Supplemental Data

Isolation of amino acid substitution F278L, which disrupts the σ^{38} region 4/β flap interaction

To identify amino acid substitutions that disrupt the σ^{38} region 4/β flap interaction we took advantage of a bacterial two-hybrid assay (Dove et al., 1997; Dove and Hochschild, 1998) that enabled us to detect the interaction between a fragment of σ^{38} encompassing region 4 and a fragment of β encompassing the flap domain (Kuznedelov et al., 2002). Using a strategy that we had successfully used to identify amino acid substitutions that disrupt the σ^{70} region 4/β flap interaction (Nickels et al., 2005), we identified a single amino acid substitution, F278L, that specifically disrupts the σ^{38} region 4/β flap interaction (see Supporting Experimental Procedures and Figure S1). To demonstrate that the F278L substitution did not simply disrupt the structural integrity of σ^{38} region 4, we took advantage of a genetic assay that we developed previously (Dove et al., 2000; Nickels et al., 2002b) to detect the ability of σ^{38} region 4 to bind a –35 element (Figure S1, panel C).

Effect of strengthening the σ^{70} region 4/β flap interaction on λQ-mediated antitermination when the TTGACT motif is disrupted

In previous work, we have shown that engagement of the paused elongation complex by λQ involves an interaction between λQ and σ^{70} region 4 that stabilizes the binding of region 4 to a DNA sequence element (TTGACT) that resembles a promoter –35 element (Nickels et al., 2002a). This TTGACT motif is separated from the pause-inducing –10-like element by just one base pair (see Figure 1A). Moreover, in the presence of λQ, regions 2 and 4 of σ^{70} can bind simultaneously to these two elements, requiring RNAP holoenzyme to adopt a conformation in which σ^{70} region 4 has been displaced from the β flap (Nickels et al., 2002a). Thus, we wondered whether displacement of σ^{70} region 4 from the β flap is required for λQ function solely because it permits σ^{70} region 4 to bind this TTGACT motif. To test this possibility, we asked whether or not altering the σ^{70} region 4/β flap interaction affected λQ function even when the binding of σ^{70} region 4 to the TTGACT motif was disrupted by mutation. To do this, we tested the effect of strengthening the σ^{70} region 4/β flap interaction on λQ-mediated antitermination in vitro using a $\lambda P_{R'}$ template containing a single base pair substitution (G–5A) that disrupts the interaction between σ^{70} region 4 and the TTGACT motif (Nickels et al., 2002a) (Figure S2). We performed the assays at a low concentration of λQ with either wild-type $\lambda P_{R'}$ or $\lambda P_{R'}$ (G–5A) and using holoenzyme reconstituted with either wild-type or mutant (T544I/D581G) σ^{70}. The results shown in Figure S2 indicate that either strengthening the σ^{70} region 4/β flap interaction (reactions performed with T544I/D581G mutant holoenzyme on a wild-type template) or disrupting the interaction between σ^{70} region 4 and the TTGACT motif (reactions performed with wild-type holoenzyme on the G–5A template) caused a ~2-fold reduction in the amount of λQ-dependent antitermination. Furthermore,

when the σ^{70} region 4/β flap interaction was strengthened and the interaction between σ^{70} region 4 and the TTGACT motif was disrupted (reactions performed with T544I/D581G mutant holoenzyme on the G–5A template) the amount of λQ-dependent antitermination was reduced by a factor of ~8. Thus, strengthening the σ^{70} region 4/β flap interaction has a significant effect on λQ-dependent antitermination in vitro even when the interaction between σ^{70} region 4 and the TTGACT motif is disrupted.

