Domain movements of the enhancer-dependent sigma factor drive DNA delivery into the RNA polymerase active site

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Introduction

Gene transcription is a tightly regulated cellular process. The basic features of the transcription machinery are conserved through all kingdoms of life, with the multi-subunit RNA polymerase (RNAP) playing a central role. Bacteria use a sequence-specific DNA-binding protein, the sigma (σ) factor, which together with five core subunits ($\alpha_2\beta\beta'\omega$) forms the RNAP holoenzyme that performs promoter recognition and DNA opening during transcription initiation. Two principal classes of σ factors are known, the σ^{70} and σ^{54}. The former mediates transcription of house-keeping genes while the latter activates gene expression in response to environmental cues. The σ^{54}-dependent transcription system encounters a kinetic barrier to open, transcriptionally active complex (RPo) formation and required Bacterial Enhancer Binding Proteins (bEBPs) for activation. These proteins, such as PspF (Phage Shock Protein F), belong to the ATPases Associated with various cellular Activities (AAA+) family, bind to upstream enhancer binding sequences and couple the energy associated with ATP hydrolysis to remodel the inactive closed complex (RPc) into RPo.

A large scale domain reorganization of σ^{54} occurs when the holoenzyme interacts with PspF in the presence of ADP.AlFx, suggesting that ATP hydrolysis is key to removal of the 'roadblock' but little is known about these well-orchestrated but largely unmapped intermediate steps. In order to map these steps we have employed complementary single molecule fluorescence (SMF) techniques, Alternating Laser Excitation (ALEX), Total Internal Reflection Fluorescence Microscopy (TIRFM) and Fluorescence Correlation Spectroscopy (FCS) to probe the domain movements in σ^{54} upon holoenzyme formation, in the closed promoter complex, upon binding to a pre-melted *nifH* promoter mimicking the open complex, upon PspF-mediated transition state of ATP hydrolysis and RPo formation, and finally upon RNA primed initiation and subsequent transcript elongation.

Results

Here, we complement the proposed multi-step kinetic model by assigning the kinetic steps to defined structural rearrangements of Regions I and III with respect to each other, and to the promoter. The irreversible, ATP hydrolysis-driven step is preceded by two reversible steps. No domain separation occurs during the first step (step II to III, Figure 1), modelled here by $E\sigma^{54}$ binding to the early-melted promoter. However, the second step, which is modelled by $E\sigma^{54}$-late-melted promoter complex formation, leads to significant separation between Regions I and III (step III to IV, Figure 1) but without DNA repositioning. Since this domain movement only happens on the partially melted promoter it is most likely driven by ssDNA recognition, as in the case of σ^{70} where sequence-specific ssDNA binding is coupled to promoter unwinding. The domain separation is further stabilized by interaction with the activator PspF even in the absence of ATP (Figure 1, IV to V). In the presence of the transition state analogue ADP:AlF_x σ^{54} domains become further separated and Region I moves towards the leading edge of the promoter (Figure 1, V to VI). However, only ATP hydrolysis makes the protein rearrangement irreversible (Figure 1, VI to VII), the domain separation decreasing to the one seen in the nucleotide-free state (V in Figure 1) but with both regions closer to the leading edge. This most likely reflects loading of the template DNA strand into the active site, resulting in bending of the promoter and polymerase ββ' clamp closure. DNA footprints of the $E\sigma^{54}$ RPc and RPo show little difference at the upstream trailing edge, whereas in RPo the downstream footprint is extended, the interaction with the downstream fork junction is changed and the start site DNA is well within RNAP. Thus, changes in trailing edge FRET with the Region III label may reflect increased upstream wrapping

of DNA in RPo, and changes in σ^{54} Region I to III separations between RPo and RPc are dominated by a movement of Region I relative to a more static Region III.

