Modulation of the Activity of Multiple Transcriptional Activation Domains by the DNA Binding Domains Mediates the Synergistic Action of Sox2 and Oct-3 on the Fibroblast Growth Factor-4 Enhancer*

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Fibroblast growth factor (FGF)-4 gene expression in the inner cell mass of the blastocyst and in EC cells requires the combined activity of two transcriptional regulators, Sox2 and Oct-3, which bind to adjacent sites on the FGF-4 enhancer DNA and synergistically activate transcription. Sox2 and Oct-3 bind cooperatively to the enhancer DNA through their DNA-binding, high mobility group and POU domains, respectively. These two domains, however, are not sufficient to activate transcription. We have analyzed a number of Sox2 and Oct-3 deletion mutants to identify the domains within each protein that contribute to the activity of the Sox2-Oct-3 complex. Within Oct-3, we have identified two activation domains, the N-terminal AD1 and the C-terminal AD2, that play a role in the activity of the Sox2-Oct-3 complex. AD1 also displays transcriptional activation functions in the absence of Sox2 while AD2 function was only detected within the Sox2-Oct-3 complex. In Sox2, we have identified three activation domains within its C terminus: R1, R2, and R3. R1 and R2 can potentiate weak activation by Sox2 in the absence of Oct-3 but their deletion has no effect on the Sox2-Oct-3 complex. In contrast, R3 function is only observed when Sox2 is complexed with Oct-3. In addition, analysis of Oct-1/Oct-3 chimeras indicates that the Oct-3 homeodomain also plays a critical role in the formation of a functional Sox2-Oct-3 complex. Our results are consistent with a model in which the synergistic action of Sox2 and Oct-3 results from two major processes. Cooperative binding of the factors to the enhancer DNA, mediated by their binding domains, stably tethers each factor to DNA and increases the activity of intrinsic activation domains within each protein. Protein-protein and protein-DNA interactions then may lead to reciprocal conformational changes that expose latent activation domains within each protein. These findings define a mechanism that may also be utilized by other Sox-POU protein complexes in gene activation.

The extraordinary complexity of gene expression patterns generated during embryonic development is accomplished by a comparatively small number of transcription factors. It has become clear that resolution of this apparent paradox lies to a great extent in the observation that cells utilize a strategy of combinatorial and synergistic interactions among heterologous transcription factors to achieve specific and diverse patterns of gene expression. Thus, a true understanding of transcriptional regulation of developmentally regulated genes requires, rather than analyses of gene activation by individual transcription factors “in isolation,” the study of the mechanisms of activation by transcription factor complexes.

Our studies have focused on defining the regulatory mechanisms that control transcription of the murine FGF-4 gene during embryogenesis. The FGF-4 gene encodes a signaling polypeptide that has been shown to play an essential role in embryonic development (1–4). In situ analyses have shown that FGF-4 RNA is only detected in the inner cell mass of the blastocyst and subsequently at other embryonic locations, including the primitive streak, myotomes, and limb bud (2, 5). FGF-4 gene expression in these distinct structures is governed by separate enhancer elements, the best characterized of which is that directing FGF-4 gene expression in the blastocyst and in EC cells (6, 7). We previously determined (8, 9) that activity of this enhancer results from the assembly of a ternary complex, termed Oct-3*, composed of the embryonic transcription factors Oct-3 (also called Oct-4) (10) and Sox 2 (11, 12). Enhancer activation requires that both Oct-3 and Sox2 bind their adjacent sites on the enhancer since expression of either protein in the absence of the other is not sufficient to confer transcriptional activation. Other octamer-binding proteins (Oct-1 (9) or Oct-6)¶ cannot substitute for Oct-3 or Sox5 functionally replace Sox2 in the Sox2-Oct-3 complex (9). Together these results predicted that the FGF-4 enhancer should only be activated in cells that express both Sox2 and Oct-3, a notion that was supported by additional evidence in vivo (7, 13).

Our previous studies demonstrated that assembly of an active Oct-3* complex requires a specific arrangement of binding sites for Sox2 and Oct-3 on the enhancer DNA since insertion of as few as 3 additional base pairs between the normally juxtaposed binding sites severely impairs enhancer function (14).

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1 The abbreviations used are: FGF, fibroblast growth factor; EC, embryonal carcinoma; HMG, high mobility group; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay; POU*, POU-specific; POUhomodomain; bp, base pair(s).
2 L. Dailey and C. Basilico, unpublished observations.
We proposed that this specific arrangement of factor binding sites facilitates protein-protein interactions between the DNA-binding POU and HMG domains of Oct-3 and Sox2, respectively, thus leading to the observed cooperative binding on the enhancer DNA (14). While these interactions are essential to proper Oct-3* complex assembly, this parameter alone could not account for the specificity of the requirement for Oct-3 in partnership with Sox2 since the POU domain of Oct-1 can also participate in direct protein-protein interaction with Sox2 (14).

To further define the molecular basis of synergistic transcriptional activation by the Oct-3* complex, we have analyzed a number of Sox2 and Oct-3 deletion mutants to identify domains within each protein that contribute to Oct-3* activity. In addition, we have studied a series of Oct-3/Oct-1 chimeric proteins to gain insight into the molecular distinction between these related transcription factors that allows Oct-3, but not Oct-1, to form a transcriptionally active ternary complex with Sox2. Our results show that multiple regions within both Sox2 and Oct-3 can contribute to transcriptional activation by the Oct-3* complex. Some of these domains participate in transcriptional activation by each of these proteins in the absence of the partner factor and thus are intrinsic activation domains. The function of other domains within Sox2 and Oct-3 is only observed in the context of the ternary complex. However, analysis of Oct-1/Oct-3 chimeras suggest that the Oct-3 homeodomain also plays a critical role in assembly of an active Oct-3* complex. Our results indicate that the synergistic action of Oct-3 and Sox2 results from two concerted steps. The first relies on cooperative binding of these factors to the enhancer DNA which is mediated by the POU domain of Oct-3 and the HMG domain of Sox2. These protein-protein and protein-DNA interactions may then lead to reciprocal conformation changes within each protein that cause the exposure of latent activation domains and activation of gene expression. As a number of complexes composed of specific POU and HMG domain partners have been described (15–19), these results may also represent a more general mechanism by which this class of transcription complexes achieve both activity and partner specificity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection—**HeLa cell transfections and CAT assays were as described previously (14). The CMV-Bgal plasmid was utilized for normalization.

