Supplementary Data for

Impact of template backbone heterogeneity on RNA polymerase II transcription

Liang Xu[1], Wei Wang[1], Lu Zhang[2], Jenny Chong[1], Xuhui Huang[2], and Dong Wang[1,*]

[1] Skaggs School of Pharmacy and Pharmaceutical Sciences, The University of California San Diego, La Jolla, California, 92093-0625, United States.

[2] Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

*To whom correspondence should be addressed. Tel: 858 822 5561; Fax: 858 822 1953; Email: *dongwang@ucsd.edu*

Supplementary Figures

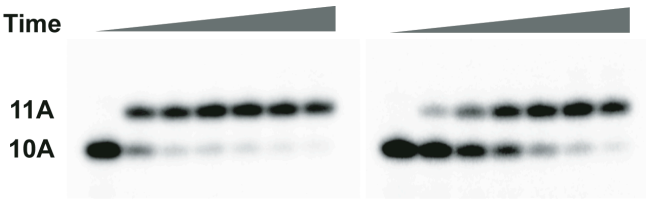

Figure S1. The ATP incorporation by pol II at the 3´-5´ dT template (left) is faster than that at 2´-5´ rU template (right) at 1 μM concentration. The scaffolds are shown in Figure S2. Time points are 0, 10s, 30s, 1 min, 2 min, 5 min and 10 min.

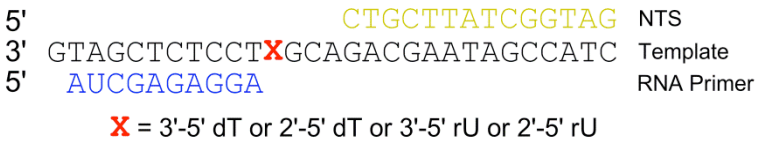

Figure S2. Scaffolds used in single turnover in vitro transcription experiments. NTS stands for non-template strand.

