

# Backtracking by RNA polymerase: single molecule experiments vs theory

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RNA polymerase II (RNAP II) is responsible for transcribing all mRNAs in eukaryotic cells in a highly regulated process that is conserved from yeast to human and that serves as a central control point for cellular function. We have investigated the transcription dynamics of single RNAP II molecules against force in a single-molecule dual-trap optical-tweezers assay. Remarkably, we found that the response of RNAP II to force is entirely determined by enzyme backtracking, a pause state that is involved in the proofreading of the RNA transcript. We found pause durations be broadly distributed and to follow a  $t^{-3/2}$  power law, giving rise to both short and long-lived pauses. To understand the mechanism behind backtracking, we modeled backtracking as a force-biased random walk. Importantly, we found that this single mechanism naturally generates two populations of pauses that are distinct both in duration and trajectory: long-time pauses with the behavior expected for random-walk backtracks, and a new class of short-time backtracks with characteristics similar to those of the ubiquitous pause. We therefore suggest that pauses that have previously been suggested to stem from distinct mechanisms are to a significant extent simply due to backtracking. Finally, we studied the role of conformational characteristics of the RNA strand in the context of the early stages of transcription, during which the polymerase is prone to premature and irreversible stalling. Specically, we examined the hypothesis that the absence of long transcripts contributes to stalling in the vicinity of the promoter.

Taken together, we have shed light on the basic physical mechanisms that underly transcription by RNA polymerase II, using a combination of experimental and theoretical approaches.