We also tested the effect of strengthening the σ^{70} region 4/β flap interaction on λQ-dependent antitermination in vivo under conditions where the interaction between σ^{70} region 4 and the TTGACT motif is disrupted (Figure S2, panel C). To do this, we used a $\lambda P_{R'}$-*lacZ* reporter construct in which the TTGACT motif bore the G–5A mutation. The wild-type or modified $\lambda P_{R'}$-*lacZ* reporter construct was introduced in single copy into strains containing either wild-type *rpoD* or *rpoD*-T544I/D581G. In the strain containing wild-type *rpoD*, disrupting the interaction between σ^{70} region 4 and the TTGACT motif caused a ~2-fold reduction in the amount of λQ-dependent antitermination at low concentrations of λQ. Thus, the effect of the G–5A mutation in the TTGACT motif is similar to the effect of the T544I/D581G mutations in *rpoD* (see also Figure 2D). Furthermore, when the σ^{70} region 4/β flap interaction was strengthened (by the T544I/D581G mutations in *rpoD*) and the interaction between σ^{70} region 4 and the TTGACT motif was disrupted, the amount of λQ-dependent antitermination was reduced ~4-fold. Therefore, consistent with the in vitro results (Figure S2, panel B), strengthening the σ^{70} region 4/β flap interaction has a significant effect on λQ-dependent antitermination in vivo even when the interaction between σ^{70} region 4 and the TTGACT motif is

disrupted. We conclude that there is a mechanistic requirement for displacement of σ^{70} region 4 from the β flap during the λQ engagement process even under circumstances that do not permit λQ to stabilize the binding of σ^{70} region 4 to the TTGACT motif (see also Figure S3).

Effect of strengthening the σ^{70} region 4/β flap interaction on λQ-mediated antitermination in the absence of NusA

In prior work, we found that the effect of disrupting the interaction between σ^{70} region 4 and the TTGACT motif was enhanced when λQ antitermination was tested under sub-obtimal reaction conditions (Nickels et al., 2002a). Among the sub-optimal conditions that exacerbated the effect of mutating the TTGACT motif was the absence of NusA in the reaction. NusA is a transcription elongation factor that stabilizes the binding of λQ to the paused early elongation complex (Yarnell and Roberts, 1992) and increases the magnitude of λQ-dependent antitermination ~4-5 fold (Grayhack et al., 1985). We therefore wondered whether the effect of strengthening the σ^{70} region 4/β flap interaction on λQ function would also be enhanced when the reactions were performed in the absence of NusA. (Note that the experiments presented in Figure 2 were performed in the presence of NusA.) The data presented in Figure S3 show the results of transcription assays performed in the absence of NusA. The results indicate that at low concentrations of λQ, strengthening the σ^{70} region 4/β flap interaction (reactions performed with T544I/D581G mutant holoenzyme) reduced λQ-dependent antitermination ~2.5 fold in the absence of

NusA (similar to the magnitude of the effect observed in the presence of NusA). Furthermore, the effect of strengthening the σ^{70} region 4/β flap interaction was overcome when the reactions were performed at saturating concentrations of λQ in the absence of NusA (again, mirroring what was observed in the presence of NusA). In marked contrast, the effect of disrupting the interaction between σ^{70} region 4 and the TTGACT motif (reactions performed with G–5A template) was not alleviated when the reactions were performed at saturating concentrations of λQ in the absence of NusA, consistent with previous observations (Nickels et al., 2002a). Thus, whereas the removal of NusA from the transcription reactions significantly enhances the effect of disrupting the binding of σ^{70} region 4 to the TTGACT motif, removal of NusA does not enhance the effect of strengthening the σ^{70} region 4/β flap interaction. These results provide further support for the conclusion, based on the data presented in Figure S2, that the mechanistic requirement for displacement of σ^{70} region 4 from the β flap during the λQ engagement process cannot be fully explained based on the observation that λQ subsequently stabilizes the binding of σ^{70} region 4 to the TTGACT motif.

Binding of 82Q to promoter complexes formed with wild-type holoenzyme

We used the exonuclease challenge assay to assess the ability of 82Q to bind to promoter complexes formed on the modified 82 $P_{R'}$ template with wild-type RNAP holoenzyme. As for the experiment of Figure 5B, we incubated the preformed promoter complexes in the presence or absence of 82Q and challenged the resultant complexes with exonuclease III. Figure S4 (panel B) shows that a weak 82Q-dependent barrier to

exonuclease digestion was detectable with wild-type RNAP holoenzyme, suggesting that 82Q binds relatively inefficiently to promoter complexes formed by the wild-type holoenzyme.