**Oligonucleotides and Oligonucleotide Primers—**The -64fgf CAT reporter plasmid was constructed by PCR amplification of the murine fgf-4 promoter region between positions -64 and +101 using the forward primer 5'-CCCCGGGACGCGGCTGGCGCCC-3' (containing a recognition site for Smal) and the reverse primer 5'-AGACTTCTGCG-GAGTTCAGACTGCT-3' (containing a recognition site for BglII). After Smal/BglII digestion, one copy of the 200-bp PCR product was inserted between the Smal and BglII sites, upstream of the CAT gene within the pcAT3-Base Vector (Promega). To obtain the 6'S/O/CAT reporter construct, six copies of annealed oligonucleotides containing the fgf-4 enhancer sequence shown in Fig. 1A were first cloned in tandem into the BamHI site of Bluescript KS plasmid. This plasmid was digested with SacI and Smal and the multimerized element was cloned up-steam of the FOP promoter in -64fgfCAT. To obtain the 6'S/lex CAT reporter plasmid, six tandem copies of the lexA operator sequence shown in Fig. 6A were cloned into the BamHI site of Bluescript KS. Multimerized binding sites were excised from the plasmid by SacI and Smal digestion and cloned in -64fgf-CAT. C- and N-terminal deletion mutants of the Sox2 coding sequence were generated by PCR using PCEP-Sox2 (9) as a template. To obtain C-terminal deletion mutants, forward primer 5'-GGTTGGAGCGTTATATAAAG-3' was used in conjunction with deletion-specific reverse primers that each contained the last 6 codons of the corresponding Sox2 sequence followed by a stop codon and a BamHI recognition site. To obtain deletion mutants Sox2 31–129 (HMG) and Sox2 31–319, forward primer 5'-GGTTGGAGCGTTATATAAAG-3' containing a NotI recognition site and the ATG start codon, was combined with reverse primers 5'-GGATCTCT-CAAGCTGTTACTATCT-3' or 5'-GGATCTCTCACATGTTGACGAGGGCCG3', respectively. All PCR products were digested with NotI and BamHI and cloned in the pCEP4 expression vector (Invitrogen). To generate the Oct-3 deletion mutants, DNA fragments were generated by PCR using the wild type pCMV-Oct-4 plasmid (21) as a template and oligonucleotide primers complementary to Oct-3 sequences upstream or downstream of the POU domain of the Oct-3 plasmid. The 5' oligonucleotide primers contained either a BamHI or EcoRI restriction site while the 3' oligonucleotide primers contained a stop codon followed by an XbaI restriction site. PCR products were enzymatically digested and cloned between the BamHI and XbaI sites of the pEVRF2 expression plasmid (20, 21). Cloning in this manner produces a fusion protein containing an N-terminal YFP (enhanced yellow fluorescent protein) derived from the Herpes simplex virus TK gene. The inclusion of this leader sequence was employed to minimize differences in expression levels or stability of Oct-3 variants lacking the natural Oct-3 N terminus. Primer combinations (see primer sequences below) used to generate the following mutants were: pCMV- Oct-3 (aa 1–352 numbering according to Ref. 22), primers 1 and 2; ΔN (aa 117–352), primers 2 and 4; and ΔC (aa 1–286), primers 2 and 3. The 5'-Oct-3 chimeras plasmids 0.1.0 and 0.3.0, encoding only the POU domains of Oct-1 or Oct-3, respectively, were generated by PCR using plasmids pCGOct-1 or pCMVOct-4, respectively, as templates. The 5' oligonucleotide primers contained restriction sites for both BamHI and KpnI while the 3' oligonucleotide primers contained sites for SalI and XbaI. 0.1.0 encodes for Oct-1 amino acids 278 to 436 (numbering according to Ref. 23) and was generated using primers 1 and 3. These 0.3.0 encodes for Oct-3 amino acids 126 to 282 was generated using primers 7 and 8. The PCR products were digested with BamHI and XbaI and inserted between the BamHI and XbaI sites of the pEVRF2 plasmid. Thus for both 0.1.0 and 0.3.0 the insert contains 5' to 3' BamHI KpnI; POU, SalI, and XbaI. The 3.1.3 Oct-1-Oct-3 chimera was constructed in a stepwise manner. The chimera pcmv-POU2–4C described by Bresem et al. (21) contains the POU domain of Oct-2 fused to the C-terminus (amino acids 283–352) of Oct-4 (Oct-3). After BamHI and SalI digestion of pcmv-POU2–4C, the POU-2 segment of this plasmid was replaced with the BamHI/SalI-digested Oct-1 fragment of 0.1.0 to create 0.1.3 (POU-1 plus the Oct-3 C terminus). Digestion of 0.1.3 with KpnI and XbaI was followed by ligation of the purified POU-1-Oct-3 C-terminal fragment into the XbaI sites of the Brehm plasmid pcmv-4N-POU2, to create 3.1.3. The "wild type" plasmid 3.3.3 was created by replacement of the POU-1 segment of 3.1.3 by that of POU-3 after digestion of 3.0.0 with KpnI and SalI and ligation of the purified POU-3 sequence into KpnI- and SalI-digested 3.1.3.

Chimeras between the Oct-1 and Oct-3 POU domains were originally created by Y. Luo of the Rockefeller University and cloned into the pSVAC expression vector. pACE2 contains the POU domain vector. POU B contains the POU and linker segments of Oct-1 (Oct-1 amino acids 278–378) fused to the POU domain of Oct-3 (Oct-3 amino acids 223–282). POU D contains the POU and linker segments of Oct-3 (amino acids 127–222) fused to the POU domain of Oct-1 (Oct-1 amino acids 379–436). These chimeric sequences were used as templates for PCRs with oligonucleotide primers 5 and 8 (for POU B) or primers 7 and 6 (POU D). The PCR products were amplified, cloned into pUC19 (KpnI-digested) and inserted into the SalI sites of 3.1.3 to replace the wild type Oct-3 POU domain with POU B or POU D. The th primers used were pr1, GGGGGAGATCTTCCAT-GGCTGGAAGAGTTGGAAGAGTTAGGTGTTAGTAATG; pr2, GGGGGAGATCTTCAGACTGCTT; pr3, CCCCTCCTAGATCAAGGAACTACTTCT; pr4, CCCCTCCTAGATCAAGGAACTACTTCT; pr5, CCCCTCCTAGATCAAGGAACTACTTCT; pr6, GGGGGAGATCTTCCATGGCTGGAAGAGTTGGAAGAGTTAGGTGTTAGTAATG.

