

Supporting Information

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SI Text

Supporting Methods

T7A1 Promoter Templates for in Vitro Transcription. Templates for promoter-specific transcription initiation (Table S3) were obtained by PCR using ssDNA templates and primers, the sequences of which are summarized in Table S2. Template (0.15 μ M), 2 μ M of each primer, and 1 mM dNTPs were combined in 100 μ L of 1 \times ThermoPol buffer (New England Biolabs), heated to 95 $^{\circ}$ C for 5 min, and 2 units of Vent DNA polymerase (New England Biolabs) was added to the reaction. PCR proceeded for 15 cycles of 30 s at 95 $^{\circ}$ C, 30 s at 55 $^{\circ}$ C, and 30 s at 72 $^{\circ}$ C. The product DNA was purified with a Qiagen PCR purification kit.

Promoter-Specific Initiation of Transcription. Transcription initiation was performed essentially as described (1, 2) with minor modifications. The open complex at bacteriophage T7A1 promoter was obtained by 5-min incubation of 5 pmol of *E. coli* RNAP holoenzyme with 5 pmol of template DNA in 5 μ L of transcription buffer [TB; 20 mM Tris-HCl (pH 7.9), 40 mM KCl, 5 mM MgCl₂] at 37 $^{\circ}$ C, followed by addition of 5 μ L of primer and substrate mixture containing 250 μ M ATP, 250 μ M GTP, and 1.25 mM ApUpC RNA primer prewarmed at 37 $^{\circ}$ C. After 5-min incubation with the primer, ATP, and GTP, the resulting TEC11 was immobilized on Ni-NTA agarose (Qiagen) by addition of 30 μ L of 50% suspension of Ni-NTA agarose, which was pretreated with 0.3 mg/mL acetylated BSA and prewashed with TB. After 10-min incubation with Ni-NTA agarose [the incubation was done with shaking at 1,000 rpm in Thermomixer (Eppendorf)], the TEC was washed twice with 1 mL of TB, incubated with 1 mL of TB containing 1 M KCl for 5–10 min, and again washed twice with 1 mL of TB. The TEC was walked along the template by 3- to 5-min incubation with 5 μ M NTP subsets followed by washing the TEC 6 times with 1 mL of TB.

TEC Assembly from RNA and DNA Oligonucleotides. Promoter-independent assembly of the TECs from DNA and RNA oligonucleotides allows for a direct comparison of transcription elongation properties of *E. coli* RNAP and *S. cerevisiae* Pol II (2–4). Assembly was performed essentially as described (3, 4) with minor modifications. *E. coli* RNAP or *S. cerevisiae* Pol II core enzyme (5 pmol) was immobilized by 10- to 15-min incubation with 50 μ L of 50% Ni-NTA agarose in TB. Ni-NTA agarose was pretreated with 0.3 mg/mL of acetylated BSA. RNA primer RNA9 (1.5 μ M) was annealed to 1.5 μ M template DNA (TDS76 or TDS85; Table S2) in TB exactly as described (4). RNA–DNA hybrid [15 pmol (10 μ L)] was added to the immobilized polymerase and incubated for 10 min. Next, 50 pmol of the nontemplate DNA strand was added for 10 min. The resulting TEC9 was washed as described for the promoter-derived TEC11. Note that assembly of the TEC from *E. coli* RNAP holoenzyme and core enzyme produced identical results.

TEC Manipulations and RNA Labeling. To obtain TEC12 labeled at C12 position, the immobilized washed TEC11 was incubated with 0.3 μ M [α -P³²] CTP (3,000 Ci/mmol; Perkin Elmer) for 5 min and washed with TB. To obtain TEC20 labeled at C12, C14, and C17 positions, 0.3 μ M [α -P³²] CTP was added to TEC11 together with Ni-NTA agarose suspension, and labeling was performed concurrent with TEC immobilization. TEC18 labeled at 17 position was obtained by walking TEC11 to C14 and 5-min incubation with 5 μ M GTP and 0.3 μ M [α -P³²] CTP. To obtain

labeled TEC9 (Fig. S1D), we used the approach described in ref. 2. TEC11 formed on D111 template was walked to G23 and incubated with 5 μ M ATP, 5 μ M UTP, and 0.3 μ M of [α -P³²] CTP. The resulting TEC39 was treated with 2 units/ μ L of RNase T1 (100 units/ μ L; Roche Molecular Biochemicals, produced in April 2001) for 20 min, cleaving the RNA between G23 and C24. The RNase was removed by washing the immobilized TEC 10 times with 1 mL of TB, and the RNA in the TEC was degraded from the 3' end to 9 nt by 30-min incubation with 2 mM pyrophosphate. Pyrophosphate was removed by washing the TEC 10 times with 1 mL of TB.

