

Mechanism of sequence-specific pausing of bacterial RNA polymerase

Maria L. Kireeva and Mikhail Kashlev¹

National Cancer Institute Center for Cancer Research, Frederick, MD 21702

Edited by Jeffrey W. Roberts, Cornell University, Ithaca, NY, and approved March 25, 2009 (received for review January 14, 2009)

Sequence-specific pausing of multisubunit RNA polymerases (RNAPs) represents a rate-limiting step during transcription elongation. Pausing occurs on average every 100 bases of DNA. Several models have been proposed to explain pausing, including backtracking of the ternary elongation complex, delay of translocation of the enzyme along DNA, or a conformational change in the active site preventing formation of the phosphodiester bond. Here, we performed biochemical characterization of previously-reported pauses of *Escherichia coli* RNAP and found that they are not associated with backtracking or a translocation delay. Instead, the paused complex contains the 3' end of the transcript in the active center and is capable of binding the next cognate NTP. However, bond formation occurs much slower in the paused complex compared with its fully-active counterpart. The pausing is dramatically decreased by a substitution of the base encoding the next incoming NTP and the base encoding the 3' end of the nascent RNA, suggesting that (mis)-alignment of the 3' end of the RNA and the incoming NTP in the active site is crucial for pausing. These pause sites are conserved between *E. coli* and *Thermus thermophilus* RNAPs, but are not recognized by *Saccharomyces cerevisiae* RNAP II, indicating that prokaryotic RNAPs might be more sensitive to the changes in the alignment of the nascent transcript and the substrate NTP in the active site.

backtracking | elongation | transcription | translocation

Transcription elongation in vivo and in vitro is interrupted by sequence-specific pauses (1, 2). Pausing is broadly involved in regulation of gene expression (reviewed in ref. 3). In prokaryotes, pausing regulates the expression of *his* (4), *trp* (5), *pyr* (6), and other operons (7). It is essential for RNA folding (8), synchronization of transcription and translation (9, 10), and ρ -dependent (11) and ρ -independent termination (12). In eukaryotes, paused RNA polymerase II (Pol II) has been detected in the promoter-proximal region of numerous genes (13); reactivation of these ternary elongation complexes (TECs) emerges as a general mechanism for rapid induction of gene expression (reviewed in ref. 14). It is not clear whether pausing occurs by a single conserved mechanism shared by the prokaryotic and eukaryotic RNA polymerases (RNAPs).

It has been firmly established that a transient catalytic inactivation of TECs may occur by reverse translocation (backtracking) of RNAP. Originally, backtracking was observed for a *Escherichia coli* RNAP TEC deprived of substrate NTPs (15, 16). Backtracking does not affect the size of the transcription bubble or the length of the RNA–DNA hybrid, but leads to extrusion of the 3' end of the nascent RNA from the active site (16). Backtracking occurs when the RNA–DNA hybrid in the upstream position is more favorable than the RNA–DNA hybrid at the 3' end of the nascent RNA thermodynamically (15) or presents a preferable binding substrate for the polymerase (17). In some stalled TECs backtracking is reversible (15). It has been suggested that limited and reversible backtracking leads to pausing (15, 18). Indeed, backtracking has been observed at the *E. coli ops* pause site (18), at the HIV transactivation response Pol II pause site (19), and in the promoter-proximal region of the adenovirus major late promoter template (20). Backtracking

resulting in sequence-specific pausing has been used to explain bursts of mRNA production and a broad distribution of transcription elongation times observed in vivo (21). Backtracking that results in a broad spectrum of pausing events lasting 1–10 s has been proposed for *Saccharomyces cerevisiae* Pol II (22) and *E. coli* RNAP (23). Modeling of transcription elongation based on the assumption that the relative efficiencies of forward translocation, reverse translocation, and backtracking of RNAP are determined by the free energy of the TEC at adjacent positions on the template predict the pausing distribution of *E. coli* RNAP with reasonable accuracy (24, 25).

However, pausing in the leader sequences of biosynthetic operons in bacteria does not involve backtracking (18, 26). It depends on formation of the secondary structure in nascent RNA (pausing hairpin) and is determined by a multipartite signal coming from the 3' end of the RNA, the RNA–DNA hybrid, and the front-end DNA duplex ahead of the hybrid (27). This hairpin-stabilized pausing is thought to involve an allosteric conformational change in the protein, causing a delay in RNAP translocation and misalignment of the 3'-terminal RNA base with the active center and the complementary base in the template DNA strand (28). It remains unclear whether all of the pausing is explained by the hairpin-stabilized and backtracking mechanisms.