To construct the lexA DNA-binding domain expression vector, the HindIII/SphI (blunted) DNA fragment encoding lexA amino acid residues 1–202 followed by one in-frame HA epitope, was isolated from the pEG202+(HA) plasmid (24) and cloned in the HindIII and BamHI (blunted) sites of pCEP4 expression vector. The resulting plasmid was used as a template for PCR with forward primer 5'-GGTTGGAGCGTTATATAAAG-3' and inserted into the SalI sites of 3.1.3 to replace a NotI recognition site follow by a stop codon and a BamHI recognition site. The resulting PCR product was then digested at the HindIII and BamHI sites and cloned into the pCEP4 vector to obtain the pCEP-lex202 expression construct. Subsequently luxA/Sox2 and luxA/Oct-3 fusion protein expression vectors were constructed by PCR amplification of DNA fragments expressing Sox2 or Oct-3 subregions were amplified by PCR using a forward (F) primer containing a NotI recognition site and a reverse (R) primer containing a stop codon followed by a BamHI recognition site. After NotI and BamHI digestion, each coding sequence was cloned in-frame with the lexA DNA-binding domain within pCEP-lex202. Forward and reverse oligonucleotide sequences for each fusion protein were as follows: lex/Oct (1–53), (F)ATAAGAATGCGGCCGCT-CTTTTCCTTCTGGCCGC; lex202. Forward and reverse oligonucleotide sequences for each fusion protein were as follows: lex/Oct (1–53), (F)ATAAGAATGCGGCCGCT-CTTTTCCTTCTGGCCGC; lex202.
RESULTS

To gain an understanding of why the Oct-3* complex composed of both Sox2 and Oct-3, but neither protein alone, can effectively activate the FGF-4 enhancer, we have analyzed Sox2 and Oct-3 deletion mutants and chimeric proteins to identify and compare the roles of domains required for independent transcriptional activation by each of these proteins with that required for function of the Oct-3* complex.

Synergy Appears to be Mostly Mediated by the DNA-binding Domains of Sox2 and Oct-3 while Transcriptional Activation Requires Additional Domains—The CAT reporter plasmid 6×(S/O)-CAT used in these and all subsequent experiments contains 6 copies of the murine FGF-4 enhancer octamer and Sox-binding elements placed upstream of a minimal murine FGF-4 promoter (Fig. 1A). As expected, the presence of the octamer and Sox-binding elements in this plasmid led to transcription of the CAT reporter gene in undifferentiated P9 cells but not in HeLa cells, since HeLa cells lack both Oct-3 and Sox2 (data not shown). Consistent with our previous results using similar reporter, the 6×(S/O)-CAT construct could only be effectively activated in HeLa cells when co-transfected with expression plasmids for both Sox2 and Oct-3 (9). As shown below, the 6×(S/O)-CAT plasmid was exquisitely sensitive to transactivation by Sox2 and Oct-3 expression, allowing the detection of weak transactivating activities that were not detectable with the previously used reporter plasmids (9, 14).

Activation of the FGF-4 enhancer by Sox2 and Oct-3 over a broad range of plasmid concentrations occurred in a synergistic manner, the level of transcriptional activation achieved by the Oct-3* complex being much greater than the sum of activation by the individual Oct-3 and Sox2 proteins (Fig. 1B). Relatively little activation of CAT gene expression was observed when Sox2 and Oct-3 were independently expressed, and then only at very high concentration of factor expressing vectors. Given our previous demonstration of a direct protein-protein interaction and cooperative DNA binding by the HMG and POU domains (14), we tested whether either of these domains is sufficient to confer synergistic activation of the 6×(S/O)-CAT plasmid when complexed with its factor partner (Oct-3 or Sox2, respectively). Activation of CAT gene expression was assessed after co-transfection of the reporter plasmid into HeLa cells with expression plasmids for the POU domain of Oct-3 (Fig. 2, POU3), or full-length Oct-3 protein (Fig. 2, Oct-3), alone or in combination with expression plasmids for Sox2 (Fig. 2, Sox2) or the Sox2 HMG domain (Fig. 2, HMG). Within the range of expression plasmid DNA used in this experiment, transfection of these constructs individually resulted in either marginal or no CAT gene activation. In contrast, coexpression of full-length Sox2 and Oct-3 proteins resulted in a 160-fold activation of CAT gene transcription (Fig. 2B) and displayed about 20-fold synergism (Fig. 2C). Coexpression of the Sox2 HMG domain with full-length Oct-3 resulted in a lower but substantial level of transcription activation of the CAT* reporter gene compared with that observed using the wild-type proteins (77-fold activation, Fig. 2B). The degree of synergism observed using the Sox2 HMG domain and Oct-3 was still considerable (16-fold). Similarly, coexpression of POU3 with wild type Sox2 resulted in a markedly lower level of overall reporter gene activation (28-fold activation) but still displayed a significant degree of synergy. These results are consistent with the notion that the DNA-binding HMG and POU domains play a major role in mediating synergy between the Oct-3 and Sox2 proteins. However, coexpression of just the POU3 and the HMG domain expression plasmids did not result in activation of the CAT
heLa cells were transiently co-transfected with 2 µg of 6×(S/O)-CAT reporter construct (shown in Fig. 1A) and various combinations of CMV expression constructs for Sox2 (500 ng), HMG (500 ng), Oct-3 (200 ng), and POU3 (200 ng) as indicated. CAT activity generated by the 6×(S/O)-CAT reporter construct alone was given the value of 1. The values shown, expressed as fold induction of the reporter construct, are from one representative experiment and show the mean of two experiments and show the mean of duplicates. C, synergy is mostly mediated by the HMG and POU domain of Sox2 and Oct-3. CAT activities from B were used to calculate the fold synergy index as described under “Experimental Procedures.”

Fig. 2. Sox2 and Oct-3 synergy is mostly mediated by their DNA-binding domains. A, schematic representation of wild-type Oct-3 and Sox2 proteins and derived truncated proteins POU3 and HMG. The location of the POU domain is depicted in black, whereas the HMG domain is shown as a hatched box. B, full activation of the FGF-4 enhancer also requires domains located outside of the DNA-binding domain of Sox2 and Oct-3. HeLa cells were transiently co-transfected with 2 µg of 6×(S/O)-CAT reporter gene (Fig. 2), demonstrating that the HMG and POU domains must act in conjunction with additional domains within Oct-3 and Sox2 to promote transcriptional activation.