The graph in Figure S4, panel B, which shows the amount of label in the 82Q-dependent band plotted as a fraction of the total label attributable to promoter complexes for the experiments of Figure 5B and Figure S4, indicates that when the assays were performed with holoenzyme lacking σ^{70} region 4, the 82Q-dependent barrier represents a greater fraction of the total promoter complexes (~0.5 at the earliest timepoint) than when the assays were performed with wild type holoenzyme (~0.1 at the earliest timepoint). Based on this comparison we infer that the removal of σ^{70} region 4 facilitates the binding of 82Q to the promoter complexes, a conclusion that is consistent with the results of the transcription antitermination assays (Fig 4).

In the case of RNAP holoenzyme lacking σ^{70} region 4, the total label attributable to promoter complexes was obtained by summing the label in the 82Q-dependent band (–56) and the RNAP-dependent band (–25) (see Figure S4 panel C, left), whereas in the case of wild-type RNAP holoenzyme, the total label attributable to promoter complexes was obtained by summing the label in the 82Q-dependent band (–56) and the two RNAP-dependent bands (–33 and –25) (see Figure S4 panel C, right). We note that the barriers at –33 and –25 correspond closely in position (relative to the RNAP active center), and presumably in origin, to the σ^{70}-dependent barriers that occur at positions –22 and –12 in the context the paused elongation complex at $\lambda P_{R'}$ (see Figure 3). Comparison of the digestion patterns on the modified 82 $P_{R'}$ template for wild-type RNAP holoenzyme

(Figure S4) and holoenzyme lacking σ^{70} region 4 (Figure 5) shows that the –33 barrier is dependent on σ^{70} region 4. Thus, in the case of wild-type holoenzyme, we infer that after the 82Q barrier is breached, exonuclease sequentially reaches the σ^{70} region 4-dependent –33 barrier and then the –25 barrier (which we suggest is caused by regions 2 and 3 of σ^{70}). However, in the case of holoenzyme lacking σ^{70} region 4, exonuclease proceeds directly to the –25 barrier after breaching the 82Q-dependent barrier. We also note that a barrier corresponding to the –33 barrier does not arise in the context of the paused elongation complex on the wild-type 82 $P_{R'}$ template (where it would be expected to occur at approximately –12), despite the fact that σ^{70} region 4 is present (data not shown); we do not know the reason for this difference.

Experimental Procedures

Table 1. Strains and plasmids used in this study.

Strains/ Plasmids	**Relevant details**	**Reference/ Source**
Strains		
PB1 *rpoD800*(ts)	Temperature sensitive allele of *rpoD*	Liebke et al., 1980
FW 102		Whipple 1998
SG 230	FW 102 with wild-type *rpoD* linked to a kanamycin resistance gene	This work
SG 233	FW 102 with *rpoD* T544I/D581G linked to a kanamycin resistance gene	This work
BN 500	FW 102 with wild-type *rpoD* linked to a kanamycin resistance gene and harboring an F' that contains $\lambda P_{R'}$-*lacZ* and a tetracycline resistance gene; strain BN 500 was constructed by mating strain BN 147 with strain SG 230	This work

