Title

Structural Changes That Drive Timed Promoter Release In Transcription Reciprocally Lead To Abortive Instability

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Abstract

RNA polymerases carry out a process, which at times carries contradictory mechanistic requirements. During initiation, an RNA polymerase should be highly sequence specific, binding only to and initiating only from specific promoter sequences. However, during processive elongation, an RNA polymerase should show no sequence specificity. Thus after sequence specific initiation, it must break initial tight binding contacts in a process known as promoter escape. An RNA polymerase must also carry out de novo initiation, starting from a 2mer, to a 3mer, etc. RNA. We have shown that elongating RNA polymerases achieve their high stability in part from a topological locking of the RNA around the DNA template strand. But a 4 base RNA transcript has insufficient length for stability from either topological or base pairing considerations; the complex must maintain substantial protein-DNA interactions. Not surprisingly then, RNA polymerases retain strong promoter contacts during synthesis of the first 8-10 bases. Also not surprisingly, short RNA (abortive) products are released along the path to a stable elongation complex. What governs the probability of abortive synthesis? How is subsequent promoter release achieved? What energetics drive that release? How is the translocational timing of release achieved? The current study builds on a model in which growth of the RNA-DNA hybrid drives a small rotation of the promoter binding domain in the protein, leading to 1) a physical separation of two components (the specificity loop and the N-terminal platform) and/or 2) disruption of the open complex stabilizing intercalating loop selectively weakening promoter binding at translocation past position +8. Probing complexes halted at specific positions, we explore a kinetic pathway in which translocating polymerases, release promoter contacts at different positions, with different rates. Current models for abortive cycling propose that the enzyme has difficulty achieving the structural transition to elongation. A mutant P266L, which shows dramatically reduced abortive cycling, has been proposed to possess a lower barrier for the transition to elongation and thus to transition sooner. We show instead that the mutant transitions to the promoter-released elongation configuration later than wild type does. We propose that the mutant shows a delay in the barrier to rotation, resulting in a delay in the transition to elongation. Conversely, lower initial barriers to rotation lead to lower destabilizing "push back" against the hybrid, leading to reduced abortive dissociation. As a direct test of this model, mutations (insertions and deletions) in the connecting C-terminal "leg" show the same phenotype (delayed transition and lower abortive dissociation) as

the initial P266L mutant, as predicted.

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