

Supplement to:

The flap domain is required for pause RNA hairpin inhibition of catalysis by RNA polymerase and can modulate intrinsic termination

Innokenti Touloukhonov and Robert Landick*

Department of Bacteriology
University of Wisconsin
Madison, WI 53706

Running title: Flap domain mediates pause hairpin action

*Corresponding author. Email: landick@bact.wisc.edu, Fax: 608 262 9865.

Strains

E. coli B strain RL1480 [relevant genotype: λ DE3 *rpoB5142*(HY526, SR543, Rif^r, SPA998) *zja::kan*] is a derivative of BL21 λ DE3 (Studier et al., 1990) that is described elsewhere (Geszvain, 2003). SPA998 is a 234-aa insertion of four repeats of the IgG-binding domain of *S. aureus* Protein A inserted at aa 998 of β (Opalka et al., 2000; Tavromina et al., 1996).

Construction of deletion mutants

To construct $\beta\Delta(890-914)\Omega\text{Gly}_3$, site-directed PCR mutagenesis was performed on the *rpoB* plasmid pRL702 (Artsimovitch et al., 2003) using oligonucleotides 1 and 2 that flanked the deleted fragment (Table S1). The shortened *rpoB* fragment located between the unique *RsrII* and *SgfI* sites was sequenced, excised, and recloned into *RsrII*, *SgfI*-cut pRL702 to give pIT109 and *RsrII*-, *SgfI*-cut pRL702*rpoB*(SF531; rif^r18) to give pIT110. To obtain overexpression plasmid encoding $\beta\Delta(890-914)\Omega\text{Gly}_3$, the mutant *rpoB* fragments were transferred to the RNAP overexpression plasmid pIA423 (Artsimovitch et al., 2003) on a *NcoI* to *SdaI* fragment.

RNAP purification

Plasmids encoding variants of *E. coli* RNAP (wild-type and mutants) were transformed into RL1480. Wild-type and mutant RNAPs were purified by chitin-affinity chromatography and intein-mediated removal of the chitin-binding domain tag after overexpression from a T7 RNAP based expression plasmid, as described (Artsimovitch et al., 2003). Fractions containing RNAP were pooled, and concentrated using Centriplus 100 or Ultrafree concentrators (Millipore) to 1-5 ml depending on total RNAP recovered. Wild-type RNAP was then dialyzed against storage buffer (10 mM Tris, pH 7.9, 50% glycerol, 0.1 mM EDTA, 0.1 mM DTT, 0.1 M NaCl) for 12-14 hours. $\beta\Delta(890-914)\Omega\text{Gly}_3$ and $\beta\Delta(900-909)$ RNAPs were subjected to additional purification to remove chromosomally encoded RNAP, taking advantage of the SPA998 tag in β from strain RL1480. The mutant RNAPs were dialyzed into 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 5 % glycerol, 0.1 mM Na-EDTA, 0.1 mM DTT (SPA buffer) and then incubated with 50-200 μ l of IgG-agarose beads (Sigma) in SPA buffer, essentially as described previously (Tavromina et al., 1996). After 30 min at 4 °C, the samples were loaded into 1 ml disposable columns (BioRad EconoPac). Flow-through fractions were collected and dialyzed against storage buffer. Wild-type and mutant RNAP holoenzymes (core $\beta'\beta\alpha_2$ plus σ^{70}) were prepared by incubating a five-fold molar excess of σ^{70} with core enzyme for 30 minutes at 30 °C.