Identification of an N-terminal Activation Domain (AD1) and a C-terminal Activation Domain (AD2) within Oct-3—The results of the previous section suggested that a component of Oct-3* complex activity must reside outside of the Oct-3 POU and Sox2 HMG domains. To identify these domains within Oct-3, and to understand whether they were uniquely required for function of the Oct-3* complex, deletion mutants of the Oct-3 proteins were created by PCR and their activity tested on the 6×(S/O)-CAT reporter plasmid in HeLa cells.

Fig. 3 shows a schematic representation of the 352-amino acid Oct-3 protein and the Oct-3 deletion mutants used in this study (numbering according to Ref. 22). EMSA (Fig. 3D) and Western blot analysis (not shown) indicated a significant variation in the expression levels of the various mutant proteins. Thus we normalized all values of CAT activity to DNA binding (i.e. expression level) for each mutant, as detailed under “Experimental Procedures.”

We first tested the ability of the Oct-3 deletion mutants to promote gene expression of the CAT reporter plasmid in the absence of Sox2. At the concentration of expression plasmid used in these experiments, Oct-3 produced an average 28-fold stimulation of CAT gene expression (Fig. 3B). Deletion of the majority of the N-terminal amino acids rendered the resulting ∆N mutant incapable of efficiently activating transcription (Fig. 3B). Only 1.5–3.5-fold activation by ∆N could be observed across a broad range of transfected expression plasmid (0.1–3 µg, data not shown). This result indicates the presence of an activation domain within the N terminus of Oct-3 that can function independently of Sox2. We will refer to this domain, broadly defined between amino acids 1 and 117 of Oct-3, as AD1. Removal of most of the amino acids C-terminal to the POU domain (Oct-3 mutant ∆C, Fig. 3B) had a negligible effect on the ability of Oct-3 to activate transcription of the reporter gene. Deletion of the N-terminal region containing AD1 from the ∆C mutant, produced a protein corresponding to the DNA-binding domain (POU3, amino acids 117–286, Fig. 3D) that failed to activate CAT gene expression. Together these results demonstrate the presence of one domain within Oct-3, AD1, that can activate transcription of the 6×(S/O)-CAT reporter gene in the absence of Sox2.

To determine the role of AD1, or perhaps additional domains, in activation by the ternary Oct-3* complex, we next tested the ability of these Oct-3 mutants to activate the reporter gene in the presence of Sox2. Coexpression of wild-type Oct-3 with Sox2 resulted in a 520-fold activation of transcription (on average) from the 6×(S/O)-CAT reporter gene (Fig. 3C). Interestingly, the ∆N mutant, which was essentially inactive in the absence of Sox2, achieved a relatively high level of activation in the presence of Sox2 (Fig. 3C). Thus, deletion of AD1 resulted in only a 50% decrease in the ability of Oct-3 to activate reporter gene transcription within the Oct-3* complex, suggesting that domains within the C-terminal region of Oct-3 are able to function in conjunction with Sox2. Coexpression of Sox2 with the Oct-3 ∆C mutant also resulted in a 50% decrease in activation of the reporter gene. Additionally, deletion of most of the C terminus from ∆N (POU3 protein) caused a further 70% reduction in the ability of this mutant to activate the reporter gene in the presence of Sox2 (Fig. 3C). EMSA analysis confirmed that this is not a result of an inability of POU3 to bind the octamer site within the enhancer DNA or efficiently form the Oct-3* complex with Sox2 (data not shown). Together these results indicate that the Oct-3 C terminus (i.e. between amino acids 286 and 352) may contribute an activation domain whose function is only apparent within the ternary Oct-3* complex since its deletion did not decrease transcriptional activation when Sox2 was not present. We designate this C-terminal domain, located between amino acids 286 and 352, as AD2.

These results are consistent with the notion that Oct-3 contains two domains that can contribute to transcriptional activation of the FGF-4 enhancer. In the absence of Sox2, only AD1 has the ability to activate the reporter gene. In contrast, the C-terminal domain AD2 only functions within the Oct-3* complex and thus is unique to the Oct-3 partnership with Sox2. Deletion of any single domain had only a slight effect on Oct-3* complex function suggesting that upon deletion of one domain, the other can still function within the ternary Oct-3* complex.

Identification of Three Activation Domains in the C-terminal...
Fig. 3. Two regions of Oct-3 contribute to Oct-3* activity. A, schematic representation of Oct-3 deletion mutants. The POU domain is shown in black and the most N-terminal and C-terminal amino acid residues are depicted to the left and right for each mutant. B, relative CAT activity induced by Oct-3 deletion mutants in the absence of Sox2. 0.05–2 µg of expression plasmid for each mutant were co-transfected with 6×(O/S)-CAT reporter DNA into HeLa cells. CAT activity was normalized to expression level (DNA binding) for each mutant as described under “Experimental Procedures” and shown as % activity of wild type Oct-3. C, relative CAT activity induced by Oct-3 mutants in the presence of Sox2. Transfection and normalization were performed as in B except that 0.5 µg of Sox2 expression plasmid was included in all samples. Results for both B and C are the averages of three to five separate experiments. D, representative EMSA using WCEs derived from transfected HeLa cells. HeLa cells were transfected with the quantity of wild type Oct-3 (WT Oct-3) or ΔC mutant expression plasmid DNA as indicated on the top of the panel. Total protein was extracted from the transfected cells and analyzed by EMSA as described under “Experimental Procedures.” Note the significant difference in expression levels of the WT and ΔC proteins. Similar variations were also found using the ΔN and POU3 constructs. Thus, CAT activities for each mutant were normalized to DNA binding data.

Region of Sox2—The results of Fig. 2C indicated that the Sox2 HMG domain is sufficient to confer synergistic activation in combination with Oct-3. However, the final degree of activation was significantly less than that achieved using full-length Sox2 in this assay, suggesting that Sox2 also harbors activation domains that contribute to Oct-3* complex function. To address this possibility, a series of Sox2 deletion mutants were created by PCR and their ability to activate transcription of the 6×(O/S)-CAT reporter construct was analyzed either in the presence or absence of Oct-3 after transient transfection in HeLa cells. Fig. 4A shows a schematic representation of the 318-amino acid Sox2 protein and of the deletion mutants used in this study. EMSA showed that all these mutants were expressed at comparable levels (data not shown). Although transfection of low levels (0.5 µg) of Sox2 expression plasmid does not lead to activation of the reporter gene in the absence of Oct-3 (Fig. 1B), overexpression of Sox2, using 5 µg of plasmid DNA, caused about 25-fold induction of CAT gene expression (Fig. 4B). This activity was completely dependent on the presence of the Sox2-binding sites in the reporter construct (data not shown). Deletion of the 64 C-terminal amino acids from Sox2 caused a progressive reduction in transcriptional activation to a level of about 15% of wt Sox2 (mutant Sox 1–255, Fig. 4B). Further deletion to amino acid 178 caused an additional reduction in Sox2 activity to essentially basal levels (mutant Sox 1–178, Fig. 4B). In contrast, deletion of amino acids from the N terminus had no effect (mutant 31–318, Fig. 4B). These results suggest that Sox2 has an Oct-3-independent transcriptional activity that is conferred by at least one broadly defined domain located within the C-terminal region between amino acids 255 and 318, and perhaps a second weaker domain localized between amino acids 178 and 216. We will refer to the 178–189 domain as region 2 (R2) and that localized between amino acid 255–318, as R1.

