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*

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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. 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 (POUS) of Oct1 does not affect the translocation mechanism but modulates the rates. Translocation involves only jumping (disassociation and reassociation) for Sox2, but both jumping and direct intersegment transfer (no dissociation into free solution) for Oct1. The dissociation ($k_{\text{off}}$, $1.5 \, \text{s}^{-1}$) and association ($k_{\text{on}}$, $5.1 \times 10^{9} \, \text{M}^{-1} \, \text{s}^{-1}$) rate constants for Sox2 are reduced 4-fold and increased 5-fold, respectively, in the presence of Oct1. $k_{\text{on}}$ for Oct1 is unaffected by Sox2, whereas $k_{\text{on}}$ ($1.3 \times 10^{9} \, \text{M}^{-1} \, \text{s}^{-1}$) is increased $\sim$13-fold. The direct intermolecular translocation rate ($k_{\text{inter}}$, $1.8 \times 10^{6} \, \text{M}^{-1} \, \text{s}^{-1}$) for the POUS domain of Oct1 is reduced 2-fold by Sox2, whereas that for the POU homeodomain (POU1Hd) of Oct1 ($k_{\text{inter}}$, $1.7 \times 10^{4} \, \text{M}^{-1} \, \text{s}^{-1}$) remains unaltered, consistent with the absence of contacts between Sox2 and POU1Hd. The data suggest a model for the sequence of binding events involved in synergistic gene regulation by Sox2 and Oct1.

**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.

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 $50–90^\circ$ (4). Oct1 comprises two major groove DNA binding domains, a POU-specific domain (POU1Hd) and a homeodomain (POU1Hd), 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 $\mu$s, 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

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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@intra.niddk.nih.gov.

3 The abbreviations used are: POU1, POU-specific domain; POU1Hd, homeodomain; TROSY, transverse relaxation optimized spectroscopy.
been used to study target searching by the homeodomain transcrip-
tion 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 <i>Hoxb1</i> 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 <i>z</i>-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 translocation between specific DNA sites proceeds entirely by jumping, a process that entails complete dissociation of Sox2 from the DNA into free solution (18). For Sox2, on the other hand, global intermolecular translocation between specific DNA sites proceeds entirely by jumping, a process that entails complete dissociation of Sox2 from the DNA into free solution (18). Direct intersegment transfer and, to a lesser extent, jumping occur with Oct1 (21). When Oct1 and Sox2 form a ternary complex on the <i>Hoxb1</i> promoter, the translocation rates between specific DNA sites are reduced by over an order of magnitude and can no longer be studied by <i>z</i>-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 <i>FGF4</i> enhancer, where the spacing between the Oct1 and Sox2 recognition sites is increased by 3 bp (22) relative to that within the <i>Hoxb1</i> regulatory element (23) (see Fig. 1A). The
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 (POUo + POU1s) 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 $^1$H/$^1$N isotopic labeling of Sox2 (residues 38–121) were expressed and purified as human Oct1 (residues 280–442) and the HMG box domain of Hoxb1-DNA complex (left 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 curves 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 KD values shown.

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 (specific binding sites for Sox2 and Oct1 are shown in 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 enhancer DNA duplexes reflect only the impact of the 3-bp insertion within 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 enhancer 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 POUh domain of Oct1 (7). The protein/protein interface on the FGF4 enhancer, on the other hand,
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A

B

DNA_a

5'-CACTGCTTCATGGGTGCTGAGTCTAGCGGCTAAGTGTGTG-3'
3'-GTCAAGGAGCGAGCTGCTTGAAGCGTTGAG AACAC-5'

DNA_b

5'-CACTGCTTCATGGGTGCTGAGTCTAGCGGCTAAGTGTGTG-3'
3'-GTCAAGGAGCGAGCTGCTTGAAGCGTTGAG AACAC-5'

DNA_c

5'-CACTGCTTCATGGGTGCTGAGTCTAGCGGCTAAGTGTGTG-3'
3'-GTCAAGGAGCGAGCTGCTTGAAGCGTTGAG AACAC-5'

