# Intra- and intermolecular translocation of the bi-domain transcription factor Oct1 characterized by liquid crystal and paramagnetic NMR

**Yuki Takayama and G. Marius Clore**[1]

*Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520*

## AUTHOR SUMMARY

To perform their function as switches that control the expression of genes, transcription factors must efficiently locate their specific targets within a sea of DNA. Three mechanisms are thought to facilitate this search process: sliding, jumping, and intersegment transfer (1). In sliding, the transcription factor protein diffuses in one dimension along the DNA. Jumping involves the complete dissociation of the protein from the DNA, followed by 3D diffusion in free solution, and reassociation of the protein at a different site on the DNA. Finally, the protein can undergo direct transfer from one site to another between segments of DNA, located either distally (>150 base pairs away) or on another DNA molecule, through the formation of a bridged intermediate in which the protein spans the two segments of DNA. The intra- and intermolecular translocation events, corresponding to sliding and intersegment transfer, respectively, can occur very rapidly, on the submillisecond to millisecond time scale, even for specific complexes that only fully dissociate from the DNA on a time scale of minutes (2). Many transcription factors comprise two or more DNA binding domains, and it is unclear whether the different DNA binding domains play distinct and complementary roles in intra- and intermolecular translocation processes. Oct1 is an example of a transcription factor with two DNA binding domains, $POU_S$ and $POU_{HD}$, connected by a flexible linker, and fulfills a critical function in cell growth, differentiation, and neural development. Here we use solution NMR spectroscopy to investigate how Oct1 locates its DNA target site.

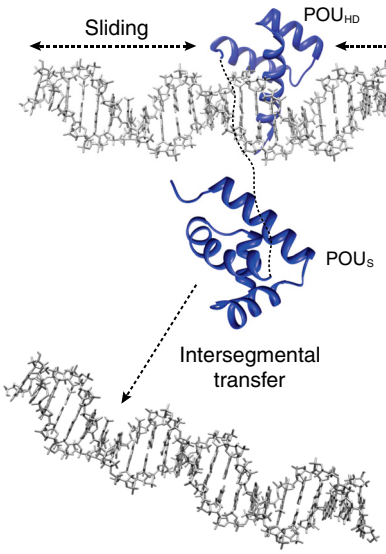


Fig. 1. Summary of the mechanism through which Oct1 searches the DNA. The $POU_{HD}$ domain predominantly undergoes sliding while the $POU_S$ domain plays a major role in initiating intermolecular translocation.

The present study relies primarily on the measurement of residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE). The RDC is a physical property used to probe the orientations of the two domains of Oct1 in specific and nonspecific complexes (3). Because translocation events involve nonspecific complexes populated at very low levels, RDCs measured on Oct1 bound to nonspecific DNA provide highly sensitive structural information on translocation intermediates. The PRE, which arises from the magnetic dipolar interaction between a proton and a paramagnetic center, was used to detect and characterize the transient, sparsely-populated species involved in translocation (2, 4). The data show that the $POU_{HD}$ domain of Oct1 sifts through the DNA primarily by rotation-coupled sliding that tracks the grooves in DNA. The $POU_S$ domain, on the other hand, serves to promote intersegment transfer by latching on to a second DNA molecule to form a bridged intermediate in which the $POU_{HD}$ domain is located on the first DNA molecule and the $POU_S$ domain on the second. The cross-talk between the two domains of Oct-1 allows the transcription factor to efficiently sift through the DNA sequence, thus facilitating the location of its target site.

Measurements were carried out both at low (0 mM) and high (150 mM) salt concentrations, because salt is known to reduce the strength of the electrostatic interactions between protein and DNA. The RDC data at low and high salt concentrations show that the orientations of the $POU_S$ and $POU_{HD}$ domains relative to the long axis of the DNA are the same in both specific and nonspecific complexes. Further, the excellent agreement between the observed RDCs and those calculated from the crystal structure of the specific Oct1-DNA complex indicate that the orientation of the two domains relative to the long axis of the DNA is the same in solution and in the crystal state. The RDC data, in conjunction with chemical shift perturbation data, indicate that, at high salt, the $POU_{HD}$ domain remains fully bound to nonspecific DNA while the $POU_S$ domain undergoes partial dissociation.