The quest to determine consensus sequences that causes transcriptional pausing started 3 decades ago. Based on the analyses of multiple *E. coli* RNAP pause sites in the early transcribed region of bacteriophage T7 and its D111 deletion variant, Aivazashvili et al. (29) proposed that the rate of each NTP incorporation depends on the nature of the base at the 3' end of the nascent transcript, and on the surrounding sequence, including the next NTP to be incorporated to the paused complex. They did not find any correlation between pausing and hairpins in the nascent RNA. Subsequently, Levin and Chamberlin (30) addressed the role of the primary sequence, base composition, RNA secondary structure, and elongation factors on transcription pausing. Their data suggested that pausing of *E. coli* RNAP occurs by several mechanisms, with some, but not all pauses correlating with formation of RNA hairpins. The possibility of RNAP backtracking at these pause sites was not addressed in these early works because the phenomenon of backtracking was discovered years later (16).

In this work, we perform systematic biochemical analyses of the *E. coli* RNAP TEC paused at previously-identified (29, 30) sequence-specific sites, A32 and C37 on the T7A1 D111 template. Two thermodynamic models (24, 25) have predicted that translocation delay or backtracking occur at these sites. Our present analysis found no evidence of a backtracking or translocation block, but, instead, revealed that efficient binding of the

Author contributions: M.L.K. and M.K. designed research; M.L.K. performed research; M.L.K. analyzed data; and M.L.K. and M.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: mkashlev@mail.ncicrf.gov.

This article contains supporting information online at www.pnas.org/cgi/content/full/0900407106/DCSupplemental.

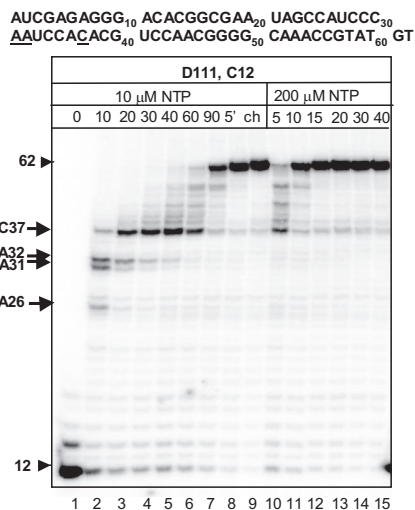


Fig. 1. Pausing of *E. coli* RNAP on the T7A1 D111 template.

next cognate NTP in the paused TEC is followed by a significant delay in phosphodiester bond formation. We conclude that at least some ubiquitous sequence-specific transcriptional pausing events are not determined by the translocation state of RNAP.

Results and Discussion

Pausing Pattern of *E. coli* RNAP on T7A1 D111 Template. The pausing pattern of *E. coli* RNAP transcribing bacteriophage T7 A1 D111 template determined in our experimental system is fully consistent with published results (29, 30). *E. coli* RNAP undergoes transient pauses at A26, A31, A32, and C37 positions (Fig. 1, lanes 1–9) at 10 μM NTP. The C37 pause appears predominant. At a higher (200 μM) concentration of NTPs, all pauses decrease, A26 and A31 pauses virtually disappear, and the C37 pause remains predominant (Fig. 1, lanes 10–15). At this site, pausing occurs after incorporation of CTP and before incorporation of ATP. As for the other pauses described (29, 30), the pausing duration depended on the concentration of substrate CTP, with the pause escape rate increasing from 0.032 s^{-1} at 10 μM CTP to 0.46 s^{-1} at 200 μM CTP (Fig. S1 A and B). C37 pausing is independent from the RNA secondary structure, because truncation of the nascent RNA in TEC37 by degradation with RNase T1 does not affect pausing in this position (Fig. S1D). Taken together, these observations suggest that C37 represents an example of a sequence-specific ubiquitous pausing site that does not depend on the nascent RNA secondary structure (31). We further characterized properties of the TEC paused at the C37 position to determine the pausing mechanism.

Pausing at C37 Is Not Associated with Backtracking or Translocation Delay.

The extent of backtracking at a given site correlates with the length of time of RNAP stalling by substrate deprivation (16, 18). To address the connection between backtracking and pausing at C37, we tested whether the escape from the pause is slowed down by stalling the TEC at the pause site, as has been reported for backtracked pauses (18, 19). RNAP was set to transcribe through the pause site at 10 μM NTP starting from position C35 (Fig. 2A, read-through) and C37 (Fig. 2A, stalled). In this setup, Pol II from C35 complex was able to proceed through the pause site without artificial stopping. The graphs in Fig. 2A show the percentage of C37 complex as a function of time. Apparently, C37 TEC stalled at the pause site for 10 min and its counterpart that transcribed through the pause site starting from the C35 position exhibited the same dynamics of escape from the pause. Backtracked pauses and arrests are suppressed by oligonucleo-

