

Impact of Protein/Protein Interactions on Global Intermolecular Translocation Rates of the Transcription Factors Sox2 and Oct1 between DNA Cognate Sites Analyzed by *z*-Exchange NMR Spectroscopy*

Received for publication, May 17, 2012, and in revised form, June 18, 2012 Published, JBC Papers in Press, June 20, 2012, DOI 10.1074/jbc.M112.382960

Yuki Takayama¹ and G. Marius Clore²

From the Laboratory of Chemical Physics, NIDDK, National Institutes of Health, Bethesda, Maryland 20892-0520

Background: Sox2 and Oct1 interact on a variety of promoters to regulate transcription.

Results: Global intermolecular translocation rates in a ternary Sox2·Oct1·*FGF4*-DNA complex have been analyzed by *z*-exchange spectroscopy.

Conclusion: Translocation is modulated by protein/protein interactions on the DNA.

Significance: The data suggest a model for the sequence of binding events involved in combinatorial control of gene regulation by Sox2 and Oct1.

Oct1 and Sox2 synergistically regulate developmental genes by binding to adjacent sites within promoters. We have investigated the kinetics of global intermolecular translocation of Sox2 and Oct1 between cognate sites located on different DNA molecules by *z*-exchange NMR spectroscopy. In the *Hoxb1* promoter, the Sox2 and Oct1 sites are immediately adjacent to one another, and the intermolecular translocation rates are too slow to be measured by *z*-exchange spectroscopy. By introducing a 3-bp insertion between the Sox2 and Oct1 sites to mimic the spacing in the *FGF4* enhancer, the interprotein contact surface is reduced, and the translocation rates are increased. Interaction between Sox2 and the POU-specific domain (POU_S) of Oct1 does not affect the translocation mechanism but modulates the rates. Translocation involves only jumping (dissociation and reassociation) for Sox2, but both jumping and direct intersegment transfer (no dissociation into free solution) for Oct1. The dissociation ($k_{\text{off}} \sim 1.5 \text{ s}^{-1}$) and association ($k_{\text{on}} \sim 5.1 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) rate constants for Sox2 are reduced 4-fold and increased 5-fold, respectively, in the presence of Oct1. $k_{\text{off}} \sim 3.5 \text{ s}^{-1}$ for Oct1 is unaffected by Sox2, whereas $k_{\text{on}} \sim 1.3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ is increased ~ 13 -fold. The direct intermolecular translocation rate ($k_{\text{inter}} \sim 1.8 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) for the POU_S domain of Oct1 is reduced 2-fold by Sox2, whereas that for the POU homeodomain (POU_{HD}) of Oct1 ($k_{\text{inter}} \sim 1.7 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$) remains unaltered, consistent with the absence of contacts between Sox2 and POU_{HD}. The data suggest a model for the sequence of binding events involved in synergistic gene regulation by Sox2 and Oct1.

In eukaryotes, combinatorial control of gene expression involves the formation of multi-transcription factor complexes that effectively integrate a wide range of signaling pathways to provide temporal and cell-specific transcription regulation (1). An example of this phenomenon is provided by members of the Sox and Oct transcription factor families that interact with a variety of DNA promoter/enhancer elements to regulate transcription during embryogenesis and neural development (2, 3). Sox2 is a member of the HMG box family of architectural factors that bind to the minor groove of DNA and bend the DNA by 50–90° (4). Oct1 comprises two major groove DNA binding domains, a POU-specific domain (POU_S)³ and a homeodomain (POU_{HD}), connected by a flexible linker (5, 6). Structures of ternary complexes of Sox2 and Oct1 bound to regulatory elements within the *Hoxb1* promoter (7) and fibroblast growth factor-4 (*FGF4*) enhancer (8), differing in the spacing between the Sox2 and Oct1 binding sites, have also been solved by NMR and crystallography, respectively. Although three-dimensional structures of these binary and ternary protein-DNA complexes have yielded a wealth of static information regarding the structural basis of protein-DNA recognition by Sox2 and Oct1, less is known of the mechanisms whereby these transcription factors locate their specific target sites within an overwhelming sea of nonspecific DNA (9–11), especially within the context of multi-transcription factor complexes.

Recently, we have made use of NMR paramagnetic relaxation enhancement measurements (12, 13) to detect and characterize transient sparsely populated, spectroscopically invisible states of protein-DNA complexes that are critical to the target search process (14–19). In the context of a specific complex, these intermediate states, which occupy nonspecific DNA sites and have lifetimes of less than 250–500 μs , are populated at less than 0.5% and are involved in both one-dimensional rotation-coupled sliding along the DNA and direct intersegment transfer from one DNA molecule to another. This methodology has

* This work was supported, in whole or in part, by the intramural program of NIDDK, National Institutes of Health and by the AIDS Targeted Antiviral Program of the Office of the Director of the National Institutes of Health (to G. M. C.).

¹ Recipient of a Japan Society for the Promotion of Science Research Fellowship for Japanese Biomedical and Behavioral Researchers at the National Institutes of Health.

² To whom correspondence should be addressed: Laboratory of Chemical Physics, Bldg. 5, NIDDK, National Institutes of Health, Bethesda, MD 20892-0520. Tel.: 301-496-0782; Fax: 301-496-0782. E-mail: mariusc@intr.niddk.nih.gov.

³ The abbreviations used are: POU_S, POU-specific domain; POU_{HD}, homeodomain; TROSY, transverse relaxation optimized spectroscopy.

