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Characterizing the Functional Role of Transcription Factor E in Archaeal Transcription

Madolyn Hofstetter
Portland State University

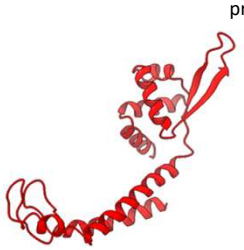
Michael Bartlett
Portland State University

Characterizing the Functional Role of TFE in Archaeal Transcription

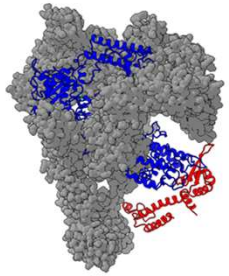
Madolyn Hofstetter, BS & Michael Bartlett, PhD
Portland State University

Background

Transcription is the first step in gene expression. In Archaea, there are two required transcription factors necessary for transcription, Transcription Factor B (TFB) and TATA Binding Protein (TBP). The two transcription factors work in tandem with RNA Polymerase (RNAP) to start the process of gene expression, separating the two strands of DNA and processing the newly build strand of RNA, one nucleotide at a time. However, there are other transcription factors that play a role in promoter opening and transcription elongation, such as Transcription Factor E (TFE). TFE binds to RNAP with high affinity and promotes opening of the active site for proper processing of the DNA. Though we know this action of TFE, we do not know where the interactions are occurring between TFE, RNAP, DNA or other potential transcription factors. In order to test this, point mutations were made in TFE's winged helix motif and surrounding areas based on previous research. The winged helix motif is rich in aromatic amino acids, which is an important conserved structure across all domains of life. Transcription assays were run to test the rate of transcription and abundance of RNA produced.

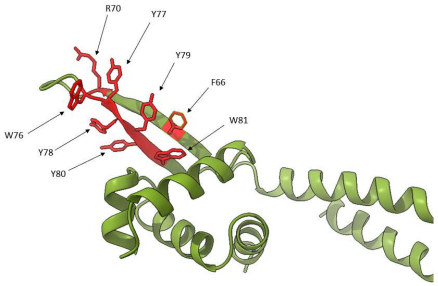


TFE



TFE-RNAP complex

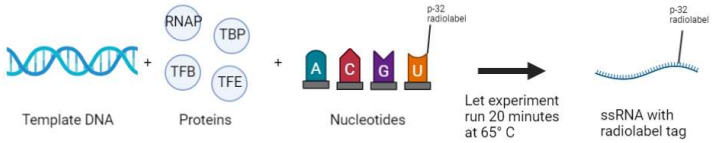
Methods

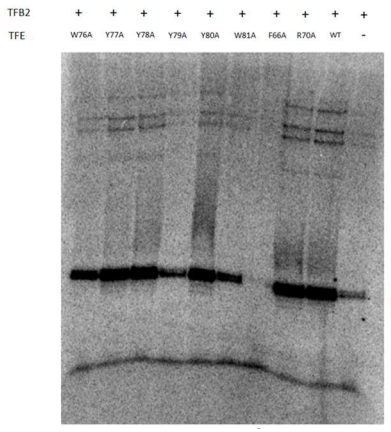


Point mutations in TFE's winged helix motif were made by mutating one single position from its existing amino acid to alanine, a small aliphatic amino acid. Transcription assays were run with RNAP, TFE (WT or point mutant), nucleotide triphosphates (NTPs), TBP, TFB, and template DNA. Radiolabeled UTP was substituted for UTP in NTPs, which allows for visualization of RNA produced by Typhoon Scanner, which is sensitive for radioactivity.

In Vitro transcription complex assembly includes:

- 250mM NaCl
- 5 mM β-mercaptoethanol
- 40 mM Na-HEPES (pH 7.3)
- 2.5 mM $MgCl_2$
- 0.1 mM EDTA
- 5% glycerol
- 0.1 mg/ml BSA
- 40 mM promoter DNA (-65 to +35)
- 120 nM TFB
- 120 nM TFE
- 240 nM TBP
- 40nM RNAP

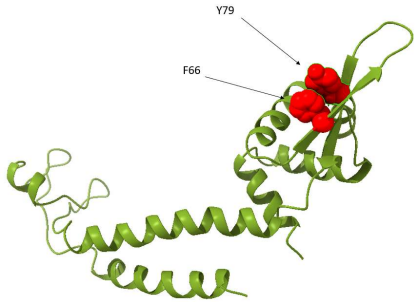




Results

14% polyacrylamide urea gels were run with reaction mixtures and scanned. RNA transcript can be seen as the 37-nucleotide band produced from each experiment. Visualization by measuring radioactivity from UTP incorporated into growing RNA strand during transcription of the template DNA strand.

The most detrimental mutations in TFE occur when Tyrosine at position 79 and Phenylalanine at position 66 are mutated to Alanine. The two amino acids appear to overlap in the center of the winged helix motif based on the 3D protein structure. I hypothesize that these two amino acids are either important for structural support of the wing tip, allowing for TFE to assist in promoter opening with RNAP, or these two amino acids protrude out and into the template DNA, facilitating in breaking bonds between the two strands, assisting in unwinding of the DNA.



Future Research

Continue characterizing the functional surfaces of TFE by testing more mutations in and around the winged helix motif. These mutations can be used to test for binding site affinity for TFE-RNAP when adding in Spt4/5 in transcription assays. The two transcription factors compete for position near RNAP's active site, and if these mutations occur in critical binding amino acids, TFE would lose affinity for RNAP, and Spt4/5 would freely be able to outcompete and bind.
To assess interactions between Y79 and F66, cross-linking experiments will be performed to explore potential interactions between these amino acids and the template DNA.

Citations

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