BACTERIAL TRANSCRIPTION

Sigma 54 minds the gap

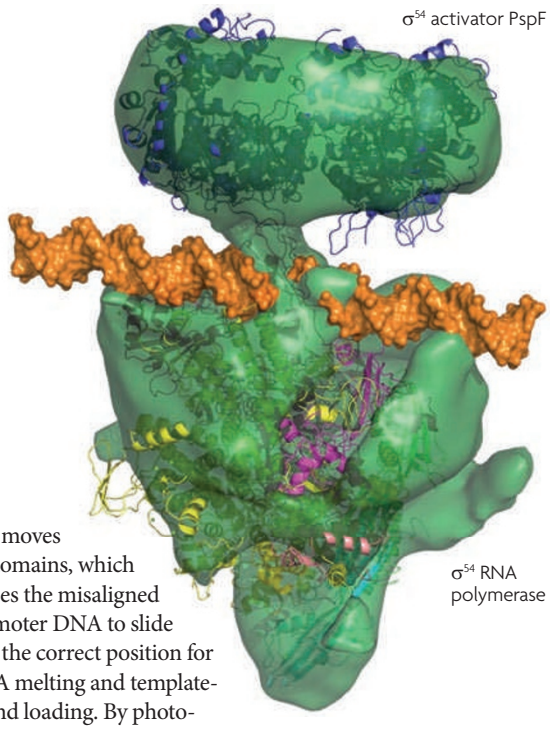
Using cryo–electron microscopy (cryo–EM) and photocrosslinking to capture the initiation of transcription by *Escherichia coli* RNA polymerase (RNAP)–σ^{54}, Bose *et al.* report in *Molecular Cell* that σ^{54} can block access of single-stranded DNA to the active site of RNAP. An interaction with an ATP-hydrolysing transcriptional activator removes this block and enables transcription to proceed.

The main bacterial sigma factor (σ^{70}) transcribes housekeeping genes, whereas the alternate sigma factor (σ^{54}) transcribes a subset of genes. Despite limited amino-acid similarity between σ^{54} and σ^{70}, these sigma factors bind to overlapping regions of the RNAP. However, whereas the RNAP–σ^{70} holoenzyme is competent for transcription, the RNAP–σ^{54} complex binds to promoter DNA, then remains stalled and unable to transcribe. When the activator protein PspF (an AAA+ family protein) binds to a bacterial enhancer-like sequence in the promoter and hydrolyses ATP, the RNAP–σ^{54} complex is converted from a 'closed' (bound but stalled) to an 'open' complex that can actively transcribe. Activation of RNAP–σ^{54} transcription by PspF is therefore analogous to activation of RNA polymerase II-mediated transcription in eukaryotes by the ATP-hydrolysing protein TFIIH.

Cryo–EM reconstruction was used to reveal interactions made between RNAP, σ^{54} and the activator protein PspF. Bose *et al.* compared the cryo–EM reconstruction of RNAP with RNAP–σ^{54}, fitted into this the crystal structure of the RNAP enzyme from *Thermus thermophilus* and imaged RNAP complexed with truncated versions of σ^{54}, or a form of σ^{54} tagged at the carboxyl terminus with nanogold, to reveal the position of the C-terminal promoter recognition domain of the sigma factor within the holoenzyme. To identify the likely position of DNA in the RNAP–σ^{54} complex, they inspected cryo–EM reconstructions of RNAP–σ^{54} and *T. thermophilus* holoenzyme complexed with DNA. Strikingly, the σ^{54} C-terminal promoter interaction domain seems to misalign promoter DNA with the active site of the RNAP.

It was important to locate the region I domain of σ^{54} because this domain not only serves to inhibit transcription in the closed complex but also directly interacts with the transcriptional activator PspF and is necessary for open-complex formation. Comparing a reconstruction of a holoenzyme complex that contained σ^{54} lacking region I with the wild-type holoenzyme showed that region I of σ^{54} occludes DNA from the active site of RNAP, further accounting for the inability of the closed complex of RNAP–σ^{54} to transcribe.

To probe how binding to the activator protein alters the interactions among the components of the transcription complex, Bose *et al.* analysed a cryo–EM reconstruction of RNAP–σ^{54}–PspF (amino acids 1–275) stabilized with a non-hydrolysable analogue of ATP. This revealed that region I of σ^{54} is rearranged after binding the activator protein PspF and no longer fully blocks the active site of the enzyme. Interaction with PspF also moves σ^{54} domains, which causes the misaligned promoter DNA to slide into the correct position for DNA melting and template-strand loading. By photocrosslinking promoter probes to the holoenzyme complex, they proved that the activator works in front of the RNAP to remodel the complex and activate transcription.



A cryo–electron microscopy reconstruction of the RNA polymerase–σ^{54}–PspF–DNA co-complex; promoter DNA is orange. Figure courtesy of D. Bose and X. Xhang, Imperial College, London, UK.

The initiation of bacterial transcription in *E. coli* serves as a model for transcription in Bacteria, Eukarya and Archaea. This is the first time that structural features of activator-driven domain rearrangements that occur in transcription initiation have been visualized. The next step is to examine the details of the nucleotide-dependent transactions that underlie this model for transcription initiation.

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ORIGINAL RESEARCH PAPER Bose, D. *et al.* Organization of an activator-bound RNA polymerase holoenzyme. *Mol. Cell* **32**, 337–346 (2008)