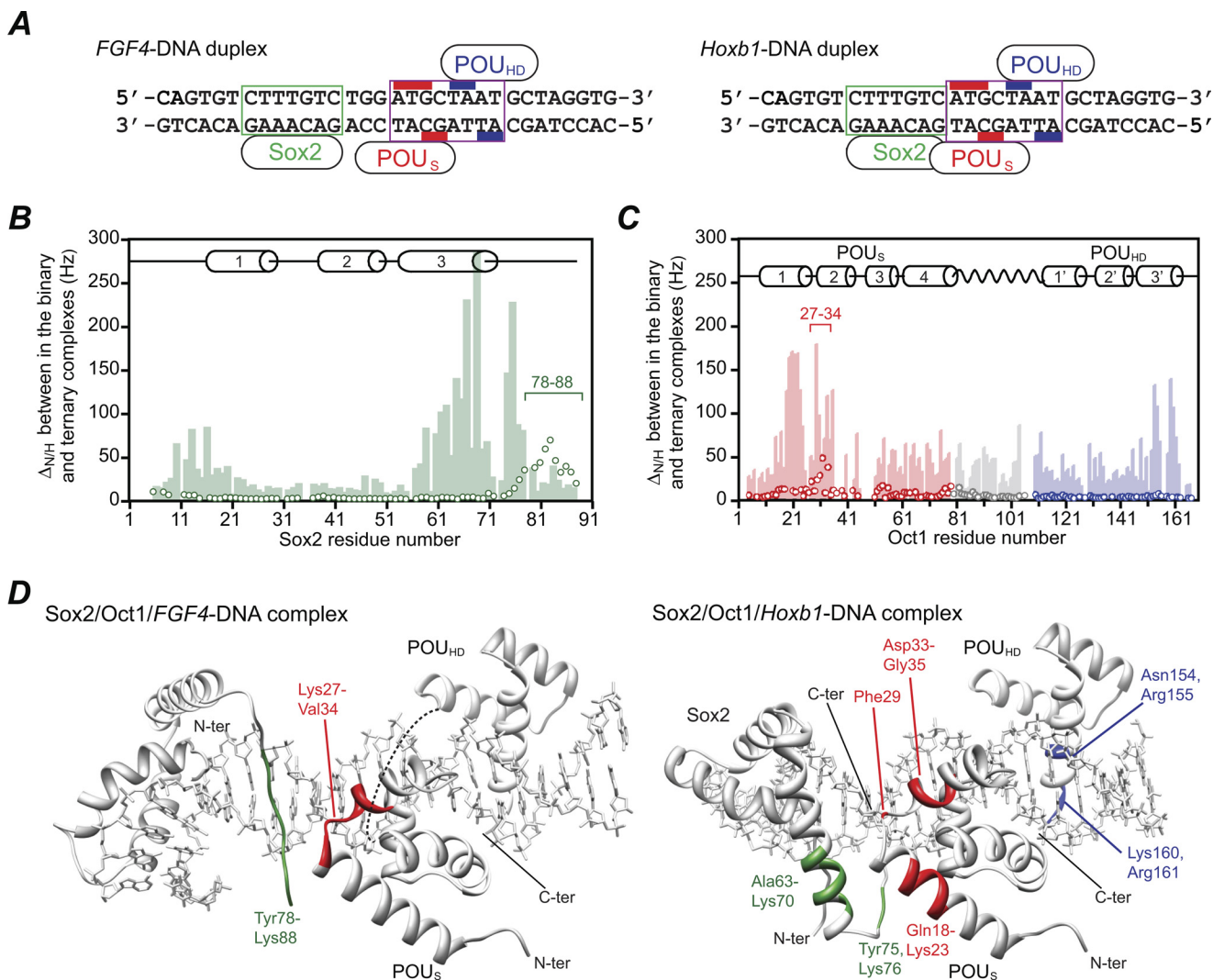


FIGURE 1. Comparison of chemical shift mapping of the protein/protein interaction surfaces at the Sox2 and Oct1 interfaces in the Sox2-Oct1-FGF4-DNA and Sox2-Oct1-Hoxb1-DNA ternary complexes. *A*, FGF4 (left) and Hoxb1 (right) DNA duplexes. The Sox2 and Oct1 binding sites are delineated by the boxes in green and purple, respectively. The bases that interact with the POU_s and POU_{HD} domains of Oct1 are indicated by the red and blue bars, respectively. The Hoxb1-DNA duplex represents the actual sequence from the Hoxb1 promoter (18). The FGF4-DNA duplex is not the actual sequence present in the FGF4 enhancer but simply represents the Hoxb1 sequence with the 3-bp insertion between the Sox2 and Oct1 sites from the FGF4 enhancer element. As explained under "Results", this was done to ensure that differences in equilibrium and kinetic rate constants reflect only the different spacing of the Sox2 and Oct1 sites. *B* and *C*, profiles of backbone $^1\text{H}/^{15}\text{N}$ chemical shift differences ($\Delta_{\text{H}/\text{N}}$) between the ternary and binary complexes on the FGF4-DNA (open circles) and Hoxb1-DNA (light bar) duplexes for Sox2 (green) (*B*) and Oct1 (red, POU_s domain; blue, POU_{HD} domain; gray, linker) (*C*). $\Delta_{\text{H}/\text{N}}$ is calculated from $((\Delta_{\text{H}})^2 + (\Delta_{\text{N}})^2)^{1/2}$ in Hz at a ^1H frequency of 600 MHz. *D*, residues showing significant $^1\text{H}/^{15}\text{N}$ chemical shift perturbations mapped onto the structures of the Sox2-Oct1-FGF4-DNA (left, $\Delta_{\text{H}/\text{N}} > 20$ Hz, Protein Data Bank (PDB) code 1gt0 (8)) and Sox2-Oct1-Hoxb1-DNA (right, $\Delta_{\text{H}/\text{N}} > 100$ Hz, PDB code 1o4x (7)) ternary complexes. C-ter, C terminus; N-ter, N terminus.

been used to study target searching by the homeodomain transcription factor HoxD9 (15, 16), the bidomain transcription factor Oct1 (17), the minor groove binding architectural factor Sox2 (18), and a ternary complex of Oct1 and Sox2 bound to the regulatory element within the Hoxb1 promoter (18).

In addition to rapid translocation events involving sparsely populated states, global intermolecular translocation of the major spectroscopically visible species between specific sites on different DNA molecules occurs on a much slower time scale (10 ms to 1 s) and can be directly observed and kinetically analyzed using two-dimensional z -exchange NMR spectroscopy (18–21). In the case of HoxD9, global intermolecular translocation occurs exclusively by direct intersegment transfer without necessitating dissociation of the protein into free solution (20). For Sox2, on the other hand, global intermolecular trans-

location between specific DNA sites proceeds entirely by jumping, a process that entails complete dissociation of Sox2 from the DNA into free solution followed by reassociation (18). Direct intersegment transfer and, to a lesser extent, jumping occur with Oct1 (21). When Oct1 and Sox2 form a ternary complex on the Hoxb1 promoter, the translocation rates between specific DNA sites are reduced by over an order of magnitude and can no longer be studied by z -exchange spectroscopy (18).

To study the mechanism and kinetics of global intermolecular translocation of the protein components within an Oct1·Sox2·DNA ternary complex, we therefore chose to focus on the FGF4 enhancer, where the spacing between the Oct1 and Sox2 recognition sites is increased by 3 bp (22) relative to that within the Hoxb1 regulatory element (23) (see Fig. 1A). The

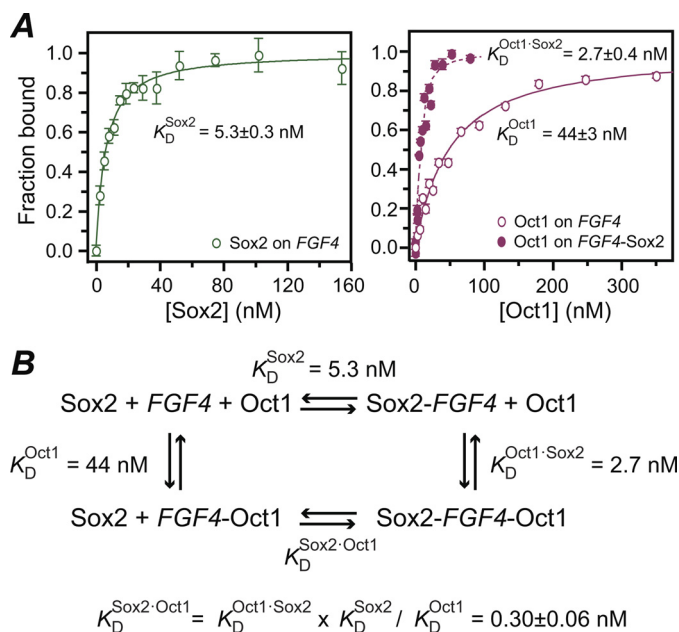


FIGURE 2. Specific binding of Sox2 to the Oct1·FGF4-DNA binary complex monitored by fluorescence anisotropy. A, titration of Sox2 into FGF4-DNA (left panel) and Oct1 into FGF4-DNA (right panel, open circles) and the Sox2·FGF4-DNA complex (right panel, filled circles). The rhodamine fluorescent label was conjugated to the 5' end of the bottom strand of the 32-bp FGF4-DNA duplex, and the excitation and emission wavelengths were set to 550 and 580 nm, respectively. The concentration of the FGF4-DNA is 1.5 nM for the Sox2 titration and 10 nM for the Oct1 titration (in the absence and presence of 100 nM Sox2). The temperature is 30 °C, and the buffer conditions are 150 mM NaCl, 10 mM PIPES, pH 6.5, 94% H₂O, 6% D₂O, identical to those used in the NMR z-exchange experiments. The experimental data points (error bars, ± 1 S.D.) are displayed as circles (open for the binary complexes and filled for the ternary complex), and the best-fit curve are displayed as solid lines (binary complexes) or dashed lines (ternary complex). B, the equilibrium dissociation constant for Sox2 in the presence of Oct1 was calculated using a thermodynamic cycle, and the three experimental K_D values shown.

