Supporting Information

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In Vivo Activity of Split T7 RNAP

We began our study by comparing the in vivo activity of full-length versus split T7 RNA polymerase (T7 RNAP). Rather than present fold induction, here we present the development of GFP fluorescence normalized to OD_{600} over time, as shown in Fig. S1. Although GFP expression with full-length T7 RNAP was found to be high even if isopropyl β-D-1-thiogalactopyranoside (IPTG) was not used to de-repress $P_{T7/lacO}$, at concentrations of arabinose above 10 mM, the expression of GFP drops precipitously. This drop in GFP expression corresponds to the appearance of a seven–base pair insertion that truncates the T7 RNAP coding sequence when plasmid isolated from cell culture postinduction was sequenced. The cultures at 100 and 200 mM arabinose reach the same final OD_{600} at the end of the experiment, suggesting the observed GFP fluorescence at 100 and 200 mM corresponds to fluorescent protein produced early on in the experiment. This contrasts the case with split T7 RNAP where GFP expression is stable and saturates at high levels of arabinose.

Pulse Chase Analysis of Full vs. Split T7 RNAP

To characterize the relative stability of full-length vs. split T7 RNAP, we performed pulse chase analysis of expression systems using either versions of the polymerase. We cotransformed pET28:GFP with either pTara:500 or pTSara into DH10B and then assayed GFP expression over time. Individual colonies were inoculated into selective LB media and grown to stationary phase. Stationary phase cell culture was inoculated in triplicate into 50 mL selective LB media containing 1 mM arabinose at 1% (vol/vol) for 5 h. Cultures were then spun down at 5,000 relative centrifugal force for 2 min twice in LB media without inducer. The samples were then incubated at 37 °C with shaking at 200 rpm for the remainder of the experiment. Every hour, 200-μL samples were taken and assayed for GFP fluorescence (ex:488, em:510) and OD_{600} (Infinite M1000, Tecan). Compared with the fluorescence associated with the full-length protein, the decrease in fluorescence with split T7 RNAP is comparable to the decrease in fluorescence with full-length T7 RNAP.

Characterizing the Promoter Specificity of Split T7 RNAP Mutants

To characterize the change in specificity resulting from single amino–acid mutations in the specificity loop, we did 30 pairwise transformations of pTSara-Mut variants with pET:NNNN:GFP variants, where Mut denotes one of the point mutations [Q758C, R756K,R756S,N748D] and NNNN denotes base pairs −11 to −8 of P_{T7} on the plasmid (Fig. S3*A*). Here we plot the normalized GFP fluorescence to OD_{600} to better characterize the magnitude of GFP expression observed in our expression systems (Fig. S3*B*).

In the case of pTSara-wt+pET:GACT:GFP, where one would expect high fold induction of GFP fluorescence, the difference between uninduced vs. induced case is still quite substantial, despite higher levels of uninduced GFP fluorescence. In addition, to demonstrate marked preference for a particular mutant P_{T7}, mutant split T7 RNAP also demonstrates different abilities to drive GFP expression, depending on the promoter it is tasked on. This may provide additional options to regulate the activity of mutant split T7 RNAP.

Characterizing the Two-Input Function of Split T7 RNAP

To characterize the two-input function of mutant split T7 RNAP, we cotransformed pTSlb-wt, pTSlb-Q758C, pTSlb-R756K, pTSlb-R756S, and pTSlb-N748D with pET:GACT:GFP, pET:GACG:GFP, pET:ACAT:GFP, pET:GCAT:GFP, and pET:CCCT:GFP, respectively, and then assayed GFP expression as a function of arabinose and IPTG (Fig. S4*A*). Here we plot the normalized GFP fluorescence to OD_{600} with no inducer, 10 mM arabinose, 10 mM IPTG, and 10 mM arabinose plus 10 mM IPTG (Fig. S4*B*).

Fig. S1. Time course plots of the comparison of the in vivo functionality of full-length and split T7 RNAP. (*A*) We first transformed pTara:500 with pET28:GFP and assayed GFP fluorescence as a function of arabinose without IPTG. (*B*) GFP normalized to OD_{600} over time in the presence of 0, 1, 10, 100, and 200 mM arabinose with the system described in (*A*). Beginning from 10 mM arabinose, the expression of GFP drops dramatically, corresponding with the appearance of a seven–base pair insertion into the T7 RNAP coding sequence that truncates the protein. (*C*) We then transformed a plasmid containing split T7 RNAP with the plasmid pET28:GFP. (*D*) GFP normalized to OD_{600} over time in the presence of 0, 1, 10, 100, and 200 mM arabinose with the system outlined in *C*. Although not as active, GFP expression using split T7 RNAP is more easily saturated and is stable even at high concentrations of arabinose.