BN 504	FW 102 with *rpoD* T544I/D581G linked to a kanamycin resistance gene and harboring an F' that contains $\lambda P_{R'}$-*lacZ* and a tetracycline resistance gene; strain BN 504 was constructed by mating strain BN 147 with strain SG 233	This work
BN 147	FW 102 harboring an F' that contains $\lambda P_{R'}$-*lacZ* and a tetracycline resistance gene	Nickels et al., 2002a
KS 1	p*lac*O_R2–62 linked to *lacZ* present in single copy on the chromosome	Dove et al., 1997
BN 317	FW 102 harboring an F' that contains test promoter p*lac*Cons–35C-*lacZ* and a kanamycin resistance gene	Nickels et al., 2002b
BN 501	FW 102 with wild-type *rpoD* linked to a kanamycin resistance gene and harboring an F' that contains $\lambda P_{R'}$ (G–5A)-*lacZ* and a tetracycline resistance gene; strain BN 501 was constructed by mating strain BN 148 with strain SG 230	This work
BN 505	FW 102 with *rpoD* T544I/D581G linked to a kanamycin resistance gene and harboring an F' that contains $\lambda P_{R'}$ (G–5A)-*lacZ* and a tetracycline resistance gene; strain BN 505 was constructed by mating strain BN 148 with strain SG 233	This work
BN 148	FW 102 harboring an F' that contains $\lambda P_{R'}$ (G–5A)-*lacZ* and a tetracycline resistance gene	Nickels et al., 2002a
Plasmids		
pLHN12-His	Vector to overexpress N-terminally His-tagged σ's under the control of a T7 RNAP-dependent promoter	Panaghie et al., 2000
pFW11^{Tet}-$\lambda P_{R'}$	contains sequence extending from –109 to +238 of $\lambda P_{R'}$ that includes the natural terminator $t_{R'}$	Nickels et al., 2002b
p82a	contains sequence extending from –100 to +116 of 82 $P_{R'}$ that includes the natural terminator t_{82}	Goliger and Roberts, 1989
pMM300	contains the open reading frame for wild-type σ^{70}, a kanamycin resistance gene and sequence complementary to the 3' end of the *rpoD* gene, all present on a KpnI-NotI restriction fragment	Nickels et al., 2002a
pMM300 (T544I/D581G)		This work
pKD46	encodes the λ red recombinase	Datsenko and Wanner, 2000
pBRλQΔ–35	directs the expression of low levels of λQ	Nickels et al., 2002a
pBRΔQ	encodes no functional λQ	Nickels et al., 2002a

pACλcI-β flap	encodes residues 1-236 of the bacteriophage λ cI protein fused via three alanine residues to residues 858-946 of the β subunit of *E. coli* RNAP under the control of the *lac*UV5 promoter	Kuznedelov et al., 2002
pBRα-σ^{38}	encodes residues 1-248 of the α subunit of *E. coli* RNAP fused to residues 243-330 of the σ^{38} subunit of *E. coli* RNAP under the control of tandem *lpp* and *lac*UV5 promoters	Dove et al., 2000
pBRα	encodes wild-type α under the control of tandem *lpp* and *lac*UV5 promoters	Dove et al., 1997

Introduction of T544I/D581G mutations into *rpoD*

Plasmid pMM300 (Nickels et al., 2002a) contains the open reading frame for wild-type σ^{70}, a kanamycin resistance gene and sequence complementary to the 3' end of the *rpoD* gene, all present on a KpnI-NotI restriction fragment. Substitutions T544I/D581G were introduced into pMM300 by site directed mutagenesis, creating plasmid pMM300 (T544I/D581G). Plasmids pMM300 and pMM300 (T544I/D581G) were digested with KpnI and NotI, and the fragments that contained the σ^{70} coding region together with the kanamycin resistance gene and sequence complementary to the 3' end of the *rpoD* gene were isolated.

Plasmid pKD46 (Datsenko and Wanner, 2000), encoding the λ red recombinase, was introduced into a PB1 strain carring *rpoD800*(ts) (Liebke et al., 1980). The plasmid-containing cells were made electrocompetent at 30^{o}C in the presence of 0.2% L-arabinose to induce production of the λ red recombinase (Datsenko and Wanner, 2000) and the cells were electroporated with the KpnI-NotI fragments isolated from pMM300 and pMM300 (T544I/D581G). Transformants were recovered for 2 hours in SOC media at 30^{o}C. Recombinants resulting from the transformation in which the *rpoD800*(ts) allele

was replaced with either a wild-type *rpoD* or *rpoD* (T544I/D581G) allele were selected on Luria agar plates containing kanamycin (50 μg/mL) at 42°C. The presence of the wild-type *rpoD* or *rpoD* (T544I /D581G) allele was confirmed by sequencing a PCR product derived from the chromosomal copy of *rpoD*. The wild-type *rpoD* or *rpoD* T544I/D581G allele was moved into strain FW102 (Whipple 1998) via P1 transduction to kanamycin resistance to create strains SG 230 (wild-type σ^{70}) and SG 233 (σ^{70} T544I/D581G). Reporter strains BN 500 ($\lambda P_{R'}$ /wild-type σ^{70}) and BN 504 ($\lambda P_{R'}$ /σ^{70} T544I/D581G) were constructed by mating strains SG 230 or SG 233 with strain BN147 (Nickels et al., 2002a), that harbors the $\lambda P_{R'}$-*lacZ* reporter on an F' episome that provides tetracycline resistance.