interaction surface between Oct1 and Sox2 in the ternary complex on the *FGF4* enhancer (8) is altered and reduced relative to that on *Hoxb1* (7), and as a result, the strength of the interaction between the two proteins is weakened, and the rate of translocation is increased sufficiently to permit the application of z-exchange spectroscopy. Here we show how protein/protein interactions between Oct1 and Sox2 on the *FGF4* promoter modulate the kinetics of global intermolecular translocation.

EXPERIMENTAL PROCEDURES

Sample Preparation—The POU region (POU_S + POU_{HD}) of human Oct1 (residues 280–442) and the HMG box domain of Sox2 (residues 38–121) were expressed and purified as described previously (7, 18). Uniform ²H/¹⁵N isotopic labeling was achieved by growing *Escherichia coli* BL21-Codon-Plus(DE3)-RIPL cells in minimal medium with 99.9% D₂O, d₇-glucose and ¹⁵NH₄Cl. Single-stranded unmodified and rhodamine-conjugated DNA oligonucleotides were purchased from Invitrogen and Midland Certified Reagents, respectively, and purified by anion-exchange chromatography on a Mono-Q (GE Healthcare) column with NaCl gradient in a buffer of 50 mM Tris-HCl, pH 7.5, and 1 mM EDTA. After annealing, DNA duplexes were further purified by anion-exchange chromatography to remove any residual single-stranded DNA (24). Fluorescence anisotropy and NMR samples were prepared in 10 mM PIPES, 150 mM NaCl, 94% H₂O, 6% D₂O, pH 6.5.

Fluorescence anisotropy and NMR samples were prepared in 10 mM PIPES, 150 mM NaCl, 94% H₂O, 6% D₂O, pH 6.5.

Fluorescence Anisotropy—The K_D for the binding of Sox2 and Oct1 to FGF4-DNA and of Oct1 to the Sox2·FGF4-DNA complex at 30 °C was determined by fluorescence anisotropy using a Jobin-Yvon FluoroMax-3 spectrometer as described previously (16). The wavelengths for excitation and emission were 550 and 580 nm, respectively. Sox2 (0–154 nM) and Oct1 (0–351 nM) were added to 1.5 and 10 nM rhodamine-conjugated 32-bp FGF4-DNA duplex, respectively. The K_D was calculated from the titration data as described previously (14). Because the difference in fluorescence anisotropy for the binding of Sox2 to the Oct1·FGF4-DNA complex is too small to permit an accurate K_D determination, the K_D for Oct1 binding to FGF4-DNA (10 nM) in the presence of Sox2 (100 nM) was measured. Under these conditions, ~95% of the DNA is present as a specific Oct1 complex, and <3% is present as a nonspecific complex. The effect of Oct1 on the K_D for Sox2 binding was then determined from a thermodynamic cycle.

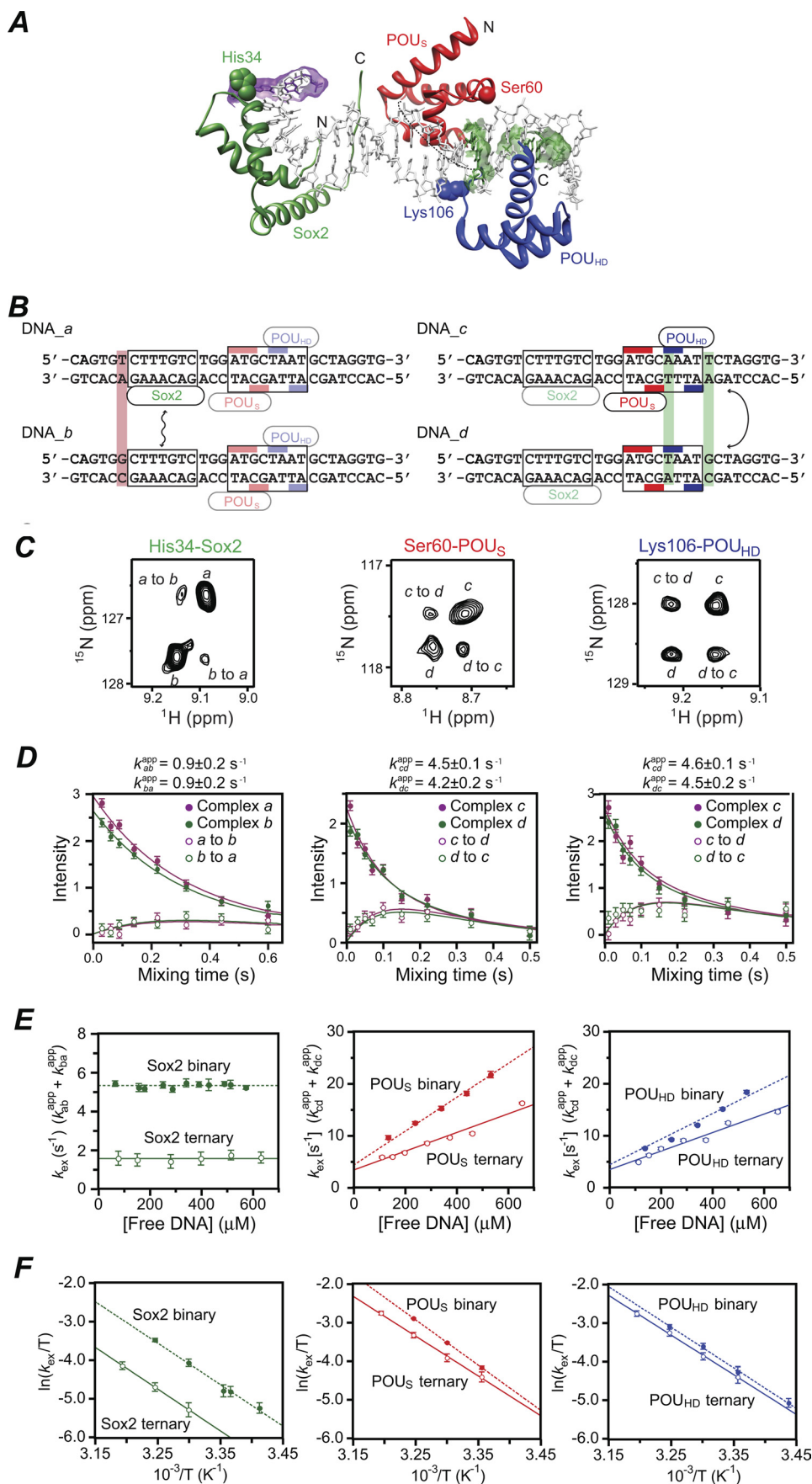
NMR Spectroscopy—All NMR experiments were carried out at 303 K on Bruker 600-MHz spectrometers equipped with z-gradient triple resonance cryoprobes. Spectra were processed using NMRPipe (25) and analyzed using the program NMRView (26).

