Crystallographic Studies of Prokaryotic Transcription

S. A. Darst, E. A. Campbell, K. Murakami
The Rockefeller University, 1230 York Avenue, New York, NY 10021 USA

Introduction

Transcription is the major control point of gene expression and RNA polymerase (RNAP) is the central enzyme of transcription. The RNAP in all cellular organisms is a complex molecular machine. In its simplest bacterial form, the enzyme comprises at least 4 subunits with a total molecular mass of about 400 kDa. The eukaryotic enzymes comprise upwards of a dozen subunits with a total molecular mass of about 500 kDa. The essential core component of the bacterial RNAP (subunits $\alpha_2\beta\beta'\omega$) is evolutionarily conserved from bacteria to man, making the bacterial RNAPs excellent model systems.

Recently, we solved the X-ray crystal structure of *Thermus aquaticus* (*Taq*) core RNAP to a resolution of 3.3 Å [1]. Our studies at SBC are aimed towards adding to our understanding of the enzyme's function and its regulation. Specifically, we performed experiments to:

1. Determine the structure of Taq core RNAP complexed with the inhibitor rifampicin.

2. Determine the structure of a complex between a promoter-specificity σ factor (*B. stearothermophilus* σ^F) and its cognate anti-σ factor SpoIIAB.

Methods and Materials

Methods and Materials are as described [1-3].

Results

1. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase [2]. Rifampicin (Rif) is one of the most potent and broad-spectrum antibiotics against bacterial pathogens and is a key component of anti-tuberculosis therapy, stemming from its inhibition of the bacterial RNAP. We determined the crystal structure of *Taq* core RNAP complexed with Rif, using native data collected at SBC-19ID. The inhibitor binds in a pocket of the RNAP β subunit deep within the active-site channel but more than 12 Å away from the active site itself. The structure, combined with biochemical results, explains the effects of Rif on RNAP function and indicates that the inhibitor acts by directly blocking the path of the elongating RNA when the transcript becomes 2 to 3 nt in length.

2. Crystal structure of the Bacillus stearothermophilus *anti-σ factor SpoIIAB with the sporulation σ factor σ^F [3].* Cell type-specific transcription during *Bacillus* sporulation is established by σ^F. Negative regulation of σ^F activity is orchestrated by SpoIIAB, which functions as an anti-σ by binding σ^F, and as a serine kinase by phosphorylating the anti-anti-σ SpoIIAA, thereby inactivating it. The crystal structure of the SpoIIAB dimer bound to σ^F in the low-affinity, ADP form of the complex has been determined at 2.9 Å resolution. High-resolution data collected at SBC-19ID was used for the structure refinements. SpoIIAB adopts the GHKL superfamily fold of ATPases and histidine kinases, with an N-terminal β/α extension that forms the dimer interface. A compact, three-helix domain of σ^F makes contacts with both SpoIIAB monomers, while 80% of the σ factor is disordered. The interaction of σ^F with the SpoIIAB dimer occludes a core RNAP binding surface on the σ, explaining the anti-σ activity of SpoIIAB. The structure also explains the specificity of SpoIIAB for its target σ factors, and provides insight into the mechanism of the anti-anti-σ activity of SpoIIAA.

Discussion

These structural studies performed at SBC-19ID have advanced our knowledge of bacterial transcription and its regulation. Current work includes structural studies of other RNAP/inhibitor complexes, and studies of further complexes in the regulatory pathway of *Bacillus* sporulation.

Acknowledgments

We thank S. Nair for assistance with data collection at SBC. We are indebted to A. Joachimiak, S.L. Ginell, F.J. Rotella, and N. Duke at the Advanced Photon Source Structural Biology Center for support during data collection. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38. K.M. was supported by a Postdoctoral Fellowship from the HFSP and a Norman and Rosita Winston postdoctoral fellowship. E.A.C. was supported by a National Research Service Award (NIH GM20470). This work was supported in part by NIH GM53759 and GM61898 to S.A.D.

References

[1] G. Zhang, E.A. Campbell, L. Minakhin, C. Richter, K. Severinov, S.A. Darst, "Crystal structure of Thermus aquaticus core RNA polymerase at 3.3 Å resolution", Cell 98, 811-824 (1999).

[2] E.A. Campbell, N. Korzheva, A. Mustaev, K. Murakami, S. Nair, A. Goldfarb, S.A. Darst, "Structural mechanism for rifampicin inhibition of bacterial RNA polymerase", Cell 104, 901-912.

[3] E.A. Campbell, S. Masuda, O. Muzzin, S. Wang, S.A. Darst, "Crystal structure of the *Bacillus stearothermophilus* anti-σ factor SpoIIAB with the sporulation σ factor σ^F", Cell 108, 796-807.