Fig. S2. Pulse chase analysis of full-length and split T7 RNAP. (*A*) To assess the relative stability of full-length versus split T7 RNAP, we transformed pET28:GFP with either pTara:500 or pTSara and then assayed GFP fluorescence after cells were first grown in media with inducer and then washed in media without inducer. (*B*) GFP fluorescence normalized to OD_{600} over time. Cells were initially grown in the presence of 1 mM arabinose for 5 h and then spun down and resuspended twice in media without arabinose.

Fig. S3. Assay of the specificity of mutant split T7 RNAP to mutant P_{T7}. The GFP fluorescence normalized to OD_{600} values used to compute the heat map in Fig. 4*B* are plotted. (*A*) A plasmid containing a mutant split T7 RNAP (wt, Q758C, R756K, R756S, and N748D) was cotransformed with another plasmid containing an unregulated P_{T7}, where base pairs −11 to −8 have been modified to GACT(wt), GACG, GCAT, ACAT, CACT, and CCCT, driving GFP expression. (*B*) GFP fluorescence normalized to OD_{600} with or without 10 mM arabinose after growing for 10 h of each pairwise combination of pTSara-Mut and pET:NNNN:GFP.

Fig. S4. Assay of the two-input function of mutant split T7 RNAP. The GFP fluorescence normalized to OD_{600} values used to compute the heat maps in Fig. 5 is plotted. (*A*) To assess the two-input function of mutant split T7 RNAP, a plasmid containing mutant split T7 RNAP where the N- and C-terminal fragments are downstream of P_{Lac} and P_{BAD}, respectively, is cotransformed with another plasmid containing mutant P_{T7} where base pairs −11 to −8 have been modified. (*B*) GFP fluorescence normalized to OD_{600} of a particular mutant split polymerase and mutant promoter pair described in *A* with no inducer, 10 mM arabinose, 10 mM IPTG, and 10 mM arabinose plus 10 mM IPTG.

Fig. S5. Plasmid maps of the constructs used in this study. All plasmids containing the T7 RNAP coding sequence were created by modifying the T7 RNAP expression vector pTara (1). All GFP reporter plasmids used in this study were derived from pET28-b. The ribosome binding site used to drive translation of the T7 RNAP coding sequence was tuned to have a strength of 500, according to the ribosome binding site calculator (2).

1. Wycuff DR, Matthews KS (2000) Generation of an AraC-araBAD promoter-regulated T7 expression system. *Anal Biochem* 277(1):67–73.
2. Salis HM, Mirsky EA, Voigt CA (2009) Automated design of synthetic ribosome binding sites to control protein expression. *Nat Biotechnol* 27(10):946–950.

Table S1. Genotypes of the plasmids used in this study

Plasmid name	ORFs	Backbone/resistance
pTara:500	P_{BAD}:T7 RNAP, P_{const}:araC	p15A,Cm^R
pTSara	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880, P_{const}:araC	p15A,Cm^R
pTSara-N	P_{BAD}:T7 RNAP 1–179, P_{const}:araC	p15A,Cm^R
pTSara-C	P_{BAD}:T7 RNAP 180–880, P_{const}:araC	p15A,Cm^R
pTSlb	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880	p15A,Cm^R
pTSara-wt	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880, P_{const}:araC	p15A,Cm^R
pTSara-Q758C	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:Q758C, P_{const}:araC	p15A,Cm^R
pTSara-R756S	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:R756S, P_{const}:araC	p15A,Cm^R
pTSara-R756K	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:R756K, P_{const}:araC	p15A,Cm^R
pTSara-N748D	P_{BAD}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:N748D, P_{const}:araC	p15A,Cm^R
pTSlb-wt	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880	p15A,Cm^R
pTSlb-Q758C	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:Q758C	p15A,Cm^R
pTSlb-R756S	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:R756S	p15A,Cm^R
pTSlb-R756K	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:R756K	p15A,Cm^R
pTSlb-N748D	P_{Lac}:T7 RNAP 1–179, P_{BAD}:T7 RNAP 180–880:N748D	p15A,Cm^R
pET28:GFP	$P_{T7/LacO}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:GACT:GFP	$P_{T7:GACT}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:GACG:GFP	$P_{T7:GACG}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:GCAT:GFP	$P_{T7:GCAT}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:CACT:GFP	$P_{T7:CACT}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:CCCT:GFP	$P_{T7:CCCT}$:GFP, P_{const}:lacI	pmb1,Kan^R
pET:ACAT:GFP	$P_{T7:ACAT}$:GFP, P_{const}:lacI	pmb1,Kan^R