We next assessed the ability of each of the Sox2 mutants to activate the 6×(O/S)-CAT reporter gene in the presence of Oct-3. For these experiments, a lower amount of Sox2 expression plasmid (0.5 µg) was used. Under these conditions none of the Sox2 proteins significantly activated transcription of the reporter gene in the absence of Oct-3. Co-transfection of both the Oct-3- and wild type Sox2-expression plasmids caused a synergistic 100-fold activation of CAT gene transcription. Analysis of the Sox2 deletion mutant proteins showed that, in contrast to the results using Sox2 in the absence of Oct-3, deletion of most of the C terminus of Sox2 had no effect on Oct-3* activity since Sox2 deletion mutant 1–178 displayed wild type levels of activation when coexpressed with Oct-3 (Fig. 4C). Thus, deletion of Sox2 domains R1 and R2, that were essential for independent activation by Sox2, did not have any measurable effect on Oct-3* complex function. Instead, a larger than 50% decrease in synergy and transcriptional activation by Oct-3* resulted from further deletion of Sox2 to amino acid 152 (mutant Sox 1–152). The activity of the domain (152–178) defined by this result is thus only observed in the context of the Oct-3* complex and will be referred to as R3.

The Oct-3 AD2 Domain Works in Conjunction with the R3 Domain within Sox2—The results of the preceding sections have shown that Oct-3 contains two activation domains that can function in the Oct-3* complex and that these domains can act in an alternate fashion, i.e. high levels of activity can be maintained upon deletion of one or the other, but not both, activation domains. The nature of these two domains differs, however, since AD1 can function at least to some extent in a Sox2-independent fashion while activation via AD2 is only detected in the presence of Sox2. Sox2 also contains activation domains of different nature. The activity of R1 or R2 was only observed when Sox2 was expressed independently of Oct-3, but was dispensable in the Oct-3* complex. In contrast, R3 appeared to behave as a complex-specific activation domain, playing a role only when Sox2 was complexed with Oct-3 on the
enhancer DNA. To gain a better understanding of the interplay among these domains within the Oct-3* complex we re-examined some of the Sox2 mutants in combination with the Oct-3 mutants. While all of the Sox mutants of Fig. 4 were analyzed in this manner, only mutant 1–178, in which the independent R1 and R2 domains have been deleted, and mutant 1–152, containing a further deletion of R3, are presented in Fig. 5.

The ability of each of these Sox2 constructs to activate the 6×S/O-CAT reporter gene in the presence or absence of wild type Oct-3 is again presented here for comparison (Fig. 5, A and B). As was shown in Fig. 4, Sox2 R1 and R2 only display a demonstrable activation function in the absence of Oct-3 (mutant 1–178, Fig. 5A), whereas R3 only displays function within the Oct3* complex (mutant 1–152, Fig. 5B).

Activation by each of these Sox2 mutants was then analyzed in the presence of the Oct-3 POU3 protein, POU3, as was also shown in Fig. 2, can promote at least some degree of synergistic activation of reporter gene transcription when combined with wild type Sox2, even though POU3 contains neither of the Oct-3 domains AD1 nor AD2 (POU3 plus WT Sox2, Fig. 5C). However, upon coexpression of either Sox2 mutant with POU3, no reporter gene activation or synergy was observed (Fig. 5C’, mutants 1–178 and 1–152). These results indicate that in the absence of AD1 and AD2, interaction between POU3 and wild type Sox2 leads to more efficient utilization of the intrinsic Sox2 domains R1 and R2 and thus to the observed synergy between WT Sox2 and POU3. The effect of R1 and R2 is most likely overshadowed by the more potent AD1 and AD2 present in wild type Oct-3, resulting in no effect on Oct-3* activity upon R1 and R2 deletion in Fig. 5B. An additional result illustrated by Fig. 5C’ is that Sox2 domain R3 did not contribute to complex activity in combination with POU3 since complexes composed of mutants 1–178 and 1–152 display the same, basal level of activation and no synergy (Fig. 5C’). Thus, the complex-specific function of Sox2 R3 must depend on additional regions outside of the Oct-3 POU domain.

Activation by the Sox2 mutants in the presence of the Oct-3 ΔC mutant, that contains AD1 but lacks AD2, is presented in Fig. 5D. The overall activation profile by this combination of mutants resembled that seen using POU3, i.e. a detectable effect on complex activity was observed upon deletion of the intrinsic Sox2 R1 and R2 domains while further deletion of R3 had no apparent effect. Thus a contribution to Oct-3* complex activity by R1 and R2 can be observed also using an Oct-3 molecule that possess AD1. In addition, the level of synergy is approximately 7 times greater for complexes containing R1 and R2 (Fig. 5D, WT Sox2 and ΔC) than those that did not (Fig. 5D, mutant 1–178 plus AD3). Deletion of R1 and R2 affected synergy to approximately the same extent in the presence of the POU3 protein (Fig. 5C), suggesting that there is probably no substantial interdomain synergy between the Oct-3 AD1 and Sox2 R1/R2 domains. R1 and R2 functions are probably enhanced as a result of cooperative interactions between the HMG and POU domains, leading to stabilized association of the complex to the DNA. These results also indicate that the region of Oct-3 important for Sox2 R3 activity does not appear to reside within the Oct-3 POU domain or N-terminal sequences (compare ΔC plus WT Sox2 with ΔC plus mutant 1–152, Fig. 5D).