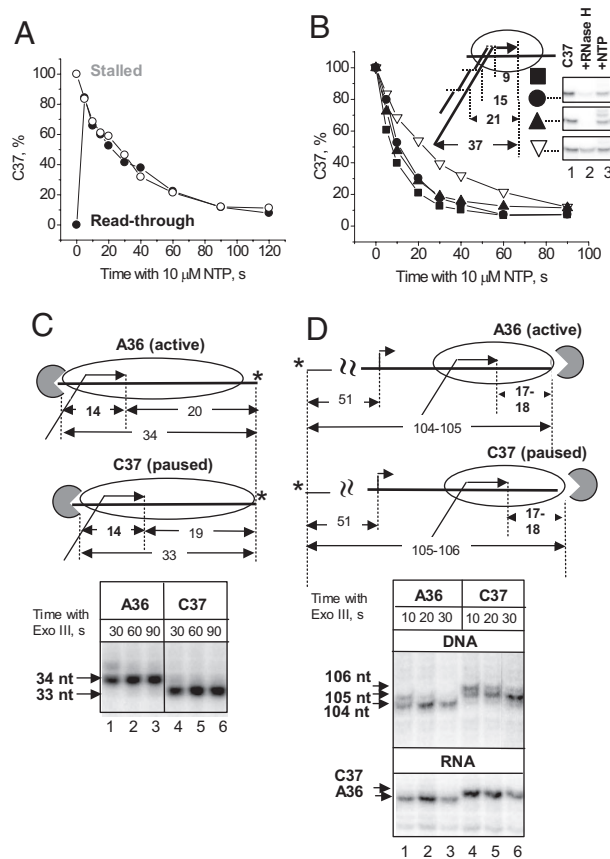


Fig. 2. Pausing at C37 is not associated with RNAP backtracking. (A) Kinetics of escape from the stall compared with reading through the pause site. TECs were stalled upstream from the pause site (solid symbols) or at the pause site (open symbols), and chased with 10 μM NTP. (B) Kinetics of escape from the pause site in the presence of antisense oligonucleotides. A36 was treated with 1 mM hexanucleotide complementary to the nascent transcript, and incubated with 10 μM CTP for 10 min. The location of hexanucleotides in respect to the 3' end of the nascent transcript in C37 is indicated in the scheme. An aliquot of each reaction (Inset, lane 1) was treated with 0.2 units/ μL of RNase H (Inset, lane 2) to confirm oligonucleotide hybridization. The remaining TEC37 was chased with 10 μM NTP and the reaction was stopped at various time points. The 5-s time point of the chase reaction is shown in lane 3 of the Inset. (C) Rear-end Exo III footprinting of A36 and C37 TECs. (D) Front-end Exo III footprinting of A36 and C37 TECs.

tides that hybridize to the transcript 12–20 bp upstream from the 3' end of the paused/arrested TEC (16, 18). We tested the effect of antisense oligonucleotides hybridized to the 5' part of 37-nt RNA on the C37 pause by walking the TEC to the A36 position, adding an antisense oligonucleotide, and advancing the TEC to the pause site by addition of CTP. Subsequently, escape from the pause site was induced by addition of all 4 NTPs. Hybridization of short DNA oligonucleotides to the nascent transcript at a distance of 9, 15, and 21 bases upstream from the 3' end of the RNA resulted in a modest (≈ 2 -fold) decrease of the C37 pause half-life (Fig. 2B). The efficient cleavage of the 37-nt transcript with RNase H confirmed the successful complementary DNA oligonucleotide hybridization to the nascent RNA (Fig. 2B Inset, compare lanes 1 and 2). The absence of sensitivity to stalling or antisense oligonucleotides by the C37 pause is not consistent with a backtracking mechanism for pausing at this site. In addition, pausing at C37 is not decreased by the transcript cleavage factor GreB (Fig. S2B), which reactivates backtracked TECs (16).

Next, we tested whether pausing at the C37 is caused by a delay of RNAP translocation. The pretranslocated and posttranslo-

cated states of the TEC differ in the position of the 3' end of the transcript relative to the active center of RNAP. The pretranslocated complex contains the 3' end of the RNA occupying the NTP binding pocket in the active center, preventing loading of the next substrate, which could be a source of pausing. The posttranslocated TEC originates from the pretranslocated TEC by a 1-bp forward shift of the enzyme along the transcript and the template leading to the clearance of the NTP binding site. In the absence of NTPs, the TEC appears to be in equilibrium between these 2 states at each template position, which could be detected by using time-resolved exonuclease III (Exo III) footprinting (28, 32). The rear-end footprint of active A36 appeared as a single band 14 bp upstream from the RNA 3' end at all times of incubation with Exo III (Fig. 2C, lanes 1–3). The 14-nt distance between the 3' end of the RNA and the rear-end boundary is characteristic of the posttranslocated TEC (28). Notably, the A36 boundary shifts 1 bp downstream upon formation of the strongly-paused C37 complex (Fig. 2C, lanes 3–6), suggesting that the translocation states of the active A36 and the paused C37 TECs are the same. Importantly, the absence of Exo III degradation products >34 nt, which would reveal C37 backtracking, provide further evidence that RNAP remains engaged with the 3' end of the RNA in the paused C37 complex. To confirm these conclusions, we performed front-end Exo III footprinting of the A36 and C37 complexes.