Exchange rates were measured using transverse relaxation optimized spectroscopy (TROSY)-based z-exchange (27) with at least eight different mixing times between 20 and 600 ms. Fitting the time dependence of the exchange and auto peaks to derive kinetic rate constants was as described in Refs. 20 and 21.

RESULTS AND DISCUSSION

Interaction of Sox2 and Oct1 on the FGF4 Promoter—In the *Hoxb1*-DNA promoter, the Sox2 and Oct1 binding sites are immediately adjacent to one another (7, 23), whereas there is a 3-bp insertion between the Sox2 and Oct1 cognate sites in the *FGF4* enhancer (8, 22). The sequence of the 32 bp FGF4-like DNA duplex (hereafter referred to as FGF4-DNA) containing the specific binding sites for Sox2 and Oct1 is shown in Fig. 1A (left). This sequence does not represent the actual sequence within the *FGF4* enhancer, but rather simply adds the 3-bp insertion (TGG) between the Sox2 and Oct1 binding sites found in the *FGF4* enhancer to the *Hoxb1* promoter sequence (Fig. 1A, right). The sequences of the Sox2- and Oct1-specific sites as well as the sequences on the 5' end of the Sox2 and 3' end of the Oct1 binding sites are thus identical to the *Hoxb1* promoter sequence. This ensures that differences in equilibrium dissociation constants and rates of intermolecular translocation between the *FGF4* enhancer and *Hoxb1* promoter DNA duplexes reflect only the impact of the 3-bp insertion between the Sox2 and Oct1 binding sites.

The different spacing of the Sox2- and Oct1-specific sites on the FGF4-DNA and *Hoxb1*-DNA duplexes alters the relative orientations of the two proteins and the protein/protein interface in the two ternary complexes (7, 8). In the *Hoxb1* ternary complex, the protein/protein interface is formed between residues Lys-59 to Lys-73 (helix 3) of Sox2 and residues Lys-14 to Thr-26 (helix 1) of the POU_S domain of Oct1 (7). The protein/protein interface on the *FGF4* enhancer, on the other hand,



involves only two residues from Sox2 (Arg-81 and Arg-82) and four residues from the POU_S domain (Ile-25, Gly-28, Thr-30, and Asp-33) (8). This is in complete agreement with the location and breadth of the corresponding ¹H_N/¹⁵N chemical shift perturbation profiles observed for the two ternary complexes relative to the binary complexes (Fig. 1, B–D). Moreover, the buried accessible surface at the Sox2/POU_S interface on the *FGF4* enhancer (~240 Å²) is approximately half that on the *Hoxb1* promoter (~540 Å²) (7, 8), which would predict larger dissociation rate constants for Sox2 and Oct1 in the *FGF4*-DNA ternary complex (see below).

Equilibrium binding of Sox2 and Oct1 to the *FGF4*-DNA was studied by fluorescence anisotropy. The equilibrium dissociation constants for sequence-specific binding of Sox2 (K_D^{Sox2}) and Oct1 (K_D^{Oct1}) are 5.3 ± 0.3 and 44 ± 3 nM, respectively, at 150 mM NaCl, 10 mM PIPES, pH 6.5, and 30 °C (corresponding exactly to the buffer and temperature conditions used in the NMR experiments) (Fig. 2A). The presence of Sox2 bound to the *FGF4*-DNA duplex increases the sequence-specific affinity of Oct1 ~15-fold; the equilibrium dissociation constant for sequence-specific binding of Oct1 to the Sox2-*FGF4*-DNA binary complex ($K_D^{\text{Oct1-Sox2}}$) determined by fluorescence anisotropy is 2.7 ± 0.4 nM (Fig. 2A, right panel, filled circles). Based on the thermodynamic cycle for the binding of Sox2 and Oct1 to DNA (Fig. 2B), the equilibrium dissociation constant for specific DNA binding of Sox2 in the presence of Oct1 ($K_D^{\text{Sox2-Oct1}}$) is calculated to be 0.3 ± 0.1 nM (Fig. 2B). By way of comparison, the increase in affinity afforded by protein/protein interactions within the ternary complex on the *Hoxb1* promoter is ~20-fold under slightly different experimental conditions (25 °C, 150 mM NaCl, 10 mM phosphate buffer) (18, 21).

Global Intermolecular Translocation of Sox2 and Oct1 on *FGF4*-DNA—To measure the rate of intermolecular translocation of Sox2 and Oct1 between cognate sites located on different DNA duplexes, we used a similar experimental design to that described in Refs. 18, 20, and 21. Single base pair mutations (Fig. 3A) were introduced in the *FGF4*-DNA duplex (DNA_a) adjacent to the recognition sites for Sox2 (DNA_b) and the POU_{HD} (DNA_c) and POU_S (DNA_d) domains of Oct1 (Fig.

3B). These single point mutations have a minimal effect on the binding affinity of Sox2 or Oct1 (18, 21), but perturb the ¹H_N/¹⁵N chemical shifts for a few backbone amide groups of Sox2 or Oct1 in the ternary complexes with DNA_a and DNA_b or DNA_c and DNA_d (referred to hereafter as complexes *a* and *b* or *c* and *d*, respectively, Fig. 3C), thereby allowing exchange rates to be measured by monitoring the time dependence of well resolved, isolated exchange and auto cross-peaks in a ¹⁵N z-exchange experiment. In the latter experiment, exchange between ¹⁵N_z magnetizations from distinct species takes place during the mixing time (which follows the ¹⁵N evolution period used to label ¹⁵N chemical shifts), giving rise to exchange cross-peaks in a two-dimensional ¹H-¹⁵N correlation spectrum (28–30). Exchange rates are obtained by simultaneously fitting the time dependence of the intensities of the exchange and auto cross-peaks as a function of the mixing time using the appropriate McConnell equations (31) for the time development of magnetization in a two-site system (20).

Selective observation of Sox2 or Oct1 was achieved by ²H,¹⁵N labeling of Sox2 for complexes *a* and *b* or Oct1 for complexes *c* and *d*. Because both Sox2 and Oct1 bind tightly to their cognate DNA sequences with equilibrium dissociation constants in the nanomolar range at 150 mM NaCl (Fig. 2), the ¹H-¹⁵N TROSY correlation spectrum for a 1:1 mixture of complexes *a* and *b* (signals from Sox2) or complexes *c* and *d* (signals from Oct1) contains cross-peaks arising from both ternary complexes (18, 21). The ratios of the cross-peak intensities for each complex in the ¹H-¹⁵N TROSY spectra of the 1:1 mixtures are very close to 1, indicating that the K_D values for both proteins to each DNA duplex are virtually identical. Examples of z-exchange spectra and fits to the time dependence of auto and exchange cross-peak intensities used to determine the exchange rates k_{ab}^{app} and k_{ba}^{app} for Sox2 or k_{cd}^{app} and k_{dc}^{app} for Oct1 are shown in Fig. 3 for His-34 of Sox2, and Ser-60 and Lys-106 of the POU_S and POU_{HD} domains, respectively, of Oct1.