The Oct-3 ΔN mutant contains AD2 but lacks AD1. Analysis of reporter gene activation upon coexpression of ΔN with the Sox2 mutants showed that, again, deletion of the Sox2 R1 and R2 domains had a detectable effect on complex activity and an approximately 7–8-fold effect on the level of synergy (Fig. 5E, mutant 1–178). Significantly, however, a reproducible, approximately 5-fold decrease in complex activity and synergy was observed upon deletion of Sox2 R3 (Fig. 5E, mutant 1–152), the only Sox2 domain that displayed a detectable function in complexes containing wild-type Oct-3 (Fig. 5E, mutant 1–152). Thus R3 activity is only observed using Oct-3 proteins that contain AD2 (i.e. wild-type Oct-3 or ΔN) but not Oct-3 proteins that lack AD2 (ΔC or POU3), suggesting that R3 works in conjunction with AD2.

In summary, these results demonstrate that the Sox2 R1 and R2 domains can be more effectively utilized within the Oct-3* complex than within Sox2 alone, and that the POU domain is sufficient to elicit this effect. However, this function is only revealed using Oct-3 molecules that lack AD1 and/or AD2 and thus do not appear to represent the major activation domains within the native Oct-3* complex. Instead, the domains that contribute most critically to Oct-3* function are Oct-3 AD1 and the complex-specific, interdependent Oct-3 AD2 and Sox2 R3 domains.

Some of the Functional Domains Identified in Sox2 and Oct-3 Are Modular, Independent Transactivation Domains—To better characterize the trans-activating properties of the Sox2 and Oct-3 domains we prepared several fusion proteins with each of the previously identified AD1, AD2, R1, R2, or R3 domains tethered to the lexA DNA-binding domain (lexA residues 1–202). The activity of each fusion protein was tested by cotransfection of each construct together with the 6×(lex)-CAT
reporter plasmid, which contains the *FGF-4* promoter placed under the control of 6 copies of the lexA-binding site (Fig. 6A). To assess AD1 function, fusion protein lexA/Oct-1–153-A containing only the N-terminal 53 amino acids of Oct-3 was constructed, because previous results (not shown) had indicated that this region was essential for AD1 activity. This fusion protein was capable of activating the CAT reporter plasmid about 80-fold above the basal level. On the other hand, the proteins containing the C-terminal Oct-3 AD2 did not show activity in this assay (Fig. 6B). Fusion proteins lexA/Oct 121–189 that contains the R2 and R3 domains of Sox2 and lexA/Oct 250–319 that contains the R1 Sox2 domain, showed a similar activity of about 50-fold over the lexA control. However, fusion protein lexA/Oct 174–255, which contains the Sox2 R2 domain, had no detectable activity (Fig. 6B).

These results indicate that at least three of the regions we have identified in Sox2 and Oct-3 (i.e. the AD1 domain of Oct-3 and R1 and R3 Sox2 domains) can work as independent modular activation domains when dissociated from their endogenous DNA-binding domain and from the context of the Sox2-Oct-3 complex. The lack of activity of AD2 in this assay could be due to a variety of reasons. On the other hand, the Sox2 R2 domain has, in general, weak activity and it could not be excluded that R3 and R2 make up a single activation domain or that the R2 function requires R3.

**The Nature of the POU Homeodomain Also Affects Oct-3**

Our previous work demonstrated that Oct-3, but not Oct-1, activates transcription in conjunction with Sox2 from reporter plasmids containing *FGF-4* enhancer sequence DNA (9). The fact that Oct-1 and Oct-3 display significant amino acid sequence homology within the conserved POU domains (10, 22, 25, 26) suggested that the differential activation properties of these two proteins would most likely be attributed to a domain(s) within the unique N- and C-terminal portion of Oct-3. Having identified the Oct-3 AD1 and AD2 activation domains, we asked whether we could create a chimeric Oct-1-Oct-3 protein that could function as an activator of the *FGF-4* enhancer in the presence of Sox2 simply by replacing Oct-1 C- and N-terminal domains with Oct-3 AD1 and AD2.

Oct-1 and Oct-3 were conceptually divided into three segments: the conserved POU domain, and the regions N-terminal or C-terminal to the POU domain. Each of these segments were amplified by PCR using oligonucleotide primers containing specific, unique restriction enzyme recognition sites that would then allow the construction of chimeras by the ligation of each of the N-terminal, C-terminal, and POU segments (see “Experimental Procedures”). As a positive control, the wild type Oct-3 protein was reconstructed from each of its amplified segments to create the 3.3.3 protein. 3.3.3 activated transcription of the 6×(S/O)-CAT reporter in a manner that was comparable to that of wild type Oct-3 in the presence of Sox2 after transfection into HeLa cells. As expected, the 0.3.0 plasmid, expressing only the POU domain of Oct-3, could activate the reporter gene only weakly in the presence of Sox2 (15% of 3.3.3, Fig. 7A). The POU domain of Oct-1, expressed from the 0.1.0 plasmid, activated reporter gene transcription to essentially the same level as did 0.3.0 (Fig. 7A). Surprisingly, however, when both the N- and C-terminal segments of Oct-3 were fused with the Oct-1 POU domain, the chimeric 3.1.3 protein was also unable to significantly activate CAT gene expression. A similar activation profile was obtained in response to these chimeric octamer-binding proteins using reporter constructs containing one or two copies of the 116-bp *FGF-4* enhancer (9) placed upstream of the *FGF-4* promoter (data not shown). This suggested that the highly conserved POU domains of Oct-1 and Oct-3 may not be functionally interchangeable and that the Oct-3 POU domain itself might facilitate or coordinate the activity of AD1 or AD2 within Oct-3 (and/or Sox2 domains).

The POU domain is composed of highly homologous subdomains designated as the POU5 domain, shared among the POU proteins, and the POU homeodomain (POU1), that is common to the more general class of homeodomain proteins, tethered by a variable linker segment (25, 27). Thus, additional chimeras were made between the POU5 and POU1 of Oct-1 and Oct-3 and tested in the context of the Oct-3 N- and C termini (chimeras 3.B.3 and 3.D.3, Fig. 7A). Replacement of the linker

