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Promoter Recognition by *E. coli* RNA Polymerase: Effects of α Subunit Interactions with Upstream DNA on Isomerization of the RNAP-Promoter Complex

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Bacterial promoters utilized by multisubunit “housekeeping” RNA polymerases are often considered to consist of appropriately spaced sequences that resemble the two consensus hexamers (-10 and -35) recognized by the *E. coli* RNA polymerase $Esigma^{70}$. However, correct identification of promoters based solely on consensus sequence information can present a difficult challenge, due to a complex array of additional promoter features and other transcription factors that influence promoter function.

Interactions between the enzyme and DNA in an RNAP-promoter complex occur over a length of ~75 bp of DNA (from ~55bp upstream to ~20bp downstream of the transcription start site at +1), and involve contributions from each of the RNAP subunits with the exception of omega. In addition to interactions of sigma with consensus sequences, these include base-specific interactions in regions outside the consensus hexamers (e.g., the UP element located upstream of the -35 hexamer with the alpha subunit CTD, the extended -10 sequence with sigma region 3.0, and the discriminator sequence located between the -10 hexamer and the transcription start site with sigma region 1.2). Additional interactions between RNAP and the DNA backbone also occur. The number and strength of these interactions, and their contributions to promoter function can vary among different promoters.

In our studies of promoter function, we have determined the contributions of some of these additional RNAP-promoter interactions to the rates of formation and decay of the RNAP-promoter complex. The association of RNA polymerase with a promoter to form a transcriptionally competent complex is characterized by an overall association rate constant, k_a, and involves at least three kinetically-significant steps that precede the binding of NTPs and formation of a transcription elongation complex (TEC). The first step, characterized by the equilibrium binding constant K_B, involves formation of a competitor-sensitive, closed complex (RP_C) with a footprint that includes DNA only from ~ -55 to ~ +1. The closed complex then undergoes conformational changes ("isomerizations"), with a composite rate constant k_i, to produce the open complex (RP_O). In the open complex the footprint extends to ~ +20, DNA from ~ -11 to ~ +3 is single-stranded, and the template strand is positioned in the RNAP

active site region.

Although several crystal structures of bacterial RNAPs provide a valuable framework for biochemical and genetic studies of the process of open complex formation, these structures do not contain information about some of the additional DNA and RNAP interactions that play critical roles in the process (e.g., the interaction between the alpha CTDs and DNA upstream of the -35 hexamer, or between sigma and DNA downstream of the -10 hexamer). The nature of the molecular interactions and conformational changes that accompany isomerization of the complex are not yet well understood.

We have focused our studies on the role of RNAP alpha subunit interactions with upstream DNA. Our results, as well as those of other labs, indicate that these interactions affect isomerization in addition to initial RNAP binding. At promoters such as *rrnB* P1 that contain a strong upstream binding site for alpha CTD (an UP element), alpha CTD-DNA interactions have a very large effect on the rate of open complex formation (>30-fold). A majority of this effect is on the initial step in the process, formation of RP_c, but there is also an effect (~4-fold) on the rate of isomerization. Since many promoters do not contain strong UP elements, but nevertheless display non-specific interactions of alpha CTD with upstream DNA, we also investigated the role of alpha CTD-DNA interactions at promoters lacking UP elements. We find that at promoters without an UP element (*lacUV5*, or lambda P_R), the non-specific alpha CTD-DNA interactions also make a surprisingly large contribution to open complex formation (~10-fold), and a majority of this effect is on the rate of isomerization of the complex.

To further understand isomerization, we have considered two general models to account for the effects of the alpha subunit on this process. In the first model, interaction of alpha CTD with non-specific DNA repositions alpha CTD such that it makes additional protein-protein interactions, for example with the alpha NTD, the flexible linker that connects these two domains, or another part of RNAP, and these interactions stabilize an intermediate on the pathway to RP_o formation. In a second model, the effect on isomerization is proposed to result from repositioning of DNA upstream of the alpha CTD binding sites such that it can interact with another region of RNAP to stabilize a kinetic intermediate.

Our determinations of upstream DNA length requirements for this effect lead us to favor the first model. We find that DNA upstream of -63, and extending to ~-85, is required for most of the effect on isomerization, and alpha CTD is known to interact with sequences extending this far upstream. We suggest that direct effects involving the alpha CTD are likely to account for stabilization of an intermediate complex. Only one of the two alpha CTDs is necessary and sufficient to confer the isomerization effect. Residues in the C-terminal tail region of alpha CTD, a region not previously implicated in effects of upstream DNA interactions, appear to play a role in the effect on isomerization. Several mutations in the alpha NTD alter UP element function in vivo. These data suggest possible models for alpha CTD interactions that may stabilize a kinetic intermediate in the process of open complex formation.