNA recognition and bending by insertions in the a2 tail of the yeast a1/a2 homeodomain heterodimer. Science **270:**290–293.
17. **Kamachi, Y., S. Sockanathan, Q. Liu, M. Breitman, R. Lovell-Badge, and H. Kondoh.** 1995. Involvement of Sox proteins in lens-specific activation of crystallin genes. EMBO J. **14:**3510–3519.
18. **Lai, J.-S., and W. Herr.** 1992. EtBr provides a simple tool for identifying genuine DNA-independent protein associations. Proc. Natl. Acad. Sci. USA **89:**6958–6962.
19. **Lai, J.-S., M. A. Cleary, and W. Herr.** 1992. A single amino acid exchange transfers VP16-induced positive control from the Oct-1 to the Oct-2 homeo domain. Genes Dev. **6:**2058–2065.
20. **Lefstin, J. A., J. R. Thomas, and K. R. Yamamoto.** 1994. Influence of a steroid receptor DNA-binding domain on transcriptional regulatory functions. Genes Dev. **8:**2842–2856.
21. **Leger, H., E. Sock, K. Renner, F. Grummt, and M. Wegner.** 1995. Functional interaction between the POU domain protein Tst-1/Oct-6 and the high-mobility-group protein HMG-I/Y. Mol. Cell. Biol. **15:**3738–3747.
22. **Luo, Y., and R. G. Roeder.** 1995. Cloning, functional characterization, and mechanism of action of the B-cell-specific transcriptional coactivator OCA-B. Mol. Cell. Biol. **15:**4115–4124.
23. **Mann, R., and S.-K. Chan.** 1996. Extra specificity from extradenticle: the partnership between HOX and PBX/EXD homeodomain proteins. Trends Genet. **12:**258–262.
24. **Niswander, L., and G. Martin.** 1992. Fgf-4 expression during gastrulation, myogenesis, limb, and tooth development in the mouse. Development **114:**755–768.
25. **Niswander, L., C. Tickel, A. Vogel, I. Booth, and G. R. Martin.** 1993. FGF-4 replaces the apical ectodermal ridge and directs outgrowth and patterning of the limb. Cell **75:**579–587.
26. **Okamoto, K., H. Okazawa, A. Okuda, M. Sakai, M. Muramatsu, and H. Hamada.** 1990. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell **60:**461–472.
27. **Perkins, N. D., N. L. Edwards, C. S. Duckett, A. B. Agranoff, R. M. Schmid, and G. J. Nabel.** 1993. A cooperative interaction between NF-κB and Sp1 is required for HIV-1 enhancer activation. EMBO J. **12:**3551–3558.
28. **Perlmann, T., P. N. Rangarajan, K. Umesono, and R. Evans.** 1993. Determinants for selective RAR and TR recognition of direct repeat HREs. Genes Dev. **7:**1411–1422.
29. **Pomerantz, J. L., T. M. Kristie, and P. A. Sharp.** 1992. Recognition of the surface of a homeo domain protein. Genes Dev. **6:**2047–2057.
30. **Scholer, H. R.** 1991. Octamania: the POU factors in mouse development. Trends Genet. **7:**323–329.
31. **Sinclair, A. H., P. Berta, M. S. Palmer, J. R. Hawkins, B. L. Griffiths, D. J. Smith, J. W. Foster, A. Frischaux, R. Lovell-Badge, and P. N. Goodfellow.** 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **346:**240–244.
32. **Stern, S., M. Tanaka, and W. Herr.** 1989. The Oct-1 homeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. Nature **341:**624–630.
33. **Strubin, M., J. W. Newell, and P. Matthias.** 1995. OBF-1, a novel B cell-specific coactivator that stimulates immunoglobulin promoter activity through association with octamer-binding proteins. Cell **80:**497–506.
34. **Sturm, R. A., G. Das, and W. Herr.** 1988. The ubiquitous octamer-binding protein Oct-1 contains a POU domain with a homeobox subdomain. Genes Dev. **2:**1582–1599.
35. **Taira, M., T. Yoshida, K. Miyagawa, H. Sakamoto, M. Terada, and T. Sugimura.** 1987. cDNA sequence of a human transforming gene hst and identification of the coding sequence required for transforming activity. Proc. Natl. Acad. Sci. USA **84:**2980–2984.
36. **Tanaka, M., J.-S. Lai, and W. Herr.** 1992. Promoter-selective activation domains in Oct-1 and Oct-2 direct differential activation of an snRNA and mRNA promoter. Cell **68:**755–767.
37. **Travis, A., A. Amsterdam, C. Belanger, and R. Grosschedl.** 1991. LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor alpha enhancer function. Genes Dev. **5:**880–894.
38. **Treisman, R., R. Marais, and J. Wynne.** 1992. Spatial flexibility in ternary complexes between SRF and its accessory proteins. EMBO J. **11:**4631–4640.
39. **Velcich, A., P. Delli-Bovi, A. Mansukhani, E. B. Ziff, and C. Basilico.** 1989. Expression of the K-fgf protooncogene is repressed during differentiation of F9 cells. Oncogene Res. **5:**31–37.
40. **Verrijzer, C. P., A. Joost, W. M. Oosterhout, and P. C. van der Vliet.** 1992. The Oct-1 POU domain mediates interactions between Oct-1 and other POU proteins. Mol. Cell. Biol. **12:**542–551.
41. **Walker, S., S. Hayes, and P. O'Hare.** 1994. Site-specific conformational alteration of the Oct-1 POU domain-DNA complex as the basis for differential recognition by Vmw65 (VP16). Cell **79:**841–852.
42. **Waterman, M., and K. Jones.** 1990. Purification of TCF-1a, a T-cell-specific transcription factor that activates the T-cell receptor gene enhancer in a context-dependent manner. New Biol. **2:**621–636.
43. **Yuan, H., N. Corbi, C. Basilico, and L. Dailey.** 1995. Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. Genes Dev. **9:**2635–2645.
44. **Zwilling, S., H. König, and T. Wirth.** 1995. High mobility group protein 2 functionally interacts with the POU domains of octamer transcription factors. EMBO J. **14:**1198–1208.