The contributions of jumping and intersegment transfer to intermolecular translocation can be dissected from the dependence of the apparent exchange rate constants on the concentration of free DNA (20, 21). The apparent rate constant k_{AB}^{app} for

FIGURE 3. Global intermolecular translocation of Sox2 and Oct1 between Sox2-Oct1-*FGF4*-DNA complexes. A, ribbon diagram of the crystal structure of the Sox2-Oct1-*FGF4*-DNA complex (PDB code 1gt0 (8)) with Sox2 in green and the POU_S and POU_{HD} domains of Oct1 in red and blue, respectively. The purple and green meshes indicate the base pairs changed in DNA_a/DNA_b and DNA_c/DNA_d, respectively. The apparent translocation exchange rates for Sox2, POU_S, and POU_{HD} were determined by fitting the time dependence of the exchange and auto cross-peaks in the z-exchange experiment for His-34, Ser-60, and Lys-106, respectively. These three residues exhibit large enough differences in ¹H_N and ¹⁵N chemical shifts between the DNA_a and DNA_b complexes (for His-34 of Sox2) and between the DNA_c and DNA_d complexes (for Ser-60 and Lys-106 of Oct1) to permit accurate quantification of both auto and exchange cross-peak intensities. (C, C terminus; N, N terminus). B, sequences of *FGF4*-DNA duplexes employed in the z-exchange experiments. DNA_a and DNA_b differ by 1 bp (ilac box) immediately 5' of the Sox2 binding site and were used to measure Sox2 translocation rates. DNA_c and DNA_d differ by 2 bp (green boxes), one immediately 3' of the POU_S site and the other immediately 3' of the POU_{HD} site, and were used to measure Oct1 translocation rates. The Sox2 and Oct1 binding sites are delineated by boxes, and the bases that contact the POU_S and POU_{HD} domains of Oct1 are indicated by the red and blue bars, respectively. C, examples of z-exchange data for His-34 of Sox2 (left), Ser-60 of POU_S (middle), and Lys-106 of POU_{HD} (right) in the Sox2-Oct1-*FGF4*-DNA ternary complex seen in ¹H-¹⁵N TROSY-based z-exchange spectra at a mixing time of 220 ms at 30 °C and 150 mM NaCl. For the Sox2 measurements, the concentrations of ²H/¹⁵N-labeled Sox2, Oct1 (at natural isotopic abundance), DNA_a, and DNA_b are 0.55, 0.7, 0.35, and 0.35 mM, respectively. For the Oct1 measurements, the concentrations of Sox2 (at natural isotopic abundance), ²H/¹⁵N-labeled Oct1, DNA_c, and DNA_d are 0.7, 0.55, 0.35, and 0.35 mM, respectively. D, time dependence of the intensities of auto (filled circles) and exchange (open circles) cross-peaks at 30 °C and 150 mM NaCl together with the best-fit curves (solid lines). The concentrations of proteins and DNA are the same as in C. E, dependence of the apparent translocation rates for Sox2 (left), POU_S (middle), and POU_{HD} (right) in the ternary complexes (open circles) on the concentration of free DNA at 30 °C and 150 mM NaCl. Also shown are the apparent translocation rates for the corresponding binary complexes (solid circles). The protein concentrations are the same as in C for the ternary complexes; for the binary complexes, the protein at natural isotopic abundance was omitted from the sample. The concentration of free DNA represents the sum of the concentrations of the two DNA duplexes present at a 1:1 ratio in the samples. Association, dissociation, and direct intersegment transfer rate constants derived from the data are summarized in Table 1. F, Eyring plots of the apparent translocation rates at 18, 20, 24, 25, 30, 35, and 40 °C for Sox2 (left), POU_S (middle), and POU_{HD} (right) in ternary (open circles) and binary (filled circles) complexes. The protein and DNA concentrations are the same as in C. Linear fits of the apparent translocation rates for the ternary and binary complexes are shown as solid and dashed lines, respectively. Activation enthalpies, entropies, and free energies derived from the data are given in Table 2. Error bars, ± 1 S.D.

transfer of a protein from site A to site B located on two different DNA molecules is given by the sum of the contributions from jumping and direct intersegment transfer (and similarly for the transfer from sites B to A). With DNA in excess over protein and $k_{\text{off}} \ll k_{\text{on}}[\text{DNA}_{\text{free}}]$, the rate-limiting step for jumping is governed by the dissociation rate constant (k_{off}). The jumping rate from A to B is therefore independent of the concentration of free DNA and is given by $k_{\text{off}}^A/2$, where k_{off}^A is the dissociation rate constant from site A (and the statistical factor of 2 arises from the fact that transfer of the protein between DNA molecules of the same sequence is of equal probability to transfer between DNA molecules of differing sequence). The direct intersegment transfer rate from A to B, on the other hand, is linearly dependent on the free concentration of the DNA containing site B and is given by $k_{\text{AB}}^{\text{inter}}[\text{DNA}_{\text{B}}^{\text{free}}]$ where $k_{\text{AB}}^{\text{inter}}$ is the second order rate constant for direct intersegment transfer from A to B.

$k_{\text{ex}}^{\text{Sox2}} (= k_{\text{ab}}^{\text{app}} + k_{\text{ba}}^{\text{app}})$, and $k_{\text{ex}}^{\text{POU}_S}$ and $k_{\text{ex}}^{\text{POU}_{\text{HD}}} (= k_{\text{cd}}^{\text{app}} + k_{\text{dc}}^{\text{app}}$ for resonances of POU_S and POU_{HD} , respectively) are plotted as a function of free DNA concentration in Fig. 3E. In the context of the *FGF4*-DNA ternary complex, the presence of Oct1 decreases the translocation rates for Sox2 and *vice versa*. Although the exchange rates are slower in the ternary complex than those in the binary complexes, the mechanism of translocation is unaffected by the presence of protein interactions on the DNA. $k_{\text{ex}}^{\text{Sox2}}$ is independent of the concentration of free DNA indicative of an exclusive jumping mechanism (Fig. 3E, *left panel*). $k_{\text{ex}}^{\text{POU}_S}$ (Fig. 3E, *middle panel*) and $k_{\text{ex}}^{\text{POU}_{\text{HD}}}$ (Fig. 3E, *right panel*), on the other hand, are linearly dependent on the concentration of free DNA with a measurable intercept at zero free

DNA concentration, indicative of the presence of both direct intersegment transfer and jumping mechanisms.

At 30 °C, $k_{\text{ex}}^{\text{Sox2}}$ is reduced from $5.3 \pm 0.2 \text{ s}^{-1}$ in the binary complex (18) to $1.5 \pm 0.3 \text{ s}^{-1}$ in the ternary complex (Fig. 3E, *left panel*). $k_{\text{ex}}^{\text{Sox2}}$ is equal to the average dissociation rate constant ($k_{\text{off}}^{\text{Sox2}}$) because $k_{\text{off}}^a = 2k_{\text{app}}^{ab}$ and $k_{\text{off}}^b = 2k_{\text{app}}^{ba}$ (and note that k_{off}^a and k_{off}^b in this instance are virtually identical). Given the measured equilibrium dissociation constants for the binding of Sox2 to DNA in the context of binary and ternary complexes (Fig. 2), the average association rate constants ($k_{\text{on}}^{\text{Sox2}} - \text{binary}$) and ($k_{\text{on}}^{\text{Sox2}} - \text{FGF4-ternary}$) are calculated to be $1.0 (\pm 0.1) \times 10^9$ and $5.1 (\pm 1.4) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, respectively (Table 1). Thus, the association rate constant for sequence-specific DNA binding of Sox2 is increased ~5-fold in the ternary complex relative to the binary one.