At short incubation times with Exo III, the front-end footprint of an active A36 complex represents a double band corresponding to both pretranslocated and posttranslocated boundaries located 17 and 18 bp downstream from the 3' end of the RNA (Fig. 2D, lane 1). Upon longer incubation with Exo III (Fig. 2D, lanes 2 and 3), the 18-bp boundary disappears, suggesting that the A36 complex easily shifts to the pretranslocated state, providing access of Exo III to an extra base in DNA. In the paused C37 TEC, the footprint shifts 1 bp downstream from its position in the A36 TEC (Fig. 2D, lanes 4–6), demonstrating that the distance between the RNAP boundary and the end of the transcript is the same in the active A36 and paused C37 TECs. Furthermore, the equilibrium between the pretranslocated and posttranslocated states in the active A36 and paused C37 TECs is nearly identical as revealed by the similar rate of disappearance of the posttranslocated boundary upon prolonged incubation with Exo III (Fig. 2D, lanes 2, 3, 5, and 6). Once again, the front-end footprint of the paused C37 TEC does not reveal the presence of backtracked RNAP, which would result in accumulation of products <105 nt. We conclude that pausing at the C37 position is not caused by delayed translocation or backtracking.

Paused TEC Binds Incoming NTP as Efficiently as an Active TEC. Next, we addressed the mechanism of pausing by testing its sequence determinants. We made a single substitution in the D111 template sequence, changing C37 to G37. This mutation practically eliminated pausing at position 37, leaving the A26, A31, and A32 pauses intact (Fig. 3A, compare lanes 1–15 and 16–30). Therefore, CMP at the RNA 3' end promoted formation of a paused conformation in the TEC with a 37-nt RNA, whereas GMP at the 3' end prevented pausing, increasing the rate of the escape from the pause 20-fold (Fig. 3B). Comparison of Exo III footprints of C37 and G37 TECs showed that the equilibrium between the posttranslocated and pretranslocated states has not been affected by the base substitution (Fig. 3C, compare lanes 1–3 and 7–9). Binding of the incoming complementary NTP to a stalled TEC can be detected by a characteristic shift of the pretranslocated/posttranslocation equilibrium to the posttranslocated state as a result of stabilization of the posttranslocated TEC by the NTP in the active center (28). Because the templates carrying paused C37 and active G37 TECs encode AMP in position 38, Exo III footprinting in the presence of nonhydrolyzable ATP analog (AMPcPP) allows comparison of the incoming NTP

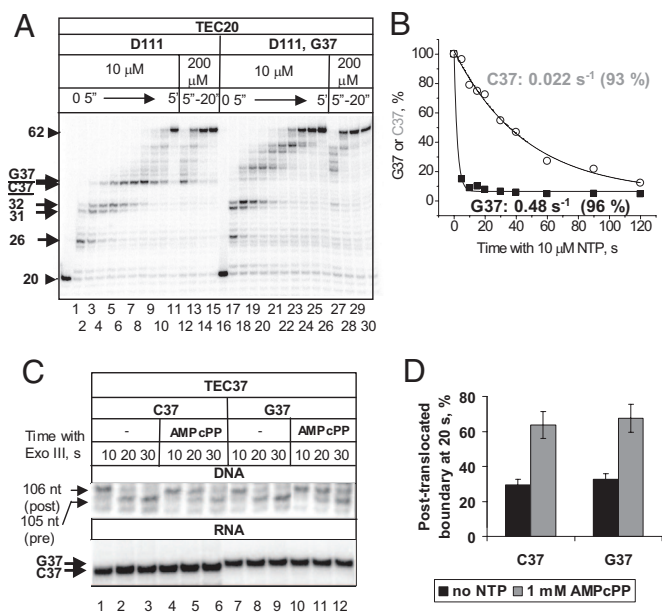


Fig. 3. The nature of NMP at the 3' end of the nascent RNA is one of the sequence determinants of pausing. (A) Mutation of C37 to G37 dramatically reduces pausing at this position. (B) Kinetics of escape from G37 and C37 sites. (C) Exo III footprint of the front-end boundary of C37 and G37 TECs and effect of the incoming NTP substrate on the translocation equilibrium. (D) Quantitative analyses of the pretranslocated and posttranslocated boundaries of RNAP. Error bars show SD for 3 independent experiments.

binding by the paused and active TECs. Fig. 3C shows that paused C37 and active G37 bind an incoming NTP in a similar manner: addition of 1 mM AMPcPP stabilized the posttranslocated conformation of both C37 and G37 TECs (compare lanes 4–6 and 10–12). Quantitative analyses of the enzyme boundary shift induced by AMPcPP (Fig. 3D) confirmed that response of the 2 TECs to the incoming complementary substrate is nearly identical. Therefore, the paused TEC binds the incoming NTP as efficiently as the active TEC does. The delay in the nucleotide addition appears to take place after the incoming NTP enters the active site. Therefore, we addressed the role of the incoming NTP in formation of the pausing intermediate.