TECs used for time-course analyses and Exo III footprinting analyses were removed from Ni-NTA agarose beads to ensure rapid mixing of the components and even distribution of the material between the time points. To this end, 0.1 mg/mL acetylated BSA was added to 50% suspension of Ni-NTA agarose containing the immobilized TEC, and the TECs were eluted by addition of 1/10th volume of 1 M imidazole (pH 7.9). After 5- to 10-min incubation, the beads were removed from the solubilized TEC by passing of the suspension through a 0.45- μ M PVDF Ultrafree-MC centrifugal filter device (Millipore). The filter was washed with 4–5 volumes of TB containing 0.1 mg/mL BSA, and the 2 filtrates were combined. Thus, the final concentration of imidazole in the TEC solution was brought <20 mM.

Transcription Time-Course Analyses. Transcription was initiated by addition of 4 NTPs or a subset of NTPs to the TEC. For incubation times of 5 s and longer, 1/4th reaction volume of 5 \times NTPs stock solution was mixed with the starting TEC, and the reaction was stopped at different time points manually, by withdrawing 5- μ L aliquots and mixing them with 10 μ L of gel-loading buffer (10 M urea, 50 mM EDTA, 0.001% bromophenol blue and xylene cyanol). For incubation times <5 s, equal volumes (15 μ L) of the TEC and 2 \times NTPs stock were mixed in a rapid quench flow instrument RQF-3 (KinTek), and the reaction was quenched with 1 M HCl at preset time intervals. The acid in the samples was neutralized by addition of 2 M Tris-HCl, pH 9, and the samples were precipitated with ethanol, and dissolved in the gel-loading buffer. The RNA products were resolved in 20% polyacrylamide gel containing 7 M urea (Promega) and 1 \times TBE. The acrylamide to methylene-bis-acrylamide ratio in all gels was 19:1.

The quantification of the radiolabeled RNA products was done with ImageQuant software (Amersham Biosciences), and the data were analyzed with OriginPro75 (OriginLab). Single exponential fits were performed by using $y = y_0 + Ae^{-kx}$; double exponential fits were performed by using $y = y_0 + A_1e^{-k_1x} + A_2e^{-k_2x}$, where x is time, and y is the amount of the RNA species, in percentage of the total radioactivity in the lane. y_0 corresponds to the amount of the RNA that fails to be extended in the time course of the experiment (usually this number is <10%); A is the fraction size, and k is the rate of the next NTP incorporation.

Exonuclease III Footprinting. Rear-end Exo III footprinting was performed by using the TECs assembled on the 5' end-labeled (2) TDS76 template DNA strand and unlabeled NDS79 nontemplate DNA strand (Table S2). This template carries a 3-nt 3' end overhang at the downstream end. The overhang prevents digestion of the nontemplate DNA strand by Exo III. The template DNA strand is susceptible to Exo III degradation, which allows for determination of the position of the upstream boundary of RNAP (5, 6). The RNA in TEC20 and TEC27 was

