Bacterial RNA Polymerase: New Insights on a Fundamental Molecular Machine

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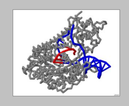
ABSTRACT

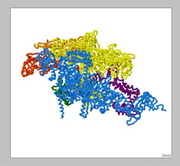
RNA polymerase (RNAP) is an information-processing molecular machine that copies DNA into RNA. It is a multi-subunit complex found in every living organism. Bacterial RNAP contains six subunits ($\beta\beta'\alpha_2\omega\sigma$). The $\beta\beta'$ subunits form several distinct functional channels that accommodate double stranded DNA and an RNA-DNA hybrid. The exit channel guides the growing RNA strand out of the complex, and the secondary channel allows nucleotides (NTPs) to enter the active site. This model focuses on the β' subunit of *Thermus themophilus* that contains the highly conserved active site sequence and several structures involved in the catalytic mechanism. The bridge helix (BH) and trigger loop (TL) work together as a "gate" to enhance the catalytic action by facilitating NTP addition. In the crystal structure of the RNAP elongation complex (EC) without NTP in the active site, the TL (β' 1236-1265) is unstructured. In the EC crystal structure with a non-hydrolysable nucleotide (AMPcPP), the TL folds into two anti-parallel helices (trigger helix, TH) that interact with the adjacent BH to create a three-helical bundle forming a catalytically active complex. The other structures that are functionally important in the β' subunit are the "lid" (β' 525-539) that cleaves the RNA-DNA hybrid, directing the newly formed RNA out through the exit channel, and the "rudder" (β' 582-602) that helps to stabilize the DNA helix and the RNA-DNA hybrid in the active site channel.

INTRODUCTION

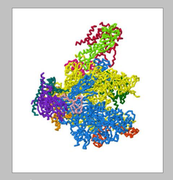
Transcription is a complex, multi-step process with RNAP playing a central role. Transcription begins when the holo-RNAP containing a specific sigma factor (σ) binds to a promoter sequence on DNA. The double-stranded DNA downstream of the promoter occupies an active site channel formed by the $\beta\beta'$ subunits. The DNA unwinds near the transcription start site in the region of the catalytic site of RNAP. Synthesis of 7-9 ribonucleotides (NTPs) stimulates promoter clearance and the transition to the elongation phase of transcription. During RNA synthesis NTPs enter the active site via the secondary channel. A recently identified structure called the "trigger helix" functions as a swinging gate that positions the incoming nucleotide into the catalytic site to facilitate nucleotide addition. The DNA-RNA hybrid structures that form during synthesis pass through the region of the β' subunit called the "lid" that is responsible for splitting the hybrid. The growing RNA chain is guided out of RNAP via the exit channel. The termination complex causes RNAP to return to its closed complex and frees DNA to dissociate from RNAP.