Pausing Depends on the Nature of the Incoming NTP. Three derivatives of the D111 template were generated, in which AMP encoded in position 38 was substituted with CMP, GMP, and UMP. All of these templates encoded CMP at position 37. Pausing patterns on the original D111 template are compared with the 3 mutant variants in Fig. 4A. Evidently, substitution of AMP by GMP only slightly decreases the pausing efficiency, but substitution of AMP to CMP or UMP practically eliminates pausing at C37. Importantly, mutation of AMP to CMP does not change the equilibrium between the pretranslocated and posttranslocated states in the C37 TEC (Fig. 4B), once again arguing against involvement of a translocation block in C37 pausing. Indeed, the dynamics of posttranslocated front-end boundaries in the C37 complex obtained on the original D111 sequence with a terminating 3' dCMP incorporated at the 3' end, and the C37 complex obtained in the same way on the D111C38 template are indistinguishable (Fig. 4B, compare lanes 1–3 and 4–6). The dramatic effect of the incoming NTP substitutions on pausing, but not on the translocation equilibrium, is consistent with the finding that paused and active TECs translocate and bind the substrate NTP with similar efficiency. It suggests that sequence-specific pausing analyzed in this work strongly depends not only

catalytically-competent configuration (36), at least some of the other states may be structurally similar to the paused conformation characterized in our present work. Importantly, the 2 TEC fractions with dramatically different elongation rates observed at the pause sites (Fig. 5A and B and Fig. S3) suggest that paused intermediate is distinct from the intermediates of the main reaction pathway (31).

The pausing model shown in Fig. 5C is based on the assumption that the incoming NTP might pair with the DNA base, which is not yet fully aligned with the 3' NMP (36). The resulting intermediate 1 gives rise to at least two distinct conformations (Fig. 5C, intermediates 2 and 3), only one of which is capable of rapid bond formation (intermediate 2). The pathway choice depends on the 3' NMP and the incoming NTP in the given sequence context. Specifically, 3' CMP supports the transition of the incoming ATP to the paused configuration (1 → 3) rather than the transition to the catalytically-competent configuration (1 → 2). In contrast, 3' GMP supports the productive alignment of ATP. Unlike ATP, incoming UTP or CTP preferentially form a catalytically competent intermediate when aligned with 3' CMP.

We cannot exclude the idea that the conformational branching to the paused intermediate occurs before NTP binding (Fig. 5D). Exceptional flexibility of the DNA base encoding the next NTP substrate in the posttranslocated TEC (36) suggests that the DNA base might acquire an active or paused conformations depending on the nature of the 3' NMP–dNMP base pair. In the case of C37 pause, the dG–rC base pair would support a paused conformation of the next dT base, and an active conformation of the next dA or dG bases. The dC–rG base pair would promote active conformation of the dT base. To be consistent with result shown in Fig. 3, this model requires that the incoming NTP pairs with the paused and active conformations of the DNA base with equal efficiency.

Evidently, a specific combination of 3' NMP and incoming NTP is necessary but not sufficient to induce pausing. *E. coli* RNAP does not pause at all at C/A or A/U junctions in DNA. The sequences of the RNA–DNA hybrid and downstream DNA affect hairpin-stabilized pauses (3, 27, 39). Similarly, the conformations of the 3' NMP and incoming NTP in the hairpin-independent pauses might depend on the local sequence context. A detailed comparison of other aspects of the hairpin-independent pausing studied in this work and hairpin-stabilized pausing is provided in *SI Text*.

Sequence-Specific Pausing Is Not Conserved Between Prokaryotic RNAPs and *S. cerevisiae* Pol II. The model for sequence-specific pausing (Fig. 5) is based mainly on the structure of the *T. thermophilus* RNAP TEC (36). The *T. thermophilus* RNAP active site shares high sequence homology with *E. coli* RNAP. These 2 RNAPs are so closely related that even promoter recognition is conserved between them. We analyzed transcription of the D111 template by *T. thermophilus* RNAP (Fig. 6A) and observed that its pausing pattern is very similar to that of *E. coli* RNAP (Fig. 1). This finding suggests that the actual structure of the paused intermediate could be resolved by using X-ray crystallography methods.