References

- Artsimovitch, I., Svetlov, V., Murakami, K., and Landick, R. (2003). Co-overexpression of *E. coli* RNA polymerase subunits allows isolation and analysis of mutant enzymes lacking lineage-specific sequence insertions. *J Biol Chem* 278, 12344-12355.
- Chan, C. L., and 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.
- Ederth, J., Artsimovitch, I., Isaksson, L., and Landick, R. (2002). The downstream DNA jaw of bacterial RNA polymerase facilitates both transcriptional initiation and pausing. *J Biol Chem* 277, 37456-37463.
- Geszvain, K. 2003. The role of the flap domain of *Escherichia coli* RNA polymerase in transcription. Ph.D. thesis. University of Wisconsin. Madison, WI.
- Lee, D. N., Phung, L., Stewart, J., and Landick, R. (1990). Transcription pausing by *Escherichia coli* RNA polymerase is modulated by downstream DNA sequences. *J Biol Chem* 265, 15145-15153.
- Opalka, N., Mooney, R. A., Richter, C., Severinov, K., Landick, R., and Darst, S. A. (2000). Direct localization of a beta-subunit domain on the three-dimensional structure of *Escherichia coli* RNA polymerase. *Proc Natl Acad Sci U S A* 97, 617-622.
- Reynolds, R., Bermúdez-Cruz, R. M., and Chamberlin, M. J. (1992). Parameters affecting transcription termination by *Escherichia coli* RNA polymerase. Analysis of 13 rho-independent terminators. *J Mol Biol* 224, 31-51.
- Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990). Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol* 185, 60-89.
- Tavromina, P., Landick, R., and Gross, C. (1996). Isolation and characterization of defective *Escherichia coli* RNA polymerase *rpoB* mutations. *J Bacteriol* 178, 5263-5371.
- Touloukhonov, I., Artsimovitch, I., and Landick, R. (2001). Allosteric control of RNA polymerase by a site that contacts nascent RNA hairpins. *Science* 292, 730-733.

Table S1. Plasmids, DNA primers , and transcription templates.

Name	Description	Source or note
<u>Plasmids</u>		
pRL702	<i>rpoB</i> with N-terminal His ₆ tag on β subunit	(Artsimovitch et al., 2003)
pRL702 <i>rpoB</i> (S531F;rif ^d 18)	<i>rpoB</i> with rifampicin resistance mutation S531F;rif ^d 18)	(Geszvain, 2003)
pIT109	deletion of β aa 890-914 in pRL702	This work
pIT110	deletion of β aa 890-914 in pRL702 <i>rpoB</i> (S531F;rif ^d 18)	This work

Table S1, continued

pIA423	expresses α^+ β + β' -CBP/intein from T7 promoter	(Artsimovitch et al., 2003)
pIT111	deletion of β aa 890-914 in pIA423	This work
pIT112	deletion of β aa 890-914(rif d18) in pIA423	This work
pIA325	expresses α^+ $\beta\Delta(900-909)$ + β' -CBP/intein from T7 promoter	(Touloukhonov et al., 2001)
pIA171	<i>his</i> pause and terminator transcription template plasmid	(Ederth et al., 2002)
pDW302	Mutant <i>his</i> pause (C44→G; G59→C) plasmid. Insertion of <i>Dra</i> I to <i>Hind</i> III fragment of pCL183 between BamHI (Mung-bean nuclease treated) and <i>Hind</i> III sites of pRL417.	This work.
pCpGT3	T3 terminator transcription template plasmid	(Reynolds et al., 1992)
pCpG λ_{R2}	λ_{R2} terminator transcription template plasmid	(Reynolds et al., 1992)
pCL183	Derivative of pCL185 containing C44→G and G59→C substitutions in the <i>his</i> pause hairpin stem.	(Chan and Landick, 1993)
pRL417	T7 A1 promoter - <i>trp</i> pause	(Lee et al., 1990)
Primers		
4463 (oligoA)	5'-GGATGATGGTGGTGGTGGT	<i>his</i> pause hairpin antisense
4464 (oligo B)	5'-ATGATGGTGGTGGTGGTGGT	upstream <i>his</i> pause hairpin antisense
4465	5'-GTTGGTAAGGTAACGCCGGGAGGA GGAGACTCTTCTCTGCGCGTA	$\beta\Delta(890-914)\Omega\text{Gly}_3$ top
4466	5'-TACGCGCAGAGAAGAGTCTCCTCCTC CCGGCGTTACCTTACCAAC	$\beta\Delta(890-914)\Omega\text{Gly}_3$ bottom
3996	5'-CACTAATTTATTCCATGTCACACTTT TCGCATCTTTTTTATGCTATAATTATTT CATCGAGAGGGACACGGGG	<i>galP1</i> upstream
645	5'-CAGTTCCTACTCTCGCATG	pIA171 downstream
4467	5'-CACTAATTTATTCCATGTCACACTTTT CGCATCTTTTTTATGCTATAATTATTTCA TCGAGAGGGACACGGCG	<i>galP1</i> upstream for pCpGT3 and pCpG λ_{R2}
4468	5'-ACCGGTCTGTATCGCGCGCAA	<i>hisT</i> reverse
4469	5'-GGTCGACCTGCAGGTCGGAACGAAG	T3 reverse
1072	5'-GCTGGAGATCGAATTTCAAAGG	pCpG λ_{R2} , downstream reverse