β-galactosidase Assays

For the assays presented in Figure S2 panel C, reporter strains cells were transformed with either plasmid pBRλQΔ–35, which directs the expression of low levels of λQ (Nickels et al., 2002a), or plasmid pBRΔQ, which encodes no functional λQ (Nickels et al., 2002a). Individual transformants were selected and grown in LB supplemented with carbenicillin (100 μg/ml), tetracycline (10 μg/ml), kanamycin (50 μg/ml) and 100 μM Isopropyl-β-D-thiogalactoside (IPTG). β-galactosidase activity was assayed as described (Dove and Hochschild, 2004).

Isolation of F278L Mutation in σ^{38} Region 4

To identify mutations in σ^{38} region 4 that disrupted the interaction between region 4 and the β flap, we mutagenized the σ^{38} moiety of the α-σ^{38} chimera using the PCR with *Taq* polymerase. A pool of mutant α-σ^{38} chimeras was transformed into strain KS1 containing plasmid pACλcI-β flap. Plating the transformation mixture onto indicator medium containing 100μM IPTG, X-gal (40 μg/ml), and the β-galactosidase inhibitor tPEG (250 μM) permitted the identification of colonies that contained α-σ^{38} chimeras unable to support λcI-β flap-dependent activation from p*lac*O_R2−62. Plasmid DNA from these colonies was isolated and the mutant α-σ^{38} chimeras were then transformed into strain BN317 and assayed for their abilities to stimulate transcription from the test promoter p*lac*Cons−35C. Mutants were sought that nearly or completely abolished λcI-β flap-dependent activation from p*lac*O_R2−62, but did not affect the ability of the α-σ^{38} to activate transcription from p*lac*Cons−35C. Out of 2,000 colonies that were analyzed, 10 contained plasmids that fit these criteria. Plasmid DNA from these colonies was isolated and the genes encoding the mutant α-σ^{38} chimeras were sequenced. Each plasmid contained a single nucleotide change in the mutagenized portion of the chimeric gene that specified the F278L substitution in the α-σ^{38} chimera.

References

Datsenko K.A. and Wanner B.L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA *97*, 6640-6645.

Dove, S.L., Joung, J.K., and Hochschild, A. (1997). Activation of prokaryotic transcription through arbitrary protein-protein contacts. Nature *386*, 627-630.

Dove, S.L., and Hochschild, A. (1998). Conversion of the ω subunit of *Escherichia coli* RNA polymerase into a transcriptional activator or an activation target. Genes Dev. *12*, 745-754.

Dove, S.L., Huang, F.W. and Hochschild, A. (2000). Mechanism for a transcriptional activator that works at the isomerization step. Proc. Natl. Acad. Sci. USA. *97*, 13215-13220.

Dove, S.L. and Hochschild, A. (2004). A bacterial two-hybrid system based on transcription activation. Methods Mol. Biol. *261*, 231-246.

Goliger J.A. and Roberts, J.W. (1989). Sequences required for antitermination by phage 82 Q protein. J. Mol. Biol. *210*, 461-471.

Grayhack, E. J., Yang, X. J., Lau, L.F., and Roberts, J.W. (1985). Phage Lambda Gene-Q Antiterminator Recognizes RNA-Polymerase Near the Promoter and Accelerates It Through a Pause Site. Cell *42*, 259-269.

Liebke, H., Gross, C., Walter,W., and Burgess, R. (1980). A new mutation, *rpoD*800, affecting the sigma subunit of *E. coli* RNA polymerase is allelic to two other sigma mutants. Mol. Gen. Genet. *177*, 277-282.