E. coli RNAP and *S. cerevisiae* Pol II share the architecture of the RNA–DNA hybrid and the transcription bubble (35, 40–43). Pol II recognizes prokaryotic intrinsic transcription terminator in vitro (44). The catalytic and fidelity mechanisms of the bacterial and yeast enzymes are also similar, relying on the mobility of the trigger loop, a small mobile element, which closes on the cognate NTP in the active site (36, 37, 42, 45). The trigger loop has been recently implicated in hairpin-stabilized pausing of *E. coli* RNAP (28). At least some sequence determinants of pausing could be conserved between *E. coli* RNAP and *S. cerevisiae* Pol II. We compared the transcription patterns by TECs assembled from DNA and RNA

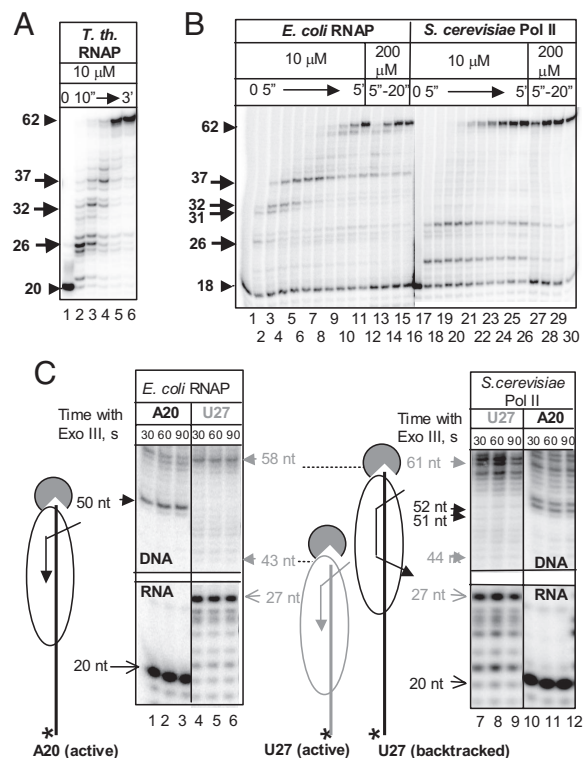


Fig. 6. Comparison of pausing patterns of *T. thermophilus* RNAP, *E. coli* RNAP, and *S. cerevisiae* Pol II. (A) Pausing of *T. thermophilus* RNAP on the D111 template. Transcription was performed at 37 °C. (B) Pausing patterns of *E. coli* RNAP and *S. cerevisiae* Pol II. Positions of the pauses recognized by both polymerases are shown by open-headed arrows. (C) Exo III footprinting of *E. coli* RNAP and *S. cerevisiae* Pol II TECs with 20- and 27-nt RNAs.

oligonucleotides by using *E. coli* RNAP core enzyme and 12-subunit yeast Pol II on the D111 template.

Contrary to our expectations, the pausing pattern of Pol II is drastically different from the pausing pattern of *E. coli* RNAP (Fig. 6B). Pol II transcribes the A31, A32 and C37 positions without detectable delay (Fig. 6B, lanes 16–26) with some pausing and irreversible arrest only at the U21 and U27 positions at 10 μM NTP (Fig. 6B, lanes 16–30). U21 and U27 also appear as minor sites of transcriptional pausing and arrest for *E. coli* RNAP (Figs. 1A and 6B, lanes 1–15). The U27 TEC is severely inactivated by stalling (Fig. S4B). Pausing at this site is eliminated by annealing of antisense oligonucleotides to the upstream segment of the nascent RNA (Fig. S4D). These properties of the U27 complex are consistent with the backtracking mechanism of pausing at this site. Indeed, the U27 TEC formed by *E. coli* RNAP served as a paradigm of an arrested TEC in a study that established backtracking as a mechanism of transcriptional arrest (16).

The involvement of backtracking in pausing at U27 was confirmed by Exo III footprinting of *E. coli* RNAP and Pol II TECs (Fig. 6C). The Exo III footprinting revealed a distinct rear-end boundary located 14 bp from the RNA 3' end of the bacterial enzyme or 15–16 bp from the RNA 3' end of Pol II (Fig. 6C, lanes 1–3 and 10–12) in the A20 TEC, but failed to detect a corresponding boundary in the U27 TEC (Fig. 6C, lanes 4–9). The RNAP boundary in the U27 TEC appears to be located ≈30 bp upstream from the RNA 3' end (Fig. 6C, lanes 4–9). Pauses/arrests of Pol II at U21 and U27 positions become less pronounced in the presence of transcript cleavage factor TFIIIS (Fig. S2A). In summary, the pauses in common between bacterial and yeast RNAPs occur by a backtracking mechanism,

suggesting that backtracking determinants are conserved between the bacterial and yeast enzymes.

The major pauses of Pol II appear to occur by backtracking. However, we cannot rule out that Pol II undergoes backtracking-independent pausing on some sequences that are distinct from the pausing signals recognized by *E. coli* RNAP. In fact, pausing of Pol II at the C12, C14, and C17 positions of the D111 template are likely to be backtracking-independent, because they are suppressed by a *rpb1-E1103G* mutation (46) that is proposed to increase the mobility of the trigger loop, but does not promote forward translocation (47). The role of backtracking-independent pausing for Pol II might be less prominent than for *E. coli* RNAP; the specific regulatory roles of the 2 pausing mechanisms of Pol II remain to be identified.

