
Interactions between RNA polymerase and specific promoter DNA sequences trigger a precise progression of conformational changes in both biomolecules. These steps constitute a mechanism of DNA opening and the start of transcription; each step provides a critical checkpoint for regulatory input. Studies of E. coli RNA polymerase demonstrate that binding free energy alone opens the initiation bubble (−11 to +2), placing the +1 template base in the active site of the enzyme. Subsequent conformational changes are required to form the stable open complex RP. Here we present recent fast footprinting experiments (hydroxyl radical, permanganate) that characterize the protection of the DNA backbone protection and the degree of thymine base unstacking in the three kinetically significant intermediates (I1, I2, I3) on the time scale of their formation. Experiments performed after mixing RNA polymerase and the λPr promoter DNA demonstrate that the DNA duplex containing the start site first loads in the RNA polymerase active site channel in duplex form to form I1, and that the subsequent step cooperatively opens the entire transcription bubble. In the back direction, [salt] upshift experiments demonstrate the existence of two unstable open intermediates, I1 and I2. Comparison of the degree of protection of the backbone in I2, I3 and RPo reveals differences between the nontemplate (NT) strand in these complexes but not the template (T) strand. We propose that the T strand is loaded first, followed by steps that reposition the nontemplate (NT) strand in these complexes but not the template (T) strand.


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