Mechanism for de novo RNA Synthesis by T7 RNA Polymerase

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DNA-directed RNA polymerases (RNAP) are distinguished by their ability to initiate *de novo* synthesis of polymer, RNA, from a promoter without the requirement for a 3'OH primer-terminus. The RNAP from bacteriophage T7 requires no accessory factors for RNA synthesis during the initiation phase of transcription. T7 RNAP is unique, however, in its requirement for *de novo* synthesis to start with the incorporation of two GTPs at the beginning of transcription.

We examined the structural and kinetic basis for *de novo* synthesis of RNA by using a series of novel DNA constructs which varied the template DNA initiation sequence and the incoming NTP analogues. Nine structures of T7 RNAP with promoter DNA and/or an incoming pair of NTPs were determined to 2.2 to 3.2 Å resolution and are *the first structural examples of de novo RNA synthesis by an RNA polymerase*. Different promoter-template DNA constructs are bound to the enzyme in virtually identical conformations. The two initiating NTPs are accommodated in the enzyme by changes to both the geometry of the active site and a novel bend in template DNA. The incoming NTPs are recognized by novel enzyme and template DNA interactions. Active site residues make a large contribution to the recognition of the initiating NTPs in addition to the specific DNA template base-pair interactions. Pre-steady state kinetic measurements support the idea that discrimination of initiating nucleotide by the enzyme plays a greater role than template specification of nucleotide selection for *de novo* synthesis of RNA by T7 RNA polymerase.

Keywords: transcription initiation, polymerase, nucleotide selection