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**Fig. 5.** Specific combinations of Sox2 and Oct-3 deletion mutants reveal intermolecular functional interactions within the complex between activation domains. A, HeLa cells were transiently transfected with 2 μg of 6×(S/O)-CAT reporter construct and 5 μg of expression construct for wild-type Sox2 or selected truncated Sox2 proteins as indicated on the left of each histogram. In each of the following panels (from panel B to panel E), the same collection of Sox2-derived proteins was co-transfected in a lower amount of expression construct (500 ng), with 200 ng of expression construct for wild-type Oct-3 or Oct-3 deletion mutants as indicated below each histogram. B, wild-type Oct-3. C, Oct-3 deletion mutant POU3. D, Oct-3 deletion mutant ΔC. E, Oct-3 deletion mutant ΔN. On the left of each panel is shown a schematic representation of Sox2 truncated proteins. In each panel the CAT activity measured for wild-type Sox2 by itself (panel A) or in combination with an Oct-3 factor (panels B–E) was set as 100%. The numbers shown at the top of each bar in the histograms represent the synergy index.
region plus the POU4 domain of Oct-1 with that of Oct-3 did not restore Oct* activity (3.D.3, Fig. 7). However, replacement of the Oct-1 homeodomain with that of Oct-3 almost completely restored Oct-3* function (3.B.3, Fig. 7). These results indicate an important role not only for AD1 and AD2, but also for the Oct-3 POU homeodomain in Oct-3* activity. The ability of each of the POUD and POU1 POU domains to support cooperative assembly of a ternary complex with Sox2 was compared with that of POU3 using EMSA. To this end, ternary complex formation by each of the bacterially expressed POU proteins was compared using two FGF-4 enhancer DNA probes: wild type, containing the normally juxtaposed octamer and Sox-binding sites, and "110," a mutant FGF-4 enhancer DNA sequence in which 10 additional base pairs have been inserted between the factor-binding sites. As we have shown previously, cooperative binding by Sox2 and Oct-3 is abrogated by increasing the distance between their respective binding sites and therefore the 110 mutant DNA probe provides a useful measure of non-cooperative complex formation for comparison. Fig. 7, B and C, show that all of the POU proteins promoted cooperative ternary complex assembly with Sox2 on the wild type enhancer DNA probe, while no cooperative binding was observed using the +10 mutant enhancer. This result indicates that the inability of the POU-1 or POU-D containing chimeras to activate transcription in the presence of Sox2 (Fig. 7A) is not due to a defect in their ability to promote cooperative assembly of the ternary complex. Thus it is possible that the Oct-3 POU domain may allow the formation of a particular conformation required for optimal interaction with the DNA target sequence and/or Sox2. These results further suggest that the key to differential activation by Oct-3 actually may lie, unexpectedly, in the POU domain rather than only in the unique portions of the protein.

**DISCUSSION**

The major impetus for undertaking the current study was to define the general features of the interaction between Oct-3 and Sox2 that result in a transcriptionally active complex and thereby gain further insight into the functional distinction between Oct-3 and Oct-1. Our results show that a major component of Oct-3/Sox2 synergy is the interaction between the DNA-binding domains of the two proteins. However, additional Oct-3 and Sox2 domains are necessary for transcriptional activation. These observations are consistent with a model in which the DNA-binding domains of these factors not only contribute to synergism by promoting cooperative DNA binding, but also coordinate complex-specific activities that are dependent on the interaction between these DNA-binding domains (Fig. 8).

**Oct-3 Activation Domains**—We have identified two broadly defined activation domains within Oct-3, named AD1 and AD2, that can function in the Sox2-Oct-3 complex. In addition to its role in Oct-3*, AD1 can also mediate transcriptional activation of Oct-3 in the absence of Sox2 or when fused to the LexA DNA-binding domain. A similar functional domain has been reported using reporter genes containing octamer elements or when fused to heterologous DNA-binding domains (21). An Oct-3 N-terminal activation domain was also required for Oct-3-mediated activation of the EC cell-specific Rex-1 gene in P19 cells in conjunction with a second, as of yet uncharacterized, factor called Rox-1 (28). Thus usage of AD1 by Oct-3 seems to be fairly general and does not depend rigorously on the nature...
of the target promoter or a specific factor partner.

AD2 function, which in our case is dependent on interaction with Sox2, has been shown to be highly variable in other contexts and depends on the distance of the octamer element from the TATA box, the DNA-binding domain to which it is tethered, and cellular environment (21, 26, 29, 30). Interestingly, AD2 function is also dependent on the nature of the factor partner cooperating with Oct-3 since AD2 is dispensable for activation of the Rex-1 promoter in conjunction with Rox-1 (28). Thus, what we have described as the "complex-specific" function AD2 can be regulated in a number of different ways. Our results suggest that interaction with Sox2 serves to "unmask" AD2 activity.

**Sox2 Activation Domains**—Our results show that Sox2 also contains multiple activation domains. Under conditions where Sox2 was overexpressed, both R1 and R2 could potentiate independent transcriptional activation of the 6×S/O reporter construct and R1 could also function when fused to the LexA DNA-binding domain. However, deletion of R1 and R2 had no detectable effect on Oct-3* activity unless the Sox2 proteins were assayed in conjunction with Oct-3 mutants lacking AD1 or AD2. Thus Sox2 R1 and R3 are dispensable in complexes containing wild type Oct-3. Interestingly, however, a less characterized Oct-3-related protein, Oct-5, may represent an actual biological counterpart of the Oct-3 N terminus (10). Thus Oct-5, which is present in ES and EC cells and unfertilized oocytes, may be an Oct-3 variant lacking a portion of the Oct-3 N terminus (10). Thus Oct-5, like Oct-3 ΔN, may well lack AD1, and the transcriptional activity of any complex formed between Oct-5 and Sox2 would be expected to be more dependent on the Sox2 C-terminal domains than those composed of Oct-3 and Sox2.

A novel Sox2 domain (R3) defined by deletion of amino acids 152–178 operates only in the Sox2-Oct-3 complex (Fig. 4). R3 function is not observed in independent Sox2 activation of transcription, but it can be detected when R3 is fused to the LexA DNA-binding domain. Thus like AD2, R3 appears to behave as a complex-dependent activation domain.

**Few other Sox2 target genes are known (19, 31, 32). Activation of the δ-crystallin gene DC5 enhancer by Sox2 requires the cooperation of a putative partner factor, δEF3, that is postulated to interact with an adjacent site within the enhancer (31). Although the identity of δEF3 is unknown, its binding site suggests that it is not a DNA-binding domain. Interestingly, deletion of the R3 domain in Sox2 has no effect on the regulation of transcription of the δ-crystallin gene and the only Sox2 domain utilized seems to be R1 (33). Thus, it appears that the definition of specific activation domains in Sox2, as in the case of Oct-3, strictly depends on the nature of its partner factor and enhancer DNA context.**

**Modulation of Sox2 and Oct-3 Activities by Their DNA-binding Domains**—Each activation domain described in the preceding sections functions less effectively (AD1, R1, and R2) or not at all (AD2 and R3) within the individual Oct-3 or Sox2 proteins compared with their activity in the Oct-3* complex. Most of them, however, can efficiently activate transcription when assayed as isolated entities fused to the LexA (AD1, R1, and R3, Fig. 6) or GAL4 (AD2, (21)) DNA-binding domain. Together these observations imply that some silencing mechanism must keep these domains in a relatively inactive state within the native proteins. The basis for this silencing is presently unclear but may involve intramolecular masking of the activation domain (34), the binding of corepressor molecules (35–37), or the existence of the activation domains in a disordered, transcriptionally inactive state in the absence of complex assembly (38).