Nickels, B.E., Roberts, C.W., Sun, H., Roberts, J.W. and Hochschild, A. (2002a). The σ^{70} subunit of RNA polymerase is contacted by the λQ antiterminator during early elongation. Mol. Cell *10*, 611-622.

Nickels, B.E., Dove, S.L., Murakami, K,S., Darst, S.A. and Hochschild A. (2002b). Protein-protein and protein-DNA interactions of σ^{70} region 4 involved in transcription activation by λcI. J. Mol. Biol. *324*, 17-34.

Panaghie, G., Aiyar, S.E., Bobb, K.L., Hayward, R.S. and de Haseth, P.L. (2000). Aromatic amino acids in region 2.3 of *Escherichia coli* σ^{70} participate collectively in the formation of an RNA polymerase-promoter open complex. J. Mol. Biol. *299*, 1217-1230.

Kuznedelov, K., Minakhin, L., Niedziela-Majka, A., Dove, S.L., Rogulja, D., Nickels, B.E., Hochschild, A., Heyduk, T., and Severinov, K. (2002). A role for interaction of the RNA polymerase flap domain with the sigma subunit in promoter recognition. Science *295*, 855-857.

Whipple, F.W. (1998). Genetic analysis of prokaryotic and eukaryotic DNA-binding proteins in *Escherichia coli*. Nucleic Acids Res. *26*, 3700-3706.

Yarnell, W.S. and Roberts, J.W. (1992). The Phage λ Gene Q Transcription Antiterminator Binds DNA in the Late Gene Promoter As It Modifies RNA-Polymerase. Cell *69*, 1181-1189.

Figure Legends

Figure S1. Amino acid substitution F278L in σ^{38} region 4 disrupts its interaction with the β flap.

A) Bacterial two-hybrid assay used to screen for substitutions in σ^{38} region 4 that alter its interaction with the β flap. Cartoon depicts how the interaction between σ^{38} region 4 (fused to the α-NTD) and the β flap (fused to the bacteriophage λ cI protein) activates transcription from test promoter p*lac*O_R2−62, which bears the λ operator O_R2 centered 62 bp upstream of the start site of the *lac* core promoter. In reporter strain KS1, test promoter p*lac*O_R2−62 is located on the chromosome and drives the expression of a linked *lacZ* gene.

B) Effect of amino acid substitution F278L in the σ^{38} moiety of the α-σ^{38} chimera on transcription from p*lac*O_R2−62 in the presence of the λcI-β flap fusion protein. KS1 cells harboring compatible plasmids directing the synthesis of the indicated proteins were grown in the presence of different concentrations of IPTG and assayed for β-galactosidase activity.

C) Schematic of test promoter p*lac*Cons–35C, which we used as a control to demonstrate that amino acid substitution F278L does not disrupt the structural integrity of the tethered σ^{38} moiety. This promoter bears a consensus –35 element (TTGACA) upstream of the core promoter elements. This consensus –35 element serves as a binding site for the tethered σ^{38} region 4 moiety. In reporter strain BN317, test promoter p*lac*Cons–35C is located on an F' episome and drives the expression of a linked *lacZ* gene.

D) Effect of amino acid substitution F278L in the σ^{38} moiety of the α-σ^{38} chimera on transcription from p*lac*Cons–35C. BN317 cells harboring plasmids directing the synthesis of the indicated proteins were grown in the presence of different concentrations of IPTG and assayed for β-galactosidase activity.

Figure S2. Effects of strengthening the σ^{70} region 4/β flap interaction and disrupting the interaction between σ^{70} region 4 and the TTGACT motif on λQ-mediated antitermination.

A) Schematic depicts the linear $\lambda P_{R'}$ template used for the in vitro transcription assays. Indicated are the promoter –10 and –35 elements, the λQ-binding element (QBE), the pause-inducing –10-like element, and the –35-like element (TTGACT motif) positioned between the QBE and the pause-inducing element.