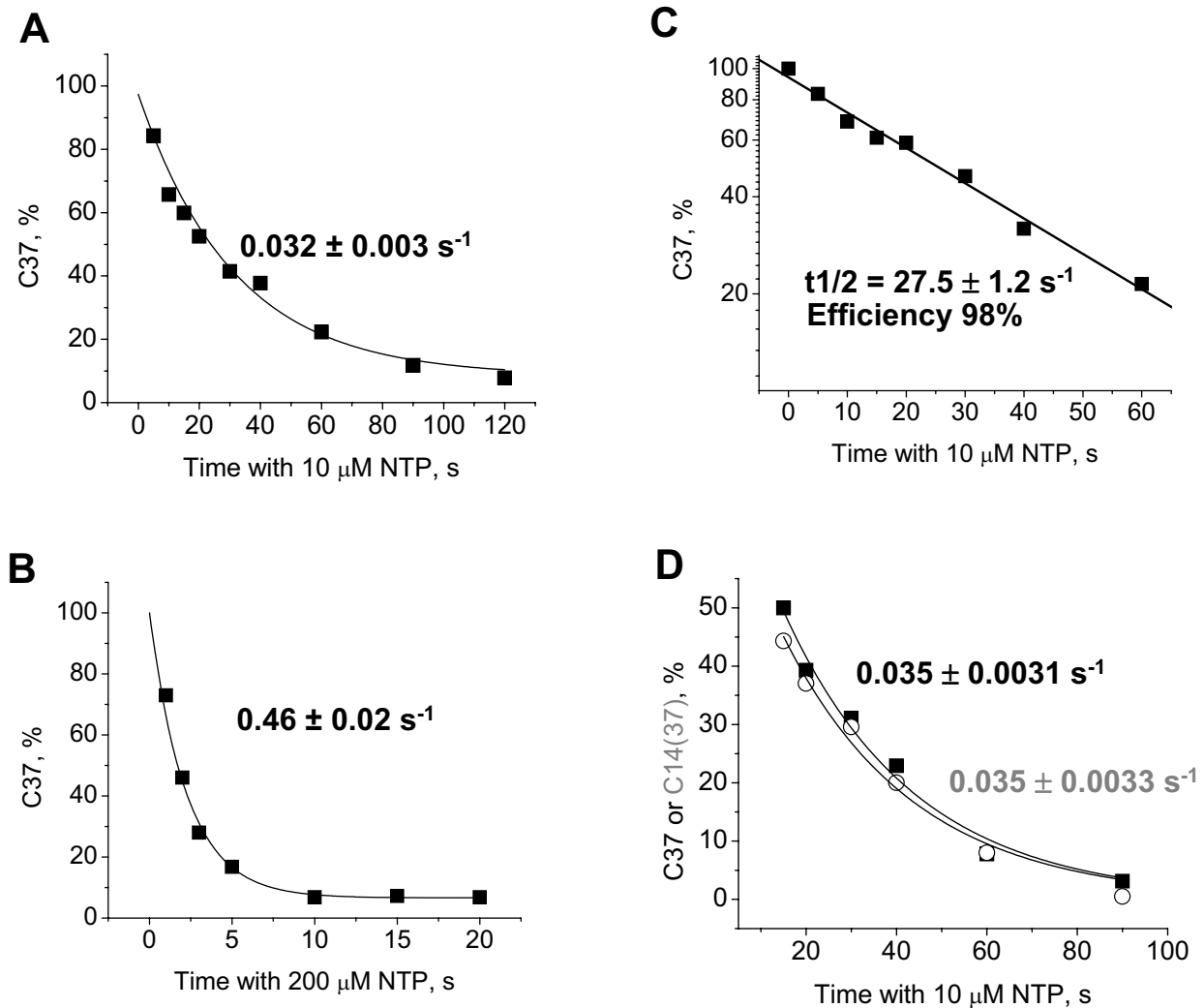


Fig. S1. Quantitative characterization of C37 pause. (A–C) To determine the rate of RNAP escape from the pause, the polymerase was brought to position A36, 1-bp upstream from the pausing site, and transcription was restarted by addition of 10 μ M (A and C) or 200 μ M (B) ATP and CTP. The amount of 37-nt RNA has been plotted against the incubation time, and the escape rate was determined by fitting the data with a single exponential equation. (D) To obtain C14 (37) TEC, the RNAP was walked to position C39, and the TEC was treated with 2 units/mL of ribonuclease T1, cleaving the RNA between G23 and C24. The RNase was removed by washing, and the RNA in the TEC was truncated from the 3' end to 9 nt by incubation with 2 mM pyrophosphate. After washing off the pyrophosphate, transcription from A9 was restarted by addition of 10 mM NTP. As a control, A32 TEC was obtained and chased with 10 μ M NTP. The amount of paused C14 and C37 was plotted and fit with a single exponential equation.

