

Structural Studies of the Elongation Complex of T7 RNA Polymerase

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Introduction

DNA-directed RNA polymerase (RNAP) catalyzes the transcription of RNA message (mRNA precursor) in two distinct phases: the initiation phase, which is characterized by *de novo* synthesis of short RNA fragments from a specific sequence of DNA template called the promoter, and the elongation phase, which is characterized by the transcription of long fragments of RNA processively in the absence of the promoter. Our laboratory has previously reported on the crystal structures of T7RNAP apo-enzyme (inhibited by T7 lysozyme) [1] and on the initiation complex of T7 RNAP [2, 3].

Methods and Materials

DNA constructs were synthesized at Yale's Keck Facilities and mixed with purified T7 RNAP for crystallization trials. Details on the procedures are currently submitted for publication.

Results

We determined the crystal structure of a T7 RNAP elongation complex containing a "transcription bubble": a 30-base duplex of DNA with a central 10-base-long noncomplementary loop and a 17-nucleotide RNA fragment with partial complementarity to the template DNA. Compared to the T7 RNAP initiation complex crystal structure [2, 3], there are major changes in the conformation and fold of the polymerase and changes in the orientation and binding of the DNA template. These changes in protein structure, mainly located in the N terminal domain, include (1) loss of the promoter binding site and gain of a novel upstream DNA binding site, (2) enlargement of the active site to accommodate longer DNA-RNA duplexes, (3) a repositioning of the specificity loop to contact the 3' end of the RNA transcript during elongation, and (4) formation of a positively charged exit tunnel for RNA. In the elongation complex, the template DNA and mRNA are splayed apart by the

thumb domain of the polymerase and the rim of the RNA exit tunnel.

The differences seen between the initiation complex and the elongation complex provide a structural basis for the high processivity of mRNA during transcription and rationalize a set of previously uninterpretable mutations in the N terminal region of T7 RNAP.

Discussion

The crystal structure of a trapped intermediate of elongation with T7 RNAP illustrates that the strict economy of sequence information available to a virus has led to the perfection of a single subunit RNA polymerase, which, instead of recruiting additional subunits for function like its bacterial homologs, refolds part of itself and reorients its substrates in order to become a processive polymerase.

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