In conclusion, our findings suggest that backtracking and translocation delay are not the main mechanisms of sequence-specific pausing by bacterial RNAPs. We have established that the paused *E. coli* RNAP TEC binds NTP as efficiently as the

active TEC. We propose that ubiquitous transcriptional pausing involves misalignment of the 3' NMP and the substrate NTP in the catalytic center of RNAP.

Materials and Methods

Purification of *E. coli* RNAP and *S. cerevisiae* Pol II, promoter initiation, and TEC assembly from oligonucleotides were done as described (41). Exo III footprinting was done as described (47). The detailed description of the methods is provided in *SI Text*. Oligonucleotides used are in [Table S2](#), and templates used for promoter-dependent initiation are in [Table S3](#).

ACKNOWLEDGMENTS. We thank Irina Artsimovitch, Sergey Borukhov, and Robert Landick for helpful comments; Zachary Burton and Dwight Nissley for critical reading of the manuscript; Irina Artsimovitch (Ohio State University, Columbus, OH) for a generous gift of *T. thermophilus* RNAP holoenzyme; and Lucyna Lubkowska for protein purification. This work was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research. The content of this publication does not necessarily reflect the views and policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

- Kassavetis GA, Chamberlin MJ (1981) Pausing and termination of transcription within the early region of bacteriophage T7 DNA in vitro. *J Biol Chem* 256:2777–2786.
- Reisbig RR, Hearst JE (1981) *Escherichia coli* deoxyribonucleic acid dependent ribonucleic acid polymerase transcriptional pause sites on SV40 DNA F1. *Biochemistry* 20:1907–1918.
- Landick R (2006) The regulatory roles and mechanism of transcriptional pausing. *Biochem Soc Trans* 34:1062–1066.
- Chan CL, Landick R (1989) The *Salmonella typhimurium* his operon leader region contains an RNA hairpin-dependent transcription pause site. Mechanistic implications of the effect on pausing of altered RNA hairpins. *J Biol Chem* 264:20796–20804.
- Landick R, Yanofsky C (1987) Isolation and structural analysis of the *Escherichia coli* trp leader paused transcription complex. *J Mol Biol* 196:363–377.
- Donahue JP, Turnbough CL, Jr (1994) Nucleotide-specific transcriptional pausing in the pyrBI leader region of *Escherichia coli* K-12. *J Biol Chem* 269:18185–18191.
- Yanofsky C (1981) Attenuation in the control of expression of bacterial operons. *Nature* 289:751–758.
- Pan T, et al. (1999) Folding of a large ribozyme during transcription and the effect of the elongation factor NusA. *Proc Natl Acad Sci USA* 96:9545–9550.
- Winkler ME, Yanofsky C (1981) Pausing of RNA polymerase during in vitro transcription of the tryptophan operon leader region. *Biochemistry* 20:3738–3744.
- Yakhnin AV, Yakhnin H, Babitzke P (2006) RNA polymerase pausing regulates translation initiation by providing additional time for TRAP-RNA interaction. *Mol Cell* 24:547–557.
- Lau LF, Roberts JW, Wu R (1983) RNA polymerase pausing and transcript release at the lambda trl terminator in vitro. *J Biol Chem* 258:9391–9397.
- Park JS, Roberts JW (2006) Role of DNA bubble rewinding in enzymatic transcription termination. *Proc Natl Acad Sci USA* 103:4870–4875.
- Muse GW, et al. (2007) RNA polymerase is poised for activation across the genome. *Nat Genet* 39:1507–1511.
- Core LJ, Lis JT (2008) Transcription regulation through promoter-proximal pausing of RNA polymerase II. *Science* 319:1791–1792.
- Komissarova N, Kashlev M (1997) RNA polymerase switches between inactivated and activated states by translocating back and forth along the DNA and the RNA. *J Biol Chem* 272:15329–15338.
- Komissarova N, Kashlev M (1997) Transcriptional arrest: *Escherichia coli* RNA polymerase translocates backward, leaving the 3' end of the RNA intact and extruded. *Proc Natl Acad Sci USA* 94:1755–1760.
- Hawryluk PJ, Ujvari A, Luse DS (2004) Characterization of a novel RNA polymerase II arrest site which lacks a weak 3' RNA-DNA hybrid. *Nucleic Acids Res* 32:1904–1916.
- Artsimovitch I, Landick R (2000) Pausing by bacterial RNA polymerase is mediated by mechanically distinct classes of signals. *Proc Natl Acad Sci USA* 97:7090–7095.
- Palangat M, Landick R (2001) Roles of RNA:DNA hybrid stability, RNA structure, and active site conformation in pausing by human RNA polymerase II. *J Mol Biol* 311:265–282.