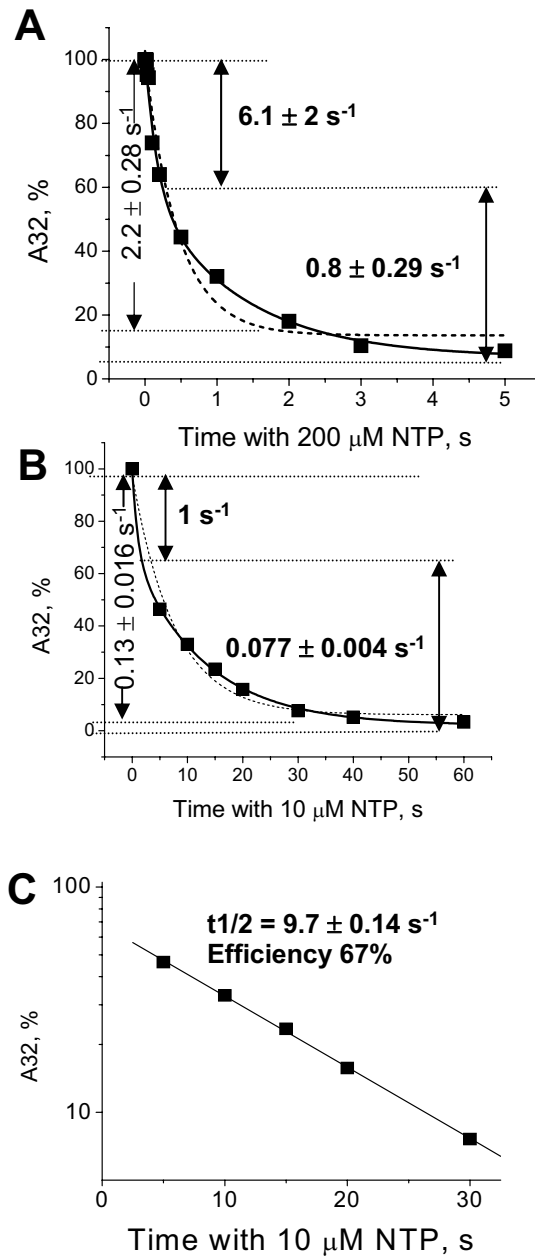


Fig. S3. Escape from A32 pause is biphasic. TEC stalled at A32 was chased with 200 μM (A) or 10 μM (B and C) NTP. The data were fit with single-exponential (broken line) or double-exponential (solid line) functions. In C, pausing half-life and efficiency were determined for the data shown in B.

