The stability of T7 RNA polymerase elongation complexes and promoter function analysis by in vitro selection

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Abstract

The forward translocation model (paused or stalled polymerase moves forward in the absence of transcription, dissociation of the complex happens when hybrid is too short to stabilize the complex) is tested by measuring the stabilities of stalled elongation complexes on DNA constructs that either favor or inhibit forward translocation. Results are consistent with the model; conditions that favor forward translocation lead to decreased stability, while conditions that disfavor forward translocation lead to increased stability. Results also show that the rewinding of the upstream edge of the transcription bubble is a major driving force for forward translocation. In separate biochemical assays, it is demonstrated that the stability of stalled complexes can also be dominated by "bumping" from a trailing polymerase transcribing from the same direction. Trailing polymerases can efficiently displace a stalled polymerase. As predicted by current models for promoter clearance, the instability caused by "bumping" is position dependent. When the first polymerase is stalled 12 bp or less from the promoter, a second polymerase is blocked from binding the promoter. When the first polymerase is stalled between 12 to 20 bp, the second polymerase can bind and make abortive transcripts, but it cannot displace the first complex since it is in an unstable initiation conformation. Only when the leading polymerase is stalled beyond 20 bp, can the second polymerase bind, initiate and displace the leading complex. The function of the promoter region that is melted in the initiation bubble (-4 to +4, relative to the transcription start site) of the T7 RNA polymerase is probed by SELEX (*in vitro* selection). Results demonstrate that there is no convergence among the selected tight binding sequences. So, those bases are not direct contributors for binding. But the selected sequences show a bias toward AT rich after many rounds of SELEX. This is consistent with the idea that bending or melting at this region facilitates promoter-polymerase binding. Finally, SELEX is also used to select for DNA sequences that will allow stably stalled elongation complex. Preliminary results are consistent with the forward translocation model.^

Subject Area

Biochemistry

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