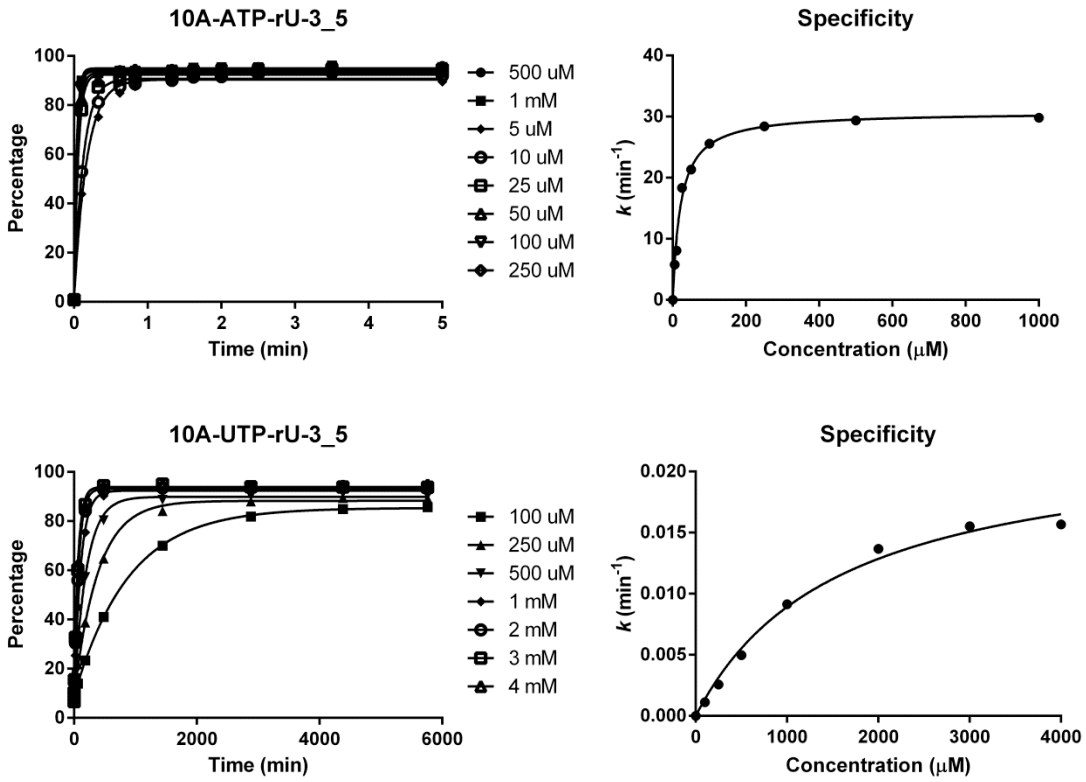

Figure S3. Representative kinetic fitting curves for ATP and UTP incorporation by pol II.

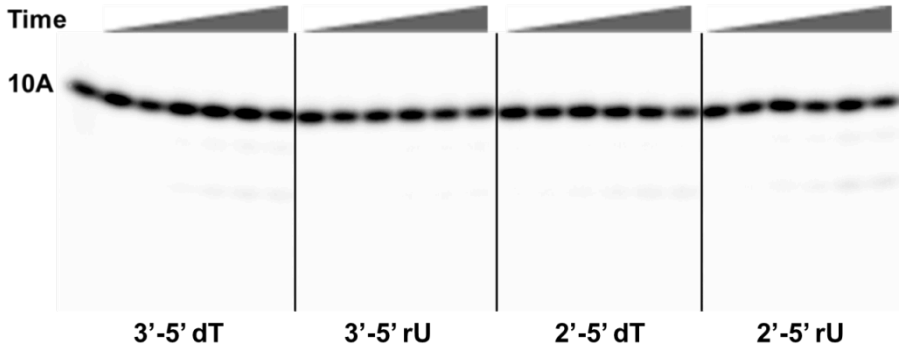

Figure S4. No obvious TFIIS-stimulated RNA transcript cleavage was observed for pol II complex containing 10mer RNA primer. The scaffolds used for TFIIS-mediated cleavage are shown in Figure S2.

