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Crystallography of RNA polymerase complex

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1 Introduction

Transcription of genomic information is the initial step required for all cellular events. DNA-dependent RNA polymerase (RNAP), a huge multi-subunit enzyme, plays a central role in this essential process. Transcription is a complex, multi-step process, consisting of the stages of initiation, elongation, and termination. To accomplish multiple tasks during transcription, RNAP is thought to change its conformation/function while forming various kinds of complexes with many transcriptional regulators. However, structural basis of such essential complexes and the mechanisms by which RNAP functions are modulated by the regulators are largely unknown.

To address these problems and comprehend the transcription mechanism, it is essential to study the structures of principal transcription complex formed during transcription. We have been performing crystallographic studies on several principal transcription complexes, consisting of RNAP and its regulatory proteins and/or nucleic acids.

2 Experiment

During transcription, RNAP frequently pauses on DNA, and sometimes undergoes backward translocation (backtracking) along DNA. The RNAP backtracking is essential for RNA cleavage reaction catalyzed by the RNAP, which is important for reactivation of the paused transcription complex, as well as for maintenance of the transcription fidelity (proofreading). To study the structural bases of the RNAP backtracking and its subsequent RNA cleavage, we crystallized a one-nucleotide backtracked transcription complex of *Thermus thermophilus* RNAP [1]. We also obtained co-crystals of the RNAP bound with a protein factor, which enhances the RNA cleavage activity of the RNAP. Diffraction data were collected from cryo-cooled crystals, using synchrotron radiation on the beamline NE3A.

Bacteriophages hijack the host transcription machinery and direct it to serve their needs. Gp39, a protein encoded by *T. thermophilus* phage P23-45 binds *T. thermophilus* RNAP and strongly inhibits the host gene transcription, without affecting the phage gene transcription. We obtained co-crystals of *T. thermophilus* RNAP holoenzyme (RNAP + initiation factor σ) and gp39. We also obtained co-crystals of gp39 variants and the β-flap domain of the RNAP [2]. We performed data collection with these crystals.

3 Results and Discussion

We succeeded in structure determination of *T. thermophilus* RNAP holoenzyme bound to gp39 [2]. The structure revealed that gp39 binds at the RNA exit site of RNAP, and dramatically relocates the C-terminal domain of the σ factor, which is responsible for recognition of the –35 promoter consensus element. The ~45 Å displacement of the C-terminal domain of the σ subunit is not compatible with a stable complex formation between the RNAP and promoter consensus sequence elements, accounting for the inhibition of transcription. The structure reveals a novel mechanism by which a phage-encoded external protein factor regulates transcription by the host RNAP, to switch gene expression in favor of phage development.

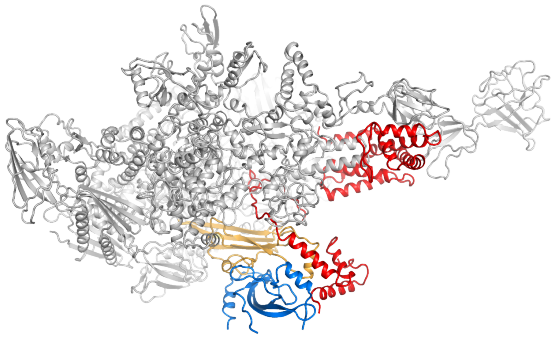

Fig. 1: Crystal structure of *Thermus thermophilus* RNAP holoenzyme bound to a phage protein gp39. The RNAP is colored gray, except for the β-flap domain colored orange. The σ factor (subunit) and gp39 are colored red and blue, respectively.

Acknowledgements

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References

[1] Murayama *et al*. *Acta Cryst*. *Sect F* **69** (2013) 174–177.
[2] Tagami *et al*. *Genes Dev*. **28** (2014) 521-531.

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