DNA_d

5'-CACTGCTTCATGGGTGCTGAGTCTAGCGGCTAAGTGTGTG-3'
3'-GTCAAGGAGCGAGCTGCTTGAAGCGTTGAG AACAC-5'

C

His34-Sox2

15 N (ppm) vs 1 H (ppm)

Ser60-POU

15 N (ppm) vs 1 H (ppm)

Lys106-POU

15 N (ppm) vs 1 H (ppm)

D

k_{on}^{a-b} = 0.94 ± 0.2 s^{-1}

k_{off}^{a-b} = 0.94 ± 0.2 s^{-1}

k_{on}^{c-d} = 4.5 ± 0.1 s^{-1}

k_{off}^{c-d} = 4.2 ± 0.1 s^{-1}

k_{on}^{d-c} = 4.2 ± 0.1 s^{-1}

k_{off}^{d-c} = 4.5 ± 0.2 s^{-1}

E

Sox2 binary

k_{on}(1) + k_{off}(1)

Sox2 ternary

k_{on}(1) + k_{off}(1)

POU binary

k_{on}(2) + k_{off}(2)

POU ternary

k_{on}(2) + k_{off}(2)

POU_HD binary

k_{on}(3) + k_{off}(3)

POU_HD ternary

k_{on}(3) + k_{off}(3)

F

In(k_{on}(1)) vs 10^{-7}T (K^2)

In(k_{off}(1)) vs 10^{-7}T (K^2)

In(k_{on}(2)) vs 10^{-7}T (K^2)

In(k_{off}(2)) vs 10^{-7}T (K^2)

In(k_{on}(3)) vs 10^{-7}T (K^2)

In(k_{off}(3)) vs 10^{-7}T (K^2)
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involves only two residues from Sox2 (Arg-81 and Arg-82) and four residues from the POUc domain (Ile-25, Gly-28, Thr-30, and Asp-33) (8). This is in complete agreement with the location and breadth of the corresponding $^{1}H_{c}/^{15}N$ 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/POUc interface on the FGF4 enhancer (≈240 Å$^{2}$) is approximately half that on the Hoxb1 promoter (≈540 Å$^{2}$) (7, 8), which would predict larger dissociation rate constants for Sox2 and Oct1 in the ternary complexes (see below).

Equilibrium binding of Sox2 and Oct1 to the FGF4–DNA was studied by fluorescence anisotropy. The equilibrium dissociation constant for sequence-specific binding of Sox2 (K$^{\text{D}}_{\text{Sox2}}$) and Oct1 (K$^{\text{D}}_{\text{Oct1}}$) 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 specific DNA binding of Sox2 in the presence of Oct1 (K$^{\text{D}}_{\text{Sox2-Oct1}}$) 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$_{1}$ (DNA$_{c}$) and POU$_{2}$ (DNA$_{d}$) domains of Oct1 (Fig. 3B). These single point mutations have a minimal effect on the FGF4–DNA ternary complex seen in Table 1. The concentrations of proteins and DNA are the same as C. E, dependence of the apparent translocation rates for Sox2 (left), POU$_{1}$ (middle), and POU$_{2}$ (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 C for the ternary complexes; for the binary complexes, the protein at natural isotopic abundance, $^{1}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{c}$ are 0.55, 0.7, 0.35, and 0.35 μM, respectively. For the Oct1 measurements, the concentrations of Sox2 (natural isotopic abundance), $^{2}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{d}$ are 0.7, 0.35, and 0.35 μM, respectively. D, time dependence of the intensities of auto (filled circles) and exchange cross-peaks at 30 °C and 150 mM NaCl. For the Sox2 measurements, the concentrations of $^{2}H_{c}/^{15}N$-labeled Sox2, Oct1 (at natural isotopic abundance), $^{1}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{b}$ are 0.55, 0.7, 0.35, and 0.35 μM, respectively. For the Oct1 measurements, the concentrations of Sox2 (natural isotopic abundance), $^{2}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{b}$ are 0.7, 0.35, and 0.35 μM, respectively. D, time dependence of the intensities of auto (filled circles) and exchange cross-peaks at 30 °C and 150 mM NaCl. For the Sox2 measurements, the concentrations of $^{2}H_{c}/^{15}N$-labeled Sox2, Oct1 (at natural isotopic abundance), $^{1}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{b}$ are 0.55, 0.7, 0.35, and 0.35 μM, respectively. For the Oct1 measurements, the concentrations of Sox2 (natural isotopic abundance), $^{2}H_{c}/^{15}N$-labeled Oct1, DNA$_{a}$, and DNA$_{b}$ are 0.7, 0.35, and 0.35 μM, respectively. D, time dependence of the intensities of auto (filled circles) and exchange cross-peaks at 30 °C and 150 mM NaCl.
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 ($k_{\text{off}}$). The jumping rate from A to B is therefore independent of the concentration of free DNA and is given by $k_{\text{off}}^{\text{AB}}/2$, where $k_{\text{off}}^{\text{AB}}$ 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 $k_{\text{inter}}^{\text{AB}}[\text{DNA}_{\text{free}}]$ where $k_{\text{inter}}^{\text{AB}}$ is the second order rate constant for direct intersegment transfer from A to B.

