

Crystallographic Studies of Prokaryotic Transcription

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Introduction

Transcription is the major control point of gene expression, and RNA polymerase (RNAP) is the central enzyme of transcription. Our long-term goal is to understand the mechanism of transcription and its regulation. Determining 3-D structures of RNAP and its complexes with DNA, RNA, and regulatory factors is an essential step. This is best accomplished with highly characterized prokaryotic RNAPs, especially because of the high degree of conservation of RNAP structure and function from bacteria to man. To this end, we bring to bear a combined biochemical and biophysical approach to understand the structure and function of the bacterial RNAP in different stages of the transcription cycle, beginning with the 3.3-Å-resolution x-ray crystal structure [1] of the 380 kDa core RNAP (subunit composition $\alpha_2\beta\beta'\omega$) from the thermophilic eubacterium *Thermus aquaticus* (*Taq*). Our studies at the Structural Biology Center (SBC) are aimed toward adding to our understanding of the enzyme's function and its regulation. Specifically, we performed experiments to determine the structure of (1) the initiating form of the bacterial RNAP, the 430 kDa holoenzyme containing core RNAP ($\alpha_2\beta\beta'\omega$) and the primary initiation-specific σ subunit [2], and (2) a complex between an alternative promoter-specificity σ factor (*Escherichia coli* σ^E) and its cognate anti- σ factor RseA [3].

Methods and Materials

Methods and materials are described in Refs. 1-3.

Results

The first result relates to the Structural basis of transcription initiation: RNA polymerase holoenzyme at 4-Å resolution [2]. The crystal structure of the initiating form of *Taq* RNAP, containing core RNAP ($\alpha_2\beta\beta'\omega$) and the promoter specificity σ subunit, was determined at 4 Å-resolution. Important structural features of the RNAP and their roles in positioning σ within the initiation complex are delineated. The two C-terminal domains of σ are separated by 45 Å on the surface of the RNAP but are linked by an extended loop. The loop winds near the RNAP active site, where it may play a role in stabilizing the initiating complex, and out through the RNA exit channel. The advancing RNA transcript must displace the loop, leading to abortive initiation and ultimately to σ release.

The second result relates to the crystal structure of *Escherichia coli* σ^E with the cytoplasmic domain of its anti- σ RseA [3]. The σ factors are the key regulators of bacterial transcription. ECF (extracytoplasmic

function) σ 's are by far the largest and most divergent group of σ^{70} -family members. ECF σ 's are normally sequestered in an inactive complex by their specific anti- σ factor, which often spans the inner membrane. Here, we determined the 2-Å-resolution crystal structure of the *E. coli* ECF σ factor σ^E in an inhibitory complex with the cytoplasmic domain of its anti- σ , RseA. Despite extensive sequence variability, the two major domains of σ^E are virtually identical in structure to the corresponding domains of other σ^{70} -family members. In combination with a model of the σ^E -holoenzyme and biochemical data, the structure reveals that RseA functions by sterically occluding the two primary binding determinants on σ^E for core RNAP.

Discussion

These structural studies performed at SBC beamline 19-ID have advanced our knowledge of bacterial transcription and its regulation. Current work includes structural studies of other transcription complexes trapped in different stages of the transcription cycle and studies of further complexes involved in σ factor regulation.

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References

- [1] G. Zhang, E.A. Campbell, L. Minakhin, C. Richter, K. Severinov, and S.A. Darst, "Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution," *Cell* 98, 811-824 (1999).
- [2] K.S. Murakami, S. Masuda, and S.A. Darst, "Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 Å resolution," *Science* 296, 1280-1284 (2002).
- [3] E.A. Campbell, J.L. Tupy, T.M. Gruber, S. Wang, M.M. Sharp, C.A. Gross, and S.A. Darst, "Crystal structure of *Escherichia coli* σ^E with the cytoplasmic domain of its anti- σ RseA," *Mol. Cell* 11, 1067-1078 (2003).