Whichever combination of these or other mechanisms hold true for Oct-3 and Sox2, our results show that these conditions must be relieved upon assembly of the Oct-3* complex. The
Factors Mediating Oct-3/Sox Synergy

model depicted in Fig. 8 proposes that this results from several processes occurring as a secondary consequence of protein-protein and protein-DNA interactions mediated by the HMG and POU domains. First, interactions between the HMG and POU domains with each other and the enhancer DNA lead to cooperative assembly of the Oct-3* complex (Fig. 8, step 1). This potentiation of complex assembly may well result in a more stable association of each factor (and their activation domains) with the DNA and in this way contribute to the synergy of Oct-3* transcriptional activation. Second, these interactions as well as dramatic distortions of the DNA-binding sites caused by the bending activities of the HMG domain (39–41), may lead to allosteric changes within the factors DNA-binding domains that are then transmitted to more distant regions of each protein. The consequences of these conformational changes could include the release and replacement of a putative corepressor with a coactivator and/or the induction of a novel structure to a previously disordered region of the protein(s), thus leading to activation of previously silent domains. We imagine these events to actually be concerted processes rather than discrete “steps” as depicted in Fig. 8.

Supporting evidence for this model can be derived from the results of Figs. 2 and 7 that demonstrate that either the Sox2 HMG domain or POU domain, in conjunction with full-length Oct-3 or Sox2, respectively, can induce the assembly of transcriptionally active Oct* complexes that display considerable synergy. Thus the DNA-binding domain of each protein is capable of transforming its factor partner (i.e. its activation domains) from a relatively inactive to a more active state and importantly, this induction appears to be reciprocal.

While the observations summarized above could simply reflect more stable tethering of factor activation domains with enhancer DNA, analyses of the Oct-1/Oct-3 chimeras suggest that additional mechanisms are needed to alleviate the relatively silent state of the activation domains. Oct-1 can also form a ternary complex with Sox2 on FGF4 enhancer DNA and yet this complex is not transcriptionally functional (9). Furthermore, the POU domain of Oct-1 can, like POU-3, also directly interact with and form a ternary complex in a cooperative manner with the Sox2 HMG domain (8, 14; Fig. 7, B and C). In fact, the results of Fig. 7A demonstrate that POU-1 can, in conjunction with Sox2, activate essentially the same low level of transcription from our reporter construct as is observed using POU-3. Thus, according to the argument above, Sox2 appears able to “respond” to either POU-1 or POU-3, presumably as a result of these cooperative interactions. However, the Oct* complex resulting from the coexpression of Sox2 and the 3.1.3 chimera has no greater transcriptional activity (i.e. 15% of wild type 3.3.3 activity) than the complex composed of Sox2 plus POU-3 or Sox2 plus POU-1. Thus, although stably tethered to the enhancer DNA, 3.1.3 cannot respond to Sox2 and the Oct-3 activation domains appear to be nonfunctional within the 3.1.3/Sox2 complex. These results thus point to a specific functional role for the Oct-3 POU domain in mediating the activities of AD1 and AD2 that cannot be fulfilled by the Oct-1 POU domain and are consistent with the notion that other mechanisms in addition to simple tethering of Oct-3 activation domains to enhancer DNA are required for their functioning. Simple replacement of the homeodomain segment of the 3.1.3 POU domain with that from POU-3 (protein 3.B.3) is sufficient to restore most, if not all, ternary complex activity, suggesting that this distinctive feature of POU-3 resides in its homeodomain.

Together these observations are consistent with the possibility that POU-3 can act first as a sort of “receptor” for a signal originating from the interaction with the HMG domain on the enhancer DNA and, second, is able to transmit this signal to other regions of the Oct-3 protein. It is thus possible that some feature of the interaction of POU-1 with Sox2 renders the former unable to undergo these alterations or to do so in an ineffective manner.

The Sox/Oct Paradigm—Studies over the last several years have provided numerous additional examples of cell type-restricted co-regulation of gene transcription by specific Octamer and Sox factor partner pairs (reviewed in Refs 44 and 45). Expression of the embryonic UTF-1 gene has also been shown to be activated by a ternary complex composed of Sox2 and Oct-3 (19), whereas Oct-3-mediated activation of the osteopontin gene is repressed by Sox2 binding (32). The organization of the Sox- and octamer-binding sites within the osteopontin gene differ from that of FGF4 and UTF-1, however, in that they are separated by 37 base pairs and no Sox2-Oct-3 ternary complex is observed (32). Recent studies have also shown that specific combinations of Sox- and octamer-binding proteins expressed in oligodendrocytes can synergistically promote transcription of a reporter gene regulated by the FGF4 Oct and Sox DNA elements (17). It is interesting to note that coregulation by Sox and POU proteins appears to be an evolutionarily conserved phenomenon. Genetic studies have shown that activity of the Sox2-related Drosophila fish-hook/Dichaete protein in embryonic segmentation and development of the midline glia of the cen-
tural nervous system, requires coexpression of a POU factor (42, 43). Moreover, flies harboring mutations of both the fish and POU domain ventral veinless genes exhibit a far more pronounced phenotype on development of the neural cord than flies containing mutation of either individual gene, consistent with the possibility that fish and ventral veinless are transcriptional coregulators (43).

Together these studies demonstrate that the coregulation of gene expression by Sox and POU domain proteins is a common cellular mechanism and that specific Sox-Oct (POU) partnerships define a transcriptional “code” that, along with instructions intrinsic to the DNA target sequence, determine transcriptional activation. Given the conservation and generality of the usage of Sox and POU protein complexes in gene transcription, we deem it likely that at least some of the features that we have defined as fundamental to activation by the Oct-3* complex on the FGF-4 enhancer, i.e. stereospecific complex assembly and mediation of activation domains by the DNA-binding domains, will also be applicable to understanding the general mechanisms underlying gene activation by other combinations of Sox and POU proteins.

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