T7 Phage RNAP

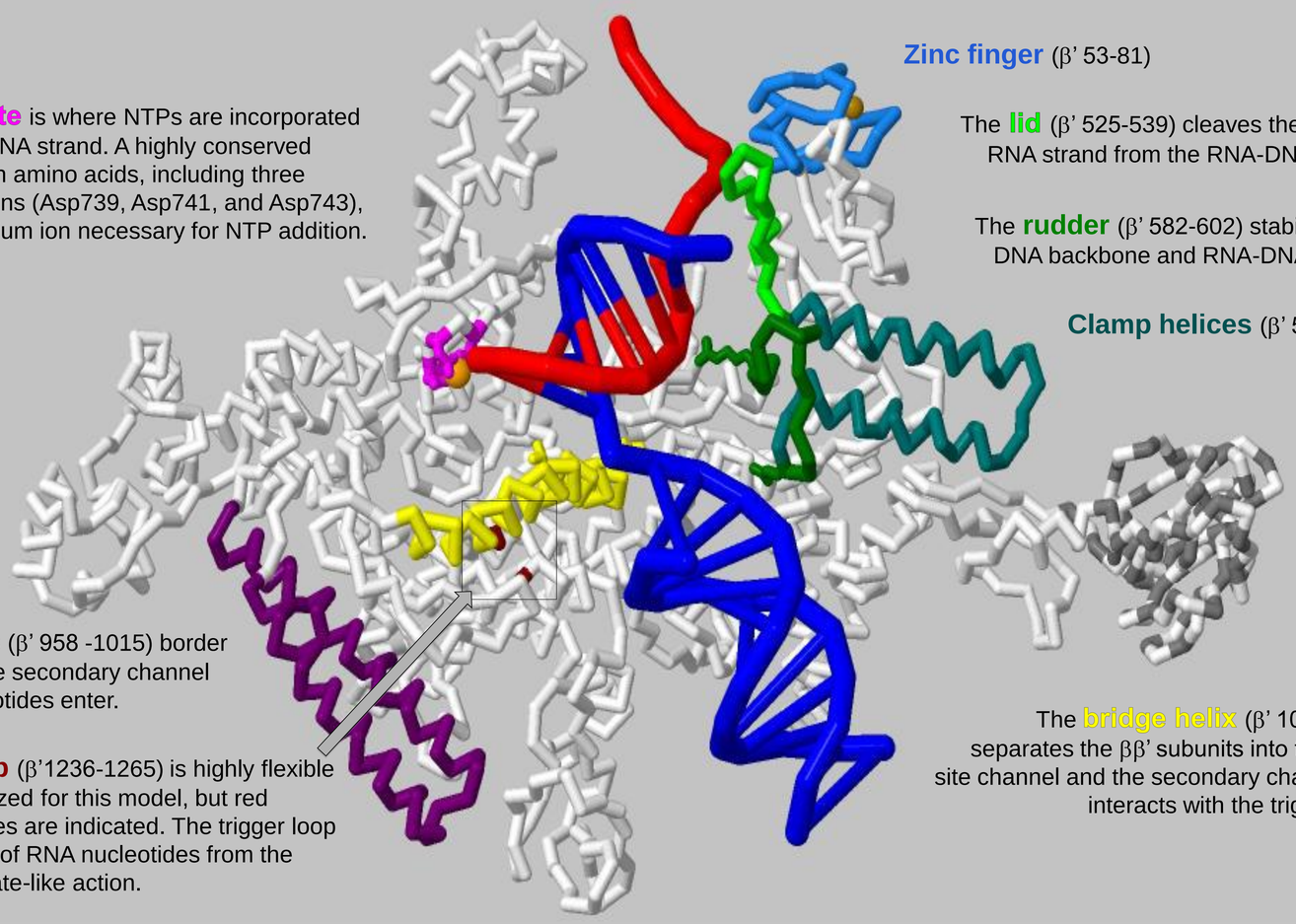


Thermus thermophilus RNAP (6 subunits)



Saccharomyces cerevisiae RNAP (12 subunits)

THE MODEL: β' SUBUNIT OF RNA POLYMERASE



Zinc finger (β' 53-81)

The **lid** (β' 525-539) cleaves the growing RNA strand from the RNA-DNA hybrid.

The **rudder** (β' 582-602) stabilizes the DNA backbone and RNA-DNA hybrid.

Clamp helices (β' 540-581)

The **catalytic site** is where NTPs are incorporated into the growing RNA strand. A highly conserved sequence of seven amino acids, including three aspartate sidechains (Asp739, Asp741, and Asp743), chelate a magnesium ion necessary for NTP addition.

The **rim helices** (β' 958 -1015) border the entrance to the secondary channel where RNA nucleotides enter.

The **trigger loop** (β'1236-1265) is highly flexible and is not crystallized for this model, but red positioning residues are indicated. The trigger loop prevents diffusion of RNA nucleotides from the active site via a gate-like action.

The **bridge helix** (β' 1067-1102) separates the $\beta\beta'$ subunits into the active site channel and the secondary channel and interacts with the trigger loop.

Jmol

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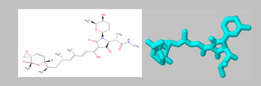
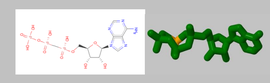
NEW INSIGHTS: RECENT RESEARCH FINDINGS

Pre-insertion State (AMPcPP & streptolydigin)

Insertion State (AMPcPP)

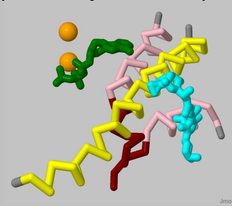


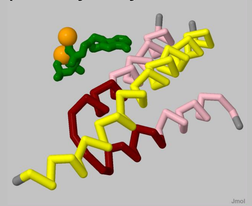
Streptolydigin (Inhibitory antibiotic)



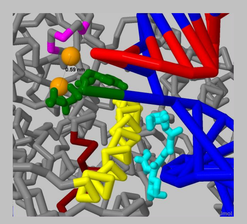
AMPcPP (Non-hydrolysable NTP)

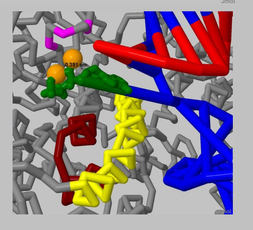
The insertion state of the TL features a distinct three helical bundle structure (TH).



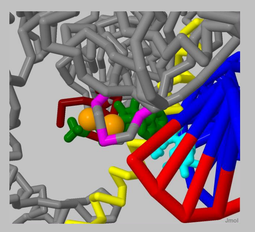


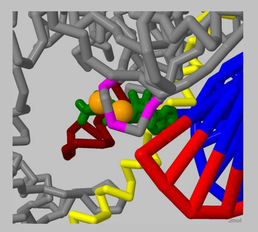
The TH orients the incoming NTP and moves the Mg^{2+} atoms closer.





The TH rotates into the secondary channel, resulting in correct NTP orientation.





SUMMARY

- RNAP is an essential multi-subunit enzyme of living organisms that carries out the process of transcription.
- RNAP operates as a molecular machine with many distinct components contributing specific functions.
- Much of the amino acid sequence of the β' subunit is highly conserved between different organisms suggesting a high degree of functional significance.
- The discovery of the trigger loop has enhanced our understanding into the mechanistic function of RNAP and has presented a possible new drug target.

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Lane, W.J.
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PDB files:

Model: 205I
T7 Phage RNAP: 1S77
Preinsertion state: 2PPB
T. thermophilus RNAP: 2A6E
Insertion state: 2O5J
S.cerevisiae RNAP: 3PO2