B) Effects of strengthening (T544I/D581G) the σ^{70} region 4/β flap interaction and/or mutating the TTGACT motif (G–5A) in vitro. Shown are the results of single-round in vitro transcription assays performed at a low concentration of λQ (7.5 nM). Graphs show the percentage of transcripts derived from terminator readthrough (readthrough/

[readthrough + terminated]) at the indicated times after transcription was initiated. Reactions were performed using holoenzyme reconstituted with either wild-type σ^{70} or σ^{70} T544I/D581G, as indicated, and either a wild-type $\lambda P_{R'}$ template or a mutant template on which the TTGACT motif was disrupted (G–5A). Assays were performed three times on separate occasions with similar results. The graph shows the data obtained from a single representative experiment.

C) Effects of strengthening (T544I/D581G) the σ^{70} region 4/β flap interaction and/or mutating the TTGACT motif (G–5A) in vivo. Reporter strain cells containing either wild-type σ^{70} or σ^{70} T544I/D581G and harboring a $\lambda P_{R'}$-lacZ reporter with either the wild-type or the mutant TTGACT motif were transformed with a plasmid that did or did not encode λQ. The cells were grown in the presence of 100 μM IPTG and assayed for β-galactosidase activity. The bar graph shows the averages of four independent measurements (and standard deviations).

Figure S3. Effects of strengthening (T544I/D581G) the σ^{70} region 4/β flap interaction or mutating the TTGACT motif (G–5A) in the absence of NusA in vitro.

A) and B) Shown are the results of single-round in vitro transcription assays performed at a low concentration of λQ (panel A, 15 nM) or at a high concentration of λQ (panel B, 500 nM). Graphs show the percentage of transcripts derived from terminator readthrough (readthrough/ [readthrough + terminated]) at the indicated times after transcription was initiated. Reactions were performed in the absence of NusA using holoenzyme

reconstituted with either wild-type σ^{70} or σ^{70} T544I/D581G and either a wild-type $\lambda P_{R'}$ template or a mutant template in which the TTGACT motif was disrupted (G–5A). Assays were performed three times on separate occasions with similar results. The graph shows the data obtained from a single representative experiment.

Figure S4. Binding of 82Q to initiation complexes formed with wild-type holoenzyme.