The average second order rate constants for direct intersegment transfer of the POU_S ($k_{\text{inter}}^{\text{POU}_S}$) and POU_{HD} ($k_{\text{inter}}^{\text{POU}_{\text{HD}}}$) domains of Oct1 in the ternary complex are $2.2 (\pm 0.2) \times 10^4$ and $1.7 (\pm 0.1) \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, respectively. $k_{\text{inter}}^{\text{POU}_S}$ is reduced by about 50% relative to its value in the binary complex ($3.4 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ (21)), whereas $k_{\text{inter}}^{\text{POU}_{\text{HD}}}$ remains unaltered ($1.8 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ in the binary complex (21)). These observations are in complete agreement with the structure of the ternary Sox2·Oct1·*FGF4*-DNA complex (8) because Sox2 interacts only with the POU_S domain of Oct1 (Figs. 1D and 3A). The average dissociation rate constant ($k_{\text{off}}^{\text{Oct1}}$) of Oct1 from the ternary complex is $3.5 \pm 0.4 \text{ s}^{-1}$, which is comparable with the value of $4.4 \pm 0.2 \text{ s}^{-1}$ measured for the binary complex (21). The average association rate constant ($k_{\text{on}}^{\text{Oct1}}$) for Oct1 binding to the Sox2·*FGF4*-DNA complex is $1.3 (\pm 0.2) \times 10^9 \text{ M}^{-1}\text{s}^{-1}$, which is ~13-fold larger than the value of $1.0 (\pm 0.1) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ for the binary complex (21).

The increases in the specific association rate constants for both Sox2 and Oct1 in the context of the ternary complex can probably be attributed to electrostatic enhancement of diffusion-controlled association (32, 33) afforded by charge/charge interactions between Sox2 and Oct1 when bound specifically to DNA. The ~3-fold smaller increase in the specific association rate constant for Sox2 when compared with Oct1 in the ternary complex can be rationalized as follows. First, the ordering of the C-terminal tail of Sox2 upon interaction with the POU_S domain of Oct1 in the ternary complex (8) (*cf.* Fig. 3A) entails an entropic penalty because the C-terminal tail is disordered in the binary Sox2·DNA complex (4, 18). For the POU_S and POU_{HD} domains of Oct1, ternary complex formation is not accompanied by any significant backbone conformational change, and

TABLE 1

Intermolecular translocation rates of Sox2 and Oct1 between cognate sites in binary and *FGF4* ternary complexes

Intermolecular translocation of Sox2 occurs solely by dissociation followed by reassociation (jumping), while for Oct1 both direct intersegment transfer and jumping occur.

	K_D	$\langle k_{\text{off}} \rangle$	$\langle k_{\text{on}} \rangle$	$\langle k_{\text{inter}} \rangle$
	<i>nM</i>	s^{-1}	$\text{M}^{-1}\text{s}^{-1}$	$\text{M}^{-1}\text{s}^{-1}$
Sox2				
Binary	5.3 ± 0.3	5.3 ± 0.2	$1.0 (\pm 0.1) \times 10^9$	ND ^a
<i>FGF4</i> ternary	0.3 ± 0.1	1.5 ± 0.3	$5.1 (\pm 1.4) \times 10^9$	ND ^a
Oct1				
Binary	44 ± 3	4.4 ± 0.2	$1.0 (\pm 0.1) \times 10^8$	$3.4 (\pm 0.2) \times 10^4$ (POU_S) $1.8 (\pm 0.4) \times 10^4$ (POU_{HD})
<i>FGF4</i> ternary	2.7 ± 0.4	3.5 ± 0.4	$1.3 (\pm 0.2) \times 10^9$	$2.2 (\pm 0.2) \times 10^4$ (POU_S) $1.7 (\pm 0.1) \times 10^4$ (POU_{HD})

^a Not detectable. From the absence of any concentration dependence in the apparent translocation rates for Sox2, one can conclude that translocation of Sox2 does not involve direct intersegment transfer in both binary and ternary complexes.

TABLE 2

Apparent activation enthalpies (ΔH^\ddagger), entropies (ΔS^\ddagger), and free energies (ΔG^\ddagger) for global intermolecular translocation of Sox2 and Oct1 between cognate sites in the binary and *FGF4* ternary complexes

	ΔH^\ddagger^a	ΔS^\ddagger^a	ΔG^\ddagger^b
	$\text{kcal} \times \text{mol}^{-1}$	$\text{cal} \times \text{mol}^{-1} \times \text{K}^{-1}$	$\text{kcal} \times \text{mol}^{-1}$
Sox2			
Binary/ <i>FGF4</i> ternary	$21.2 \pm 1.9/21.3 \pm 1.8$	$15.0 \pm 6.5/13.1 \pm 5.8$	$16.6 \pm 2.8/17.3 \pm 2.5$
Oct1-POU_S domain			
Binary/ <i>FGF4</i> ternary	$23.1 \pm 1.8/20.4 \pm 0.8$	$22.6 \pm 4.0/12.7 \pm 2.4$	$16.3 \pm 2.2/16.5 \pm 1.1$
Oct1-POU_{HD} domain			
Binary/ <i>FGF4</i> ternary	$20.8 \pm 1.2/20.3 \pm 0.5$	$14.7 \pm 4.1/12.7 \pm 1.6$	$16.4 \pm 1.7/16.5 \pm 0.7$

^a Values were derived by least squares fitting of the Eyring plots shown in Fig. 3F.

^b ΔG^\ddagger is calculated from $\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$ at 303 K.

Sox2/Oct1 Interaction Impact on Intermolecular Translocation

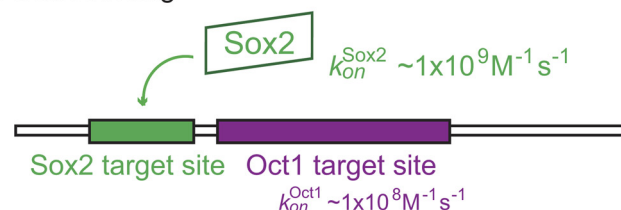
therefore does not entail any additional entropic cost. (Note that the flexible linker connecting the POU_S and POU_{HD} domains remains largely disordered when bound to DNA (5, 7, 8)). Second, in the context of the DNA duplexes employed (Fig. 3B), the number of available nonspecific sites to which Sox2 can bind and subsequently slide to its specific site is reduced by the presence of Oct1. Although nonspecific binding sites available to Oct1 are occluded by the presence of Sox2, there are still a substantial number of nonspecific DNA binding sites 3' of the Sox2 binding site (*cf.* Fig. 3, A and B) from which sliding of Oct1 can occur.

Eyring plots of the temperature dependence of the apparent translocation exchange rates (Fig. 3F) provide estimates of the activation enthalpy (ΔH^\ddagger), entropy ($T\Delta S^\ddagger$ at 30 °C), and by deduction, free energy. The activation free energies (ΔG^\ddagger) for intermolecular translocation in the ternary and binary complexes on the *FGF4* promoter are comparable (~ 16 – 17 kcal \times mol⁻¹; Table 2), indicative of similar energy barriers that are unaffected by the presence of a second protein. These data indicate that protein/protein interactions between Sox2 and Oct1 on the DNA modulate translocation rates without perturbing the activation free energies. By inference, this likely holds true for the Sox2-Oct1-*Hoxb1*-DNA ternary complex as well, where more extensive protein/protein interactions reduce the translocation rates to levels that are too slow to be measured by *z*-exchange spectroscopy.