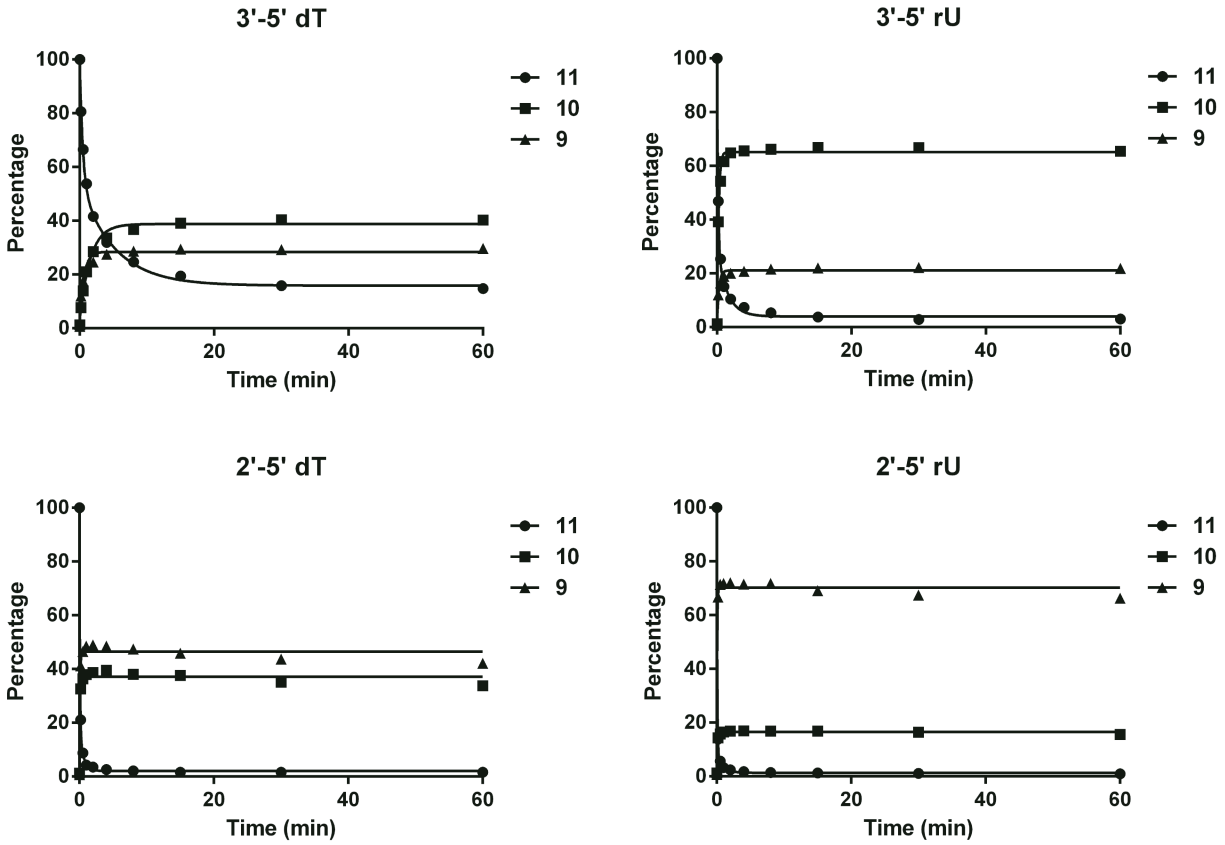

Figure S5. TFIIS-mediated cleavage products by pol II containing 11mer primer. The 10mer product corresponds to the pre-translocation cleavage product in this condition, and the 9mer product corresponds to the backtracked cleavage product.

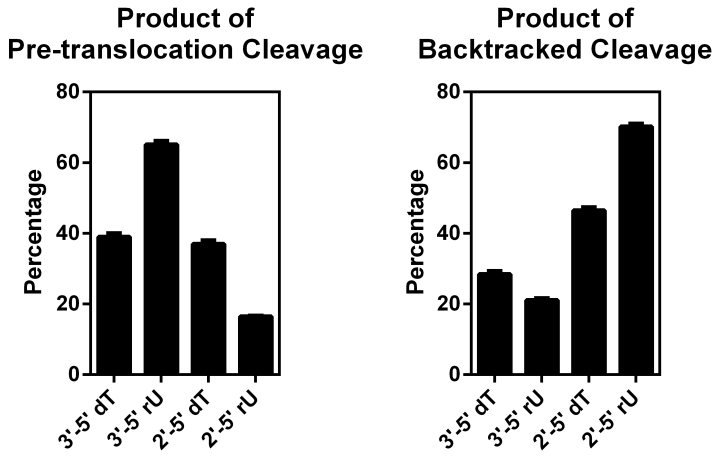

Figure S6. Analysis of TFIIS-mediated pol II cleavage patterns with different backbone heterogeneities. Left: 10mer cleavage products from the pol II complex at pre-translocation state (11mer to 10 mer); right: 9mer cleavage products from the pol II complex at backtracked state (11mer to 9 mer).