- Samkurashvili I, Luse DS (1996) Translocation and transcriptional arrest during transcript elongation by RNA polymerase II. *J Biol Chem* 271:23495–23505.
- Voliotis M, Cohen N, Molina-Paris C, Liverpool TB (2008) Fluctuations, pauses, and backtracking in DNA transcription. *Biophys J* 94:334–348.
- Galburt EA, et al. (2007) Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. *Nature* 446:820–823.
- Mejia YX, Mao H, Forde NR, Bustamante C (2008) Thermal probing of *E. coli* RNA polymerase off-pathway mechanisms. *J Mol Biol* 382:628–637.
- Bai L, Shundrovsky A, Wang MD (2004) Sequence-dependent kinetic model for transcription elongation by RNA polymerase. *J Mol Biol* 344:335–349.
- Tagidgotla VR, et al. (2006) Thermodynamic and kinetic modeling of transcriptional pausing. *Proc Natl Acad Sci USA* 103:4439–4444.
- Wang D, et al. (1995) Discontinuous movements of DNA and RNA in RNA polymerase accompany formation of a paused transcription complex. *Cell* 81:341–350.
- Chan CL, Landick R (1993) Dissection of the his leader pause site by base substitution reveals a multipartite signal that includes a pause RNA hairpin. *J Mol Biol* 233:25–42.
- Touloukhonov I, Zhang J, Palangat M, Landick R (2007) A central role of the RNA polymerase trigger loop in active-site rearrangement during transcriptional pausing. *Mol Cell* 27:406–419.
- Aivazashvili VA, Bibilashvili RS, Vartikian RM, Kutateladze TA (1981) Effect of the primary structure of RNA on the pulse character of RNA elongation in vitro by *Escherichia coli* RNA polymerase: A model. *Mol Biol (Moscow)* 15:915–929.
- Levin JR, Chamberlin MJ (1987) Mapping and characterization of transcriptional pause sites in the early genetic region of bacteriophage T7. *J Mol Biol* 196:61–84.
- Herbert KM, et al. (2006) Sequence-resolved detection of pausing by single RNA polymerase molecules. *Cell* 125:1083–1094.
- Bar-Nahum G, et al. (2005) A ratchet mechanism of transcription elongation and its control. *Cell* 120:183–193.
- Yang W, Lee JY, Nowotny M (2006) Making and breaking nucleic acids: Two-Mg²⁺ ion catalysis and substrate specificity. *Mol Cell* 22:5–13.
- Rhodes G, Chamberlin MJ (1974) Ribonucleic acid chain elongation by *Escherichia coli* ribonucleic acid polymerase. I. Isolation of ternary complexes and the kinetics of elongation. *J Biol Chem* 249:6675–6683.
- Kettenberger H, Armache KJ, Cramer P (2004) Complete RNA polymerase II elongation complex structure and its interactions with NTP and TFIIIS. *Mol Cell* 16:955–965.
- Vassilyev DG, et al. (2007) Structural basis for substrate loading in bacterial RNA polymerase. *Nature* 448:163–168.
- Wang D, et al. (2006) Structural basis of transcription: Role of the trigger loop in substrate specificity and catalysis. *Cell* 127:941–954.
- Westover KD, Bushnell DA, Kornberg RD (2004) Structural basis of transcription: Nucleotide selection by rotation in the RNA polymerase II active center. *Cell* 119:481–489.
- Palangat M, Hittinger CT, Landick R (2004) Downstream DNA selectively affects a paused conformation of human RNA polymerase II. *J Mol Biol* 341:429–442.
- Kireeva ML, Komissarova N, Waugh DS, Kashlev M (2000) The 8-nucleotide-long RNA:DNA hybrid is a primary stability determinant of the RNA polymerase II elongation complex. *J Biol Chem* 275:6530–6536.
- Sidorenkov I, Komissarova N, Kashlev M (1998) Crucial role of the RNA:DNA hybrid in the processivity of transcription. *Mol Cell* 2:55–64.
- Vassilyev DG, et al. (2007) Structural basis for transcription elongation by bacterial RNA polymerase. *Nature* 448:157–162.
- Westover KD, Bushnell DA, Kornberg RD (2004) Structural basis of transcription: Separation of RNA from DNA by RNA polymerase II. *Science* 303:1014–1016.
- Komissarova N, et al. (2002) Shortening of RNA:DNA hybrid in the elongation complex of RNA polymerase is a prerequisite for transcription termination. *Mol Cell* 10:1151–1162.
- Kaplan CD, Larsson KM, Kornberg RD (2008) The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by α -amanitin. *Mol Cell* 30:547–556.
- Malagon F, et al. (2006) Mutations in the *Saccharomyces cerevisiae* RPB1 gene conferring hypersensitivity to 6-azauracil. *Genetics* 172:2201–2209.
- Kireeva ML, et al. (2008) Transient reversal of RNA polymerase II active site closing controls fidelity of transcription elongation. *Mol Cell* 30:557–566.