

## **Towards the understanding of transcription initiation: Insights into the function of transcription factor E**

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Abstract:

The transcription cycle can be divided into three phases: initiation, elongation and termination. During each phase different regulatory proteins interact with RNA polymerase (RNAP) and thereby control gene expression. The archaeal transcription machinery is closely related to eukaryotic RNAPII-system and it is a highly attractive model system because of its superior biochemical tractability.

Initiation of transcription in the archaeal system involves recognition of promoter sequences by TBP (TATA binding protein) that is followed by the binding of TFB (transcription factor B) leading to a stabilisation of the TBP-DNA complex. The ternary DNA-TBP-TFB complex is able to recruit RNAP resulting in transcription initiation. A third additional transcription factor E (TFE) completes the initiation complex. TFE is involved in but not strictly required for transcription initiation *in vitro*. The *modus operandi* of TBP and TFB have been characterised in great detail - but our understanding of TFE action is disappointingly lacking. In archaeal extracts TFE has been shown to interact with both TBP and RNAP. TFE stimulates transcription moderately, most likely by stabilising the initiation complex and promoting open complex formation. TFE consists of two structurally discrete domains, a Winged Helix and a Zn-ribbon domain complemented by two flexible regions, the linker and tail.

In this study we focus on the molecular mechanisms of *Methanocaldococcus jannaschii* TFE by applying a wholly recombinant archaeal transcription system. In order to understand how the individual TFE domains are involved in the interaction network of the initiation complex and thereby influence transcription, we made use of TFE deletion variants lacking either of the domains. In summary, the integrity of the Winged Helix domain, the Zn-ribbon and the flexible linker region are required for TFE function *in vitro* whereas the C-terminal tail domain is dispensable. TFE stabilises the DNA-TBP-TFB-RNAP complex in a manner that is strictly dependent on RNAP subunits F/E and influenced by the topology of the promoter template. Our results suggest that both TFE domains cooperate to facilitate the stabilisation of the DNA-TBP-TFB-TFE-RNAP initiation complex and the closed-to-open complex transition during transcription initiation.