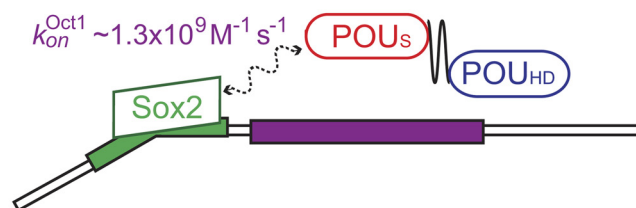
Concluding Remarks—The kinetic data on global intermolecular translocation of Oct1 and Sox2 between adjacent specific sites located on different DNA molecules presented here complement our previous work that made use of paramagnetic relaxation enhancement measurements (12, 15) to examine the interplay between these two transcription factors in translocation events involving sparsely populated (<1%), highly transient, spectroscopically invisible states (18). The latter comprise an ensemble of nonspecifically bound species in rapid exchange with the specific complex and participate both in one-dimensional sliding along the DNA (intramolecular translocation), as well as the formation of bridged intermediates spanning two DNA molecules that precedes intermolecular translocation. The events probed by paramagnetic relaxation enhancement occur on a time scale less than 250–500 μ s, although paramagnetic relaxation enhancement measurements do not afford any further characterization of the kinetics of these processes (12, 13, 15). Global (or bulk) intermolecular translocation between specific sites on different DNA molecules, on the other hand, occurs on a much slower overall time scale (0.1–1 s; *cf.* Table 1) and involves the major spectroscopically visible species (*i.e.* the specific complexes), and the rate constants from the *z*-exchange experiments pertain directly to the rate-limiting steps in this process (20, 21). The interaction of Sox2 and Oct1 on the DNA modulates the translocation mechanisms involving sparsely populated states (18), as well as the kinetics of global intermolecular translocation between specific sites as shown here. The pathways of global intermolecular translocation, however, are unaffected by the interaction between Sox2 and Oct1.

Based on the kinetic data for global intermolecular translocation presented in this study, we propose the following model

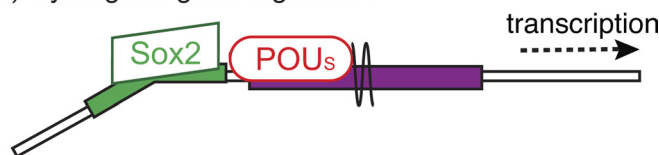
(1) Sox2 binding



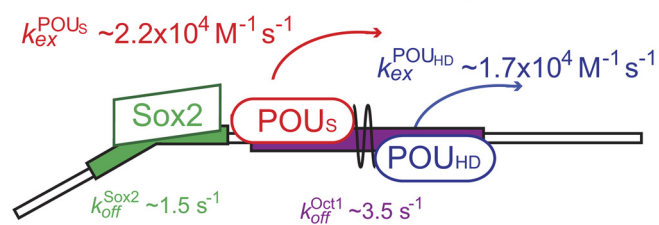
(2) Oct1 binding (interaction with Sox2)



(3) Synergistic gene regulation



(4) Oct1 dissociation via direct intersegment transfer



(5) Sox2 dissociation into free solution

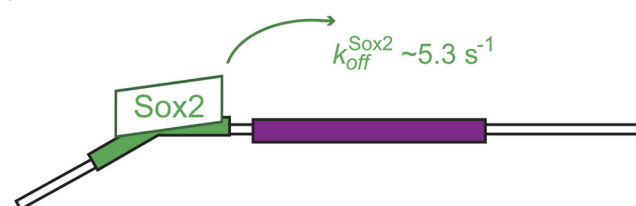


FIGURE 4. Model for the sequence of binding, intersegment transfer, and dissociation events involved in synergistic transcription regulation by Sox2 and Oct1. The initial event (step 1) involves the binding of Sox2 to its specific DNA target site followed by binding of Oct1 to form a specific ternary complex (step 2). The latter binding event is accelerated by the presence of specifically bound Sox2. Once formation of the ternary Sox2-Oct1-DNA complex has occurred on the promoter or enhancer, transcription of the relevant gene is activated (step 3). Subsequently, Oct1 dissociates from the DNA largely by direct intersegment transfer to another DNA site (step 4). Finally, Sox2 dissociates from its specific site into free solution (step 5). (See "Results" for more details.)

for the sequence of binding, intersegment transfer and dissociation events involved in combinatorial control of gene regulation by Sox2 and Oct1 (Fig. 4). The initial step involves the binding of Sox2 to its specific DNA target site. This is supported by the observation of a 10-fold larger association rate constant for the formation of the binary Sox2-DNA complex *versus* the

Oct1·DNA complex (Table 1), as well as the fact that translocation of Sox2 is a slow process involving only full dissociation (*i.e.* jumping), whereas Oct1 can undergo rapid global intermolecular translocation at the high DNA concentrations present *in vivo* (~150 mM on a base pair basis). In addition, Sox2 is localized in the cell nucleus (34), whereas Oct1, which is widely expressed in both adult and embryonic tissues (35, 36), is found in both the nucleus and the cytoplasm (37). The presence of Sox2 bound to its specific site on the promoter accelerates the binding of Oct1 by ~13-fold to a target site adjacent to the Sox2 site, and the ternary complex is further stabilized by protein/protein interactions, predominantly electrostatic in nature, between Sox2 and Oct1. Once the specific Sox2·Oct1·DNA ternary complex is formed, Oct1 and Sox2 activate transcription synergistically. Oct1 subsequently dissociates from the ternary complex largely via direct intersegment transfer, which, at the high DNA concentrations present *in vivo* will be significantly faster than dissociation into free solution. Intersegment transfer can occur to another specific site on a different promoter or simply to a nonspecific site located either on a different DNA molecule or, if on the same DNA, at a widely separated (>150 bp) location through DNA bridging. Finally, Sox2 dissociates from its specific DNA site slowly, and subsequent DNA binding of Sox2 can only occur via a second order reassociation event either to another specific site or to nonspecific sites.

Acknowledgments—We thank Drs. Garrett, Baber, and Ying for technical support.