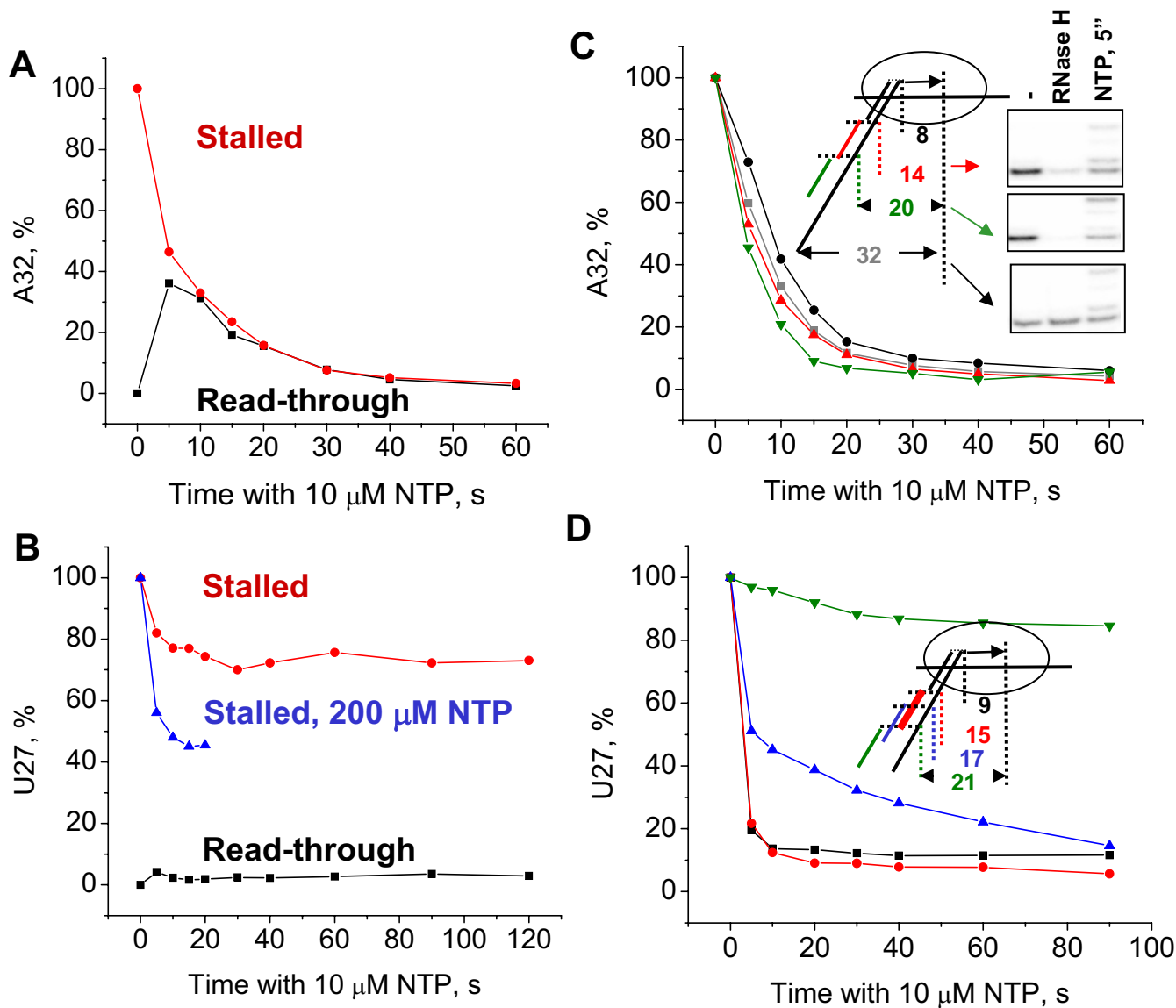


Fig. S4. Effect of transcript cleavage factors on transcription by *S. cerevisiae* Pol II (A) and *E. coli* RNAP (B). The experiment was done as described in Figs. 6A and 3A, respectively, but TFIS or GreB were added together with NTPs where indicated.

Table S1. Catalytic properties of C37 TECs formed on 4 variants of D111 template

Template	Fraction size, %	Rate, s ⁻¹
C37A38	87 ± 3	0.027 ± 0.002
C37G38	83 ± 3	0.059 ± 0.005
C37C38	86 ± 8	0.51 ± 0.08
	7 ± 5	0.04 ± 0.012
C37U38	70 ± 12	0.7 ± 0.06
	21 ± 11	0.06 ± 0.04

The experiment was done as described in Fig. 4A, and the data were fitted with single or double exponential equations.

Table S2. RNA and DNA oligonucleotides

Oligonucleotide	Sequence (5' to 3')
RNA9	AUC GAG AGG
NDS79	CCT ATA GGA TAC TTA CAG CCA TCG AGA GGG ACA CGG CGA ATA GCC ATC CCA ATC CAC ACG TCC AAC GGG GCA AAC CGT A
TDS76	GGT TTG CCC CGT TGG ACG TGT GGA TTG GGA TGG CTA TTC GCC GTG TCC CTC TCG ATG GCT GTA AGT ATC CTA TAG G
TDS76G34	GGT TTG CCC CGT TGG ACG TGT GCA TTG GGA TGG CTA TTC GCC GTG TCC CTC TCG ATG GCT GTA AGT ATC CTA TAG G
NDS82	CCT ATA GGA TAC TTA CAG CCA TCG AGA GGG ACA CGG CGA ATA GCC ATC CCA ATG CAC ACG TCC AAC GGG GCA ACC GTA TGT A
TDS85	TAC ATA CGG TTG CCC CGT TGG ACG TGT GGA TTG GCA TGG CTA TTC GCC GTG TCC CTC TCG ATG GCT GTA AGT ATC CTA TAG GTG T
TDS85G37	TAC ATA CGG TTG CCC CGT TGG ACG TCT GGA TTG GGA TGG CTA TTC GCC GTG TCC CTC TCG ATG GCT GTA AGT ATC CTA TAG GTG T
T7A1 upstream	TTA TCA AAA AGA GTA TTG ACT TAA AGT CTA ACC TAT AGG ATA C
D111 downstream	TAC ATA CGG TTG CCC
C37A38EcoRV	GGG TGG GAT ATC TAC ATA CGG TTG CCC CGT TGG ACG TGT GCA TTG GG
C37C38EcoRV	GGG TGG GAT ATC TAC ATA CGG TTG CCC CGT TGG ACG GGT GCA TTG GG
C37G38EcoRV	GGG TGG GAT ATC TAC ATA CGG TTG CCC CGT TGG ACG CGT GCA TTG GG
C37U38EcoRV	GGG TGG GAT ATC TAC ATA CGG TTG CCC CGT TGG ACG AGT GCA TTG GG
G37A38EcoRV	GGG TGG GAT ATC TAC ATA CGG TTG CCC CGT TGG ACG TCT GCA TTG GG

Table S3. Templates for promoter-dependent initiation

Transcription template	PCR template	Downstream PCR primer	Experiments in which the template was used
D111	TDS76	D111 downstream	Bulk elongation, quantitative analyses of pausing
D111G37	TDS85G37	D111 downstream	Bulk elongation, quantitative analyses of pausing
D111exoIII	TDS85	C37A38EcoRV	Quantitative analyses of pausing, front-end Exo III footprinting
D111G37exoIII	TDS76G37	C37A38EcoRV	Quantitative analyses of pausing, front-end Exo III footprinting
D111C37C38exoIII	TDS85	C37C38EcoRV	Quantitative analyses of pausing, front-end Exo III footprinting
D111C37G38exoIII	TDS85	C37G38EcoRV	Quantitative analyses of pausing
D111C37U38exoIII	TDS85	C37U38EcoRV	Quantitative analyses of pausing

All templates were amplified by using T7A1upstream oligonucleotide ([Table S2](#)) as an upstream PCR primer.