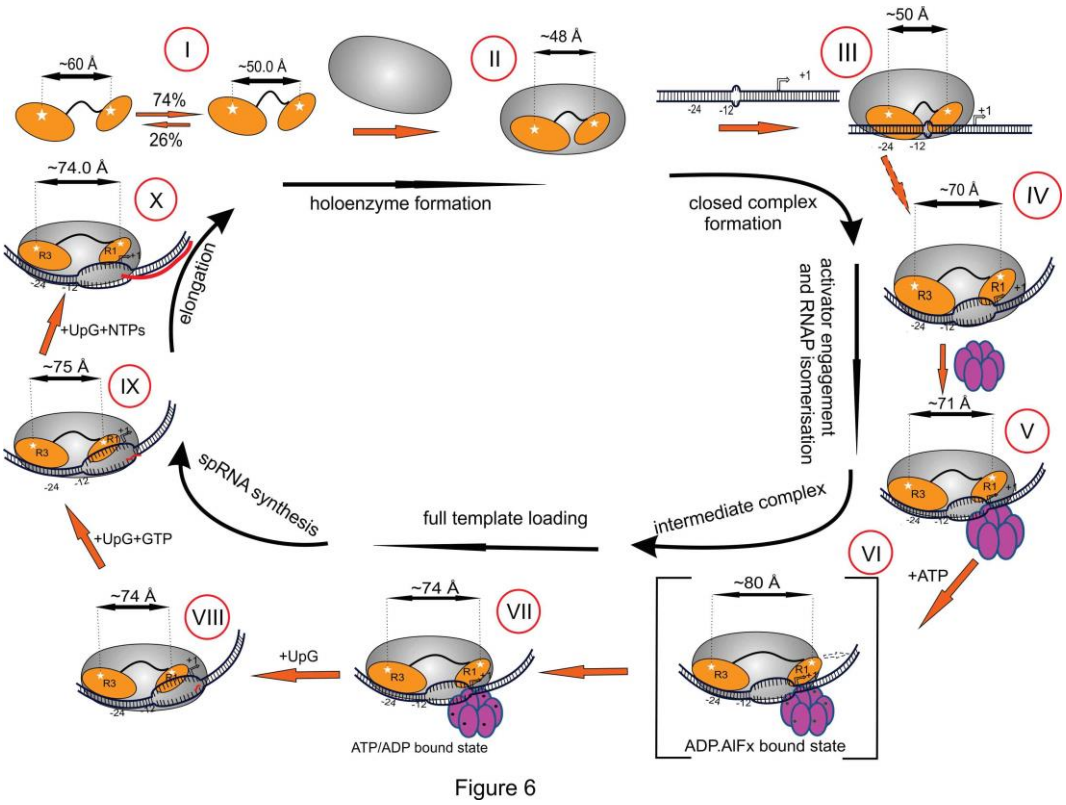

Figure 6

Figure 1: Tracking conformation dynamics of σ^{54} during the transcription cycle. Model shows the domain disposition adopted by the Region I and III of σ^{54} with respect to each other and also in the context of the *nifH* promoter DNA beginning from free σ^{54} in solution (stage I), holoenzyme formation (stage II), closed promoter complex (RPc,stage III), activator engagement and RNAP isomerisation (stage IV-V), intermediate complex (RPi) formation (stage VI), full template loading (stage VII),initial transcript formation (stage VIII-IX), elongation complex (stage X).

Our results demonstrate that there are fundamental differences between the initiation at σ^{70} and σ^{54} promoters. Whereas for σ^{70} proteins where the Region1.1 is repositioned from the RNAP DNA-binding channel to allow RPo formation, σ^{54} Region I is actively relocated by the ATPase during hydrolysis. In contrast, for σ^{70} RPo formation signals from DNA may induce opening and closing of the RNAP clamp, causing Region 1.1 to move away from the RNAP main channel. Hence for σ^{54} RPo formation is coupled to its cognate enzymatic ATPase, for σ^{70} to promoter DNA motions.

Publications

Sharma, A., Leach, R., Gell, C., Zhang, N., Burrows, P., Shepherd, D., Wigneshweraraj, S., Smith, D., Zhang, X., Buck, M., Stockley, P. & Tuma, R. (2014) Domain movements of the enhancer-dependent sigma factor drive DNA delivery into the RNA polymerase active site: insights from single molecule studies. *Nucleic Acids Res.* **42**: 5177-5190.

Funding

This work was funded by the BBSRC.

Collaborators

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