REFERENCES

1. Wolberger, C. (1999) Multiprotein·DNA complexes in transcriptional regulation. *Annu. Rev. Biophys. Biomol. Struct.* **28**, 29–56
2. Kamachi, Y., Uchikawa, M., and Kondoh, H. (2000) Pairing SOX off: with partners in the regulation of embryonic development. *Trends Genet.* **16**, 182–187
3. Dailey, L., and Basilio, C. (2001) Coevolution of HMG domains and homeodomains and the generation of transcriptional regulation by Sox/POU complexes. *J. Cell Physiol.* **186**, 315–328
4. Murphy, E. C., Zhurkin, V. B., Louis, J. M., Cornilescu, G., and Clore, G. M. (2001) Structural basis for SRY-dependent 46-X,Y sex reversal: modulation of DNA bending by a naturally occurring point mutation. *J. Mol. Biol.* **312**, 481–499
5. Klemm, J. D., Rould, M. A., Aurora, R., Herr, W., and Pabo, C. O. (1994) Crystal structure of the Oct1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. *Cell* **77**, 21–32
6. Reményi, A., Tomilin, A., Pohl, E., Lins, K., Philippsen, A., Reinbold, R., Schöler, H. R., and Wilmanns, M. (2001) Differential dimer activities of the transcription factor Oct1 by DNA-induced interface swapping. *Mol. Cell* **8**, 569–580
7. Williams, D. C., Jr., Cai, M., and Clore, G. M. (2004) Molecular basis for synergistic transcriptional activation by Oct1 and Sox2 revealed from the solution structure of the 42-kDa Oct1·Sox2·Hoxb1-DNA ternary transcription factor complex. *J. Biol. Chem.* **279**, 1449–1457
8. Reményi, A., Lins, K., Nissen, L. J., Reinbold, R., Schöler, H. R., and Wilmanns, M. (2003) Crystal structure of a POU·HMG·DNA ternary complex suggests differential assembly of Oct4 and Sox2 on two enhancers. *Genes Dev.* **17**, 2048–2059
9. Berg, O. G., and von Hippel, P. H. (1985) Diffusion-controlled macromolecular interactions. *Annu. Rev. Biophys. Biomol. Struct.* **14**, 131–160
10. von Hippel, P. H., and Berg, O. G. (1989) Facilitated target location in biological systems. *J. Biol. Chem.* **264**, 675–678
11. Halford, S. E., and Marko, J. F. (2004) How do site-specific DNA-binding proteins find their targets? *Nucleic Acids Res.* **32**, 3040–3052
12. Clore, G. M., and Iwahara, J. (2009) Theory, practice, and applications of paramagnetic relaxation enhancement for the characterization of transient low population states of biological macromolecules and their complexes. *Chem. Rev.* **109**, 4108–4139
13. Clore, G. M. (2011) Exploring sparsely populated states of macromolecules by diamagnetic and paramagnetic NMR relaxation. *Protein Sci.* **20**, 229–246
14. Iwahara, J., Schwieters, C. D., and Clore, G. M. (2004) Characterization of nonspecific protein/DNA interactions by ¹H paramagnetic relaxation enhancement. *J. Am. Chem. Soc.* **126**, 12800–12808
15. Iwahara, J., and Clore, G. M. (2006) Detecting transient intermediates in macromolecular binding by paramagnetic NMR. *Nature* **440**, 1227–1230
16. Iwahara, J., Zweckstetter, M., and Clore, G. M. (2006) NMR structural and kinetic characterization of a homeodomain diffusing and hopping on non-specific DNA. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 15062–15067
17. Takayama, Y., and Clore, G. M. (2011) Intra- and intermolecular translocation of the bidomain transcription factor Oct1 characterized by liquid crystal and paramagnetic NMR. *Proc. Natl. Acad. Sci. U.S.A.* **108**, E169–E176
18. Takayama, Y., and Clore, G. M. (2012) Interplay between minor and major groove-binding transcription factors Sox2 and Oct1 in translocation on DNA studied by paramagnetic and diamagnetic NMR. *J. Biol. Chem.* **287**, 14349–14363
19. Clore, G. M. (2011) Exploring translocation of proteins on DNA by NMR. *J. Biomol. NMR* **51**, 209–219
20. Iwahara, J., and Clore, G. M. (2006) Direct observation of enhanced translocation of a homeodomain between DNA cognate sites by NMR exchange spectroscopy. *J. Am. Chem. Soc.* **128**, 404–405
21. Doucleff, M., and Clore, G. M. (2008) Global jumping and domain-specific intersegment transfer between DNA cognate sites of the multidomain transcription factor Oct1. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 13871–13876
22. Ambrosetti, D. C., Basilio, C., and Dailey, L. (1997) Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct3 depends on protein/protein interactions facilitated by a specific spatial arrangement of factor binding sites. *Mol. Cell Biol.* **17**, 6321–6329
23. Di Rocco, G., Gavalas, A., Popperl, H., Krumlauf, R., Mavilio, F., and Zappavigna, V. (2001) The recruitment of SOX·OCT complexes and the differential activity of HOXA1 and HOXB1 modulate the Hoxb1 auto-regulatory enhancer function. *J. Biol. Chem.* **276**, 20506–20515
24. Iwahara, J., Anderson, D. E., Murphy, E. C., and Clore, G. M. (2003) EDTA-derivatized deoxythymidine as a tool for rapid determination of protein binding polarity to DNA by intermolecular paramagnetic relaxation enhancement. *J. Am. Chem. Soc.* **125**, 6634–6635
25. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **6**, 277–293
26. Johnson, B. A., and Blevins, R. A. (1994) NMR View: A computer program for the visualization and analysis of NMR data. *J. Biomol. NMR* **4**, 603–614
27. Sahu, D., Clore, G. M., and Iwahara, J. (2007) TROSY-based z-exchange spectroscopy: application to the determination of the activation energy for intermolecular protein translocation between specific sites on different DNA molecules. *J. Am. Chem. Soc.* **129**, 13232–13237
28. Montelione, G. T., and Wagner, G. (1989) Two-dimensional chemical exchange NMR spectroscopy by proton-detected heteronuclear correlation. *J. Am. Chem. Soc.* **111**, 3096–3098
29. Perrin, C. L., and Dwyer, T. J. (1990) Application of two-dimensional NMR to kinetics of chemical exchange. *Chem. Rev.* **90**, 935–967
30. Farrow, N. A., Zhang, O., Forman-Kay, J. D., and Kay, L. E. (1994) A heteronuclear correlation experiment for simultaneous determination of ¹⁵N longitudinal decay and chemical exchange rates of systems in slow equilibrium. *J. Biomol. NMR* **4**, 727–734
31. McConnell, H. M. (1958) Reaction rates by nuclear magnetic resonance. *J. Chem. Phys.* **28**, 430–431
32. Vijayakumar, M., Wong, K. Y., Schreiber, G., Fersht, A. R., Szabo, A., and Zhou, H. X. (1998) Electrostatic enhancement of diffusion-controlled protein/protein association: comparison of theory and experiment on barnase and barstar. *J. Mol. Biol.* **278**, 1015–1024

Sox2/Oct1 Interaction Impact on Intermolecular Translocation

33. Zhou, H. X., and Szabo, A. (2004) Enhancement of association rates by nonspecific binding to DNA and cell membranes. *Phys. Rev. Lett.* **93**, 178101
34. Zhang, J., Chang, D. Y., Mercado-Urbe, I., and Liu, J. (March 9, 2012) Sex-determining region Y-box 2 expression predicts poor prognosis in human ovarian carcinoma. *Hum. Pathol.* 10.1016/j.humpath.2011.10.016
35. Kang, J., Shakya, A., and Tantin, D. (2009) Stem cells, stress, metabolism, and cancer: a drama in two Octs. *Trends Biochem. Sci.* **34**, 491–499
36. Herr, W., Sturm, R. A., Clerc, R. G., Corcoran, L. M., Baltimore, D., Sharp, P. A., Ingraham, H. A., Rosenfeld, M. G., Finney, M., Ruvkun, G., et al. (1988) The POU domain: a large conserved region in the mammalian *pit-1*, *oct-1*, *oct-2*, and *Caenorhabditis elegans unc-86* gene products. *Genes Dev.* **2**, 1513–1516
37. Izadpanah, R., Trygg, C., Patel, B., Kriedt, C., Dufour, J., Gimble, J. M., and Bunnell, B. A. (2006) Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. *J. Cell Biochem.* **99**, 1285–1297