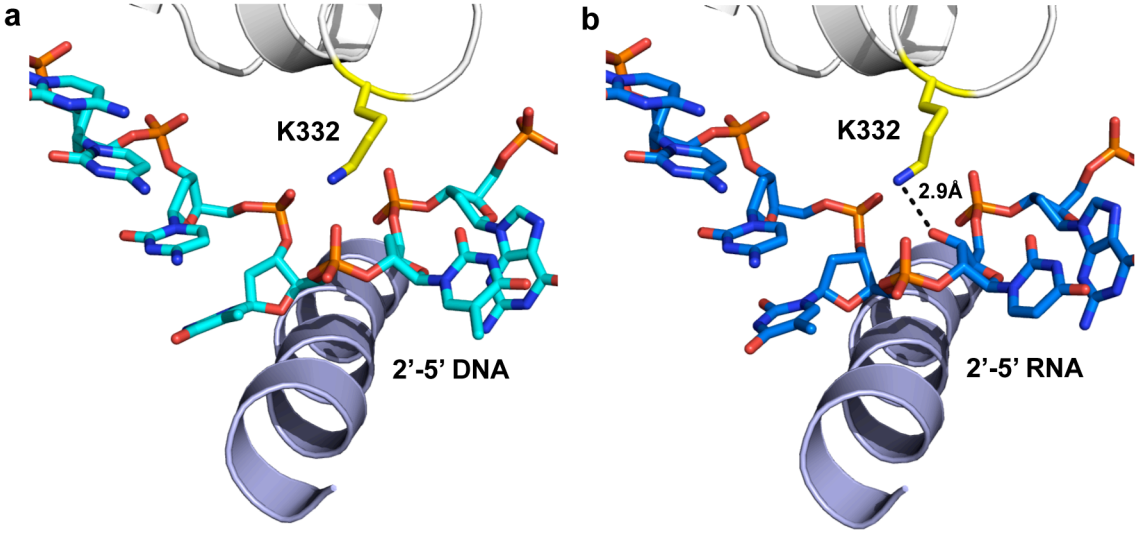

Figure S7. Modeling of structure of pol II elongation complex with a 2´-5´phosphodiester linkage connecting i+1 and i+2 nucleobases. In this backtracked model, the 3´-OH of 2´-5´ RNA can potentially form hydrogen bond with the K332 residue of Rpb 1 in the switch II region of RNA pol II. This hydrogen bond may stabilize the backtracked conformation in the 2´-5´ RNA template, and it may cause the increased backtrack percentage as we experimentally observed in Figure 5.

Supplementary Tables

Table S1. Kinetic data of AMP incorporation opposite four template backbone variants by pol II.

Template backbone	k_{pol} (min^{-1})	$K_{d, app}$ (μM)	$k_{pol}/K_{d, app}$ ($\mu M^{-1} \cdot min^{-1}$)	Relative efficiency
3′-5′ dT	750 ± 210	90 ± 20	8.3 ± 3.0	1.0 ± 0.3
2′-5′ dT	17 ± 1	530 ± 60	0.032 ± 0.004	0.0039 ± 0.0005
3′-5′ rU	31 ± 1	21 ± 2	1.5 ± 0.1	0.18 ± 0.01
2′-5′ rU	0.25 ± 0.01	890 ± 70	0.00028 ± 0.00001	0.000034 ± 0.000002

Table S2. Kinetic data of UMP misincorporation opposite four template backbone variants by pol II.

Template backbone	k_{pol} (min^{-1})	$K_{d, app}$ (μM)	$k_{pol}/K_{d, app}$ ($\mu M^{-1} \cdot min^{-1}$)	Relative efficiency
3′-5′ dT	0.015 ± 0.003	800 ± 60	$(1.9 \pm 0.4) \times 10^{-5}$	1 ± 0.2
2′-5′ dT	0.13 ± 0.01	3700 ± 700	$(3.5 \pm 0.7) \times 10^{-5}$	1.8 ± 0.5
3′-5′ rU	0.023 ± 0.002	1600 ± 300	$(1.4 \pm 0.3) \times 10^{-5}$	0.74 ± 0.22
2′-5′ rU	0.0085 ± 0.0006	1600 ± 300	$(0.53 \pm 0.01) \times 10^{-5}$	0.28 ± 0.06

Table S3. TFIIS-mediated RNA transcript cleavage rates for pol II with different template backbone variants.

Template backbone	Cleavage (min^{-1})	Relative efficiency
3′-5′ dT	0.69 ± 0.13	1.0 ± 0.2
2′-5′ dT	11 ± 1	15 ± 3
3′-5′ rU	4.4 ± 0.8	6.3 ± 1.6
2′-5′ rU	14 ± 1	20 ± 4