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The average second order rate constants for direct intersegment transfer of the POUS ($k_{\text{POUS}}^{\text{inter}}$) and POUHD ($k_{\text{POUHD}}^{\text{inter}}$) domains of Oct1 in the ternary complex are $2.2 (\pm 0.2) \times 10^4$ and $1.7 (\pm 0.1) \times 10^3 \text{M}^{-1}\text{s}^{-1}$, respectively. $k_{\text{inter}}^{\text{AB}}$ is reduced by about 50% relative to its value in the binary complex ($3.4 \times 10^4 \text{M}^{-1}\text{s}^{-1}$), whereas $k_{\text{POUS}}^{\text{inter}}$ remains unaltered ($1.8 \times 10^4 \text{M}^{-1}\text{s}^{-1}$ in the binary complex). These observations are in complete agreement with the structure of the ternary Sox2-Oct1:FGF4-DNA complex (8) because Sox2 interacts only with the POUS 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 $1.3 (\pm 0.2) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, 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 POUS 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 POUS and POUHD 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 |
| | $k_{\text{on}}$ | $k_{\text{off}}$ |
| **Sox2** | | |
| Binary | $5.3 \pm 0.3 \text{ s}^{-1}$ | $3.2 \pm 0.2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ |
| FGF4 ternary | $0.3 \pm 0.1 \text{ s}^{-1}$ | $3.1 \pm 1.4 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ |
| **Oct1** | | |
| Binary | $44 \pm 3 \text{ s}^{-1}$ | $1.0 (\pm 0.1) \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ |
| FGF4 ternary | $2.7 \pm 0.4 \text{ s}^{-1}$ | $1.3 (\pm 0.2) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ |

*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 ($\Delta H^\circ$), entropies ($\Delta S^\circ$), and free energies ($\Delta G^\circ$) for global intermolecular translocation of Sox2 and Oct1 between cognate sites in the binary and FGF4 ternary complexes |
| | $\Delta H^\circ$ | $\Delta S^\circ$ | $\Delta G^\circ$ |
| **Sox2** | | | |
| Binary/FGF4 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-POUS** domain | | | |
| Binary/FGF4 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-POUHD** domain | | | |
| Binary/FGF4 ternary | $20.8 \pm 1.2/20.3 \pm 0.5$ | $14.7 \pm 4.1/12.7 \pm 1.6$ | $16.4 \pm 1.7/16.5 \pm 0.7$ |

*Values were derived by least squares fitting of the Eyring plots shown in Fig. 3F.  
* $\Delta G^\circ$ is calculated from $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ at 303 K.
Therefore, the kinetic data 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 (intra-molecular 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 μs, 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 located on different DNA molecules presented here, 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 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 for the sequence of binding, intersegment transfer and dissociation events involved in synergistic transcription regulation by Sox2 and Oct1 (Fig. 4). The initial step (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.)
Sox2 bound to its specific site on the promoter accelerates the high DNA concentrations present in vivo. The ternary complex is formed, Oct1 and Sox2 activate transcription between Sox2 and Oct1. Once the specific Sox2 DNA site is bound, 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.

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