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**The Transition Pathway from Nonspecific to Specific Complex of DNA with a DNA-Bending Protein** SERGUEI KUZNETSOV, PAULA VIVAS, YOGAMBIGAI VELMURUGU, ANJUM ANSARI, University of Illinois at Chicago — Integration host factor (IHF) from *E. coli* is a DNA-bending protein that recognizes and binds to its specific sites primarily by the indirect read-out mechanism, in which sequence-dependent DNA dynamics and flexibility play an important role. The crystal structure of IHF bound to a 35-bp long cognate site H' indicates that the DNA is kinked at two sites separated by  $\sim 9$  bp, resulting in a “U-turn” bend of the DNA. To probe the DNA bending dynamics, we use a laser T-jump, and time-resolved FRET on end-labeled DNA substrates. Our results show that DNA bending occurs on the same time-scales as thermal disruption of single base-pairs in B-DNA, suggesting that spontaneous kinking may be the rate-limiting step. To test this hypothesis, we modified the DNA at the site of the kinks by introducing (i) a nick in the sugar-phosphate backbone, and (ii) mismatches to create internal loops. For each of these substrates, the 4-20 fold increase in the binding affinity is reflected in a corresponding increase in the bending rates. Furthermore, the DNA bending rates are independent of the salt concentration. These results indicate that in the transition state ensemble the DNA is kinked, but specific protein-DNA interactions involving ion release have not occurred.

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