For PRE measurements on specifically-bound Oct1, DNA oligonucleotides were labeled with dT-EDTA-$Mn^{2+}$, a paramagnetic label, at several sites (one per DNA duplex). Under conditions where the species present in solution rapidly

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[1]To whom correspondence should be addressed. E-mail: mariusc@mail.nih.gov.

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interconvert between one another, the observed PREs are exquisitely sensitive to the presence of minor states, even at populations as low as 0.5–1% (4). Thus, although only the NMR spectrum of the specific Oct1-DNA complex is observed, the PRE rates reflect translocation events involving transient, sparsely-populated intermediates.

At low salt, with the paramagnetic label located on the $POU_S$ side of the DNA (referred to as site 1), the PREs for the $POU_S$ domain are in excellent agreement with the crystal structure, but small PRE effects are also observed for the $POU_{HD}$ domain although none are predicted from the structure. Similarly, when the paramagnetic label is placed on the $POU_{HD}$ side of the DNA (referred to as site 2), the PRE profile for the $POU_{HD}$ domain is in excellent agreement with that predicted from the crystal structure, but large PRE effects are observed for the $POU_S$ domain where none are predicted. Thus, the $POU_{HD}$ and $POU_S$ domains must sample states in close proximity to the site 1 and 2 labels, respectively, with the population of the latter being significantly larger than the former.

At higher salt concentrations, the exchange rates between the specific complex and the sparsely-populated states sampled by sliding and intersegment transfer are increased. As a result the PRE profiles are enhanced in magnitude allowing one to obtain further insights into the nature of the translocation intermediates. These data demonstrate the existence of states in which the orientations of the $POU_{HD}$ and $POU_S$ domains are flipped by 180° relative to those in the specific complex.

To analyze the contributions to the PRE profiles from intramolecular sliding and intermolecular translocation, a series of experiments were carried out on samples comprising an equal mixture of specific and nonspecific DNA duplexes. In the first set of experiments, the paramagnetic label was placed on the nonspecific DNA so that any observed PREs in the spectrum of the specific complex can only arise from intermolecular translocation involving transient excursions of a domain from the specific DNA to the nonspecific DNA and back to the specific DNA. In the second set of experiments, two samples were prepared with the paramagnetic label placed on the specific DNA duplex, either on the $POU_S$ or $POU_{HD}$ sides, and the nonspecific DNA unlabeled so that PREs arise from both sliding and intermolecular translocation (via intersegment transfer between DNA molecules bearing the specific site), as well as from the specific complex itself. The combined PRE data obtained from both sets of experiments demonstrate that translocation of the $POU_{HD}$ domain is predominantly intramolecular, while translocation of the $POU_S$ domain is largely intermolecular.

Additional PRE experiments using only paramagnetically labeled nonspecific DNA indicate that both domains sift through numerous binding sites on the DNA in two approximately equally populated orientations related by a 180° rotation with respect to the long axis of the DNA.

In conclusion, there is rapid interconversion between the specific Oct1-DNA complex and sparsely-populated, transient translocation intermediates that occupy nonspecific DNA binding sites. From the ratio of the equilibrium dissociation constants for specific and nonspecific DNA binding of Oct1, the population of the sparsely-populated states is estimated as $<1\%$. The key finding is that the transitions from the specific Oct1-DNA complex to the transient states largely involve intramolecular translocation by rotation-coupled sliding for the $POU_{HD}$ domain but intermolecular translocation through intersegment transfer for the $POU_S$ domain.

Taken together, the data presented here suggest the following model whereby Oct1 efficiently explores the DNA landscape to locate its target site within a sea of DNA (Fig. 1). The $POU_{HD}$ domain largely searches the DNA by sliding, while the $POU_S$ domain acts as an antenna to promote intersegment transfer. Transfer from one DNA molecule (or distantly located DNA segment) to another occurs via a bridged intermediate that is formed by the $POU_S$ domain latching on to the second DNA molecule. Once this intermediate is formed, the probability of completing intermolecular translocation of Oct1 by dissociation of the $POU_{HD}$ domain from the first DNA molecule followed by association to the second DNA molecule is significantly enhanced. Thus, cross-talk between the $POU_S$ and $POU_{HD}$ domains of Oct1, each performing distinct and mutually complementary roles in the search process, is essential for efficient transcription.

In summary, our findings have helped illuminate how a transcription factor with several DNA binding domains efficiently locates its target within a stretch of DNA. The findings help illustrate how transcription factors balance the opposing constraints of speed and specificity.

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