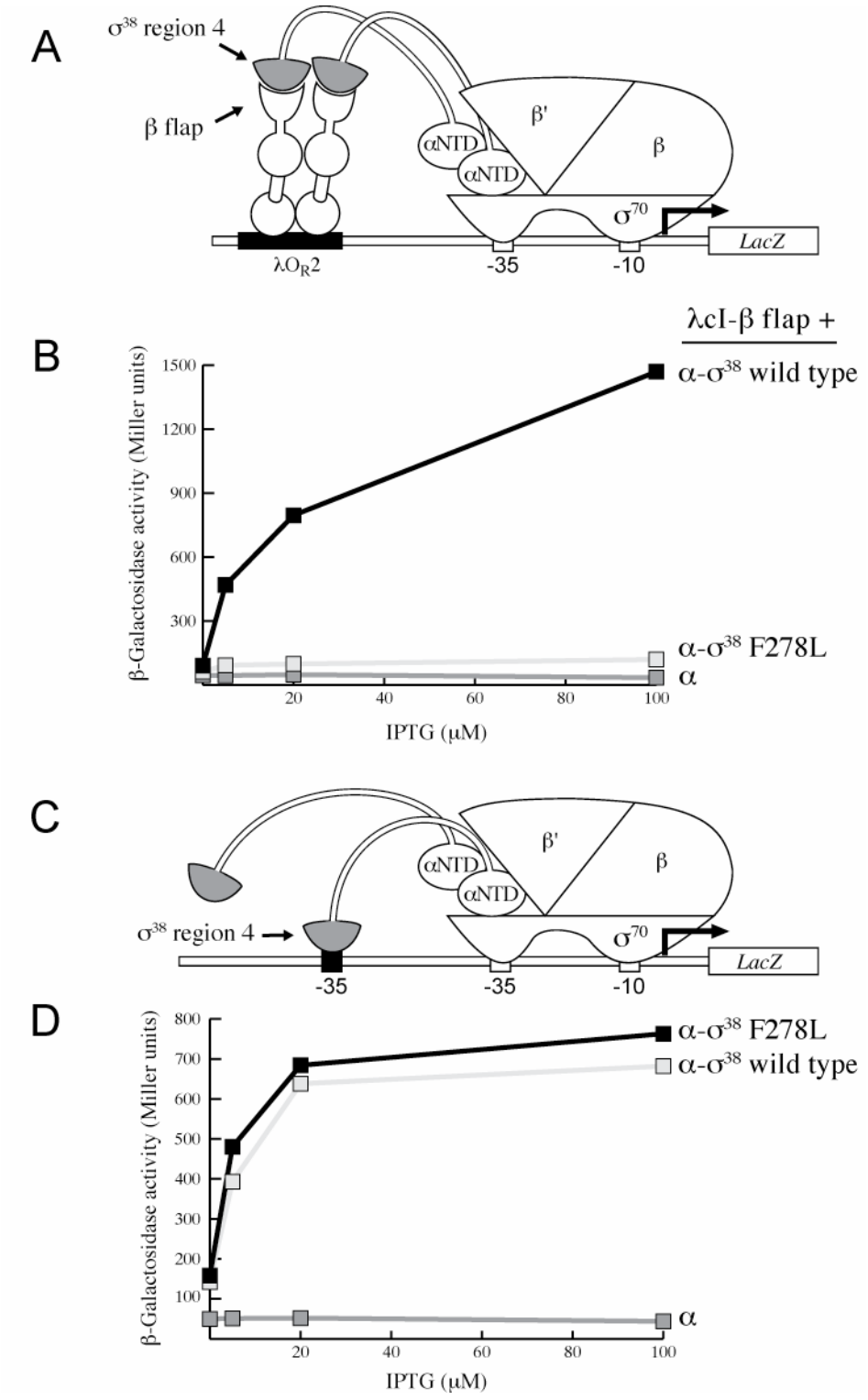
A) Depicted is the 82Q-engaged transcription initiation complex (formed with wild-type holoenzyme). The modified phage 82 $P_{R'}$ template used in the assays is end-labeled at the 5' end of the template (bottom) strand, as indicated. Also indicated are the positions (relative to the transcription start site) at which the progress of exonuclease III digestion is blocked by 82Q (–56) and by RNAP (–33 and –25). The red DNA segment is the consensus extended –10 element; σ^{70} region 2 is shown bound to the non-template strand. The RNAP outline is dashed to indicate that it does not depict a barrier to exonuclease digestion.

B) Exonuclease challenge assays. Initiation complexes were formed with wild-type RNAP. These complexes were then incubated with 20 nM 82Q (lanes 1-5) or no 82Q (lanes 6-10) and challenged with exonuclease III for the indicated times. Lane 11 contains an A+G sequencing ladder. The graph on the right shows a comparison of experiments performed either with RNAP holoenzyme lacking σ^{70} region 4 (RNAP σ^{70} 1-529) (see Figure 5B) or with wild-type RNAP holoenzyme (RNAP wt). The exonuclease barriers were quantified with Imagequant, and label in the 82Q-dependent band at –56 was plotted

as a fraction of the sum of label in the bands at –56 and –25 (in the case of reactions performed with RNAP σ^{70} 1-529) and –56, –33 and –25 (in the case of reactions performed with RNAP wt).

C) Quantification of the exonuclease barriers obtained with 82Q and RNAP σ^{70} 1-529 (left) or 82Q and RNAP wt (right).

Figure S1.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σ38 region 4
β flap
αNTD
αNTD
β'
β
σ70
LacZ
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-35
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B
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α-σ38 wild type
α-σ38 F278L
α
β-Galactosidase activity (Miller units)
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1200
900
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300
20
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60
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100
IPTG (μM)
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αNTD
αNTD
β'
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σ38 region 4
σ70
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-35
-10
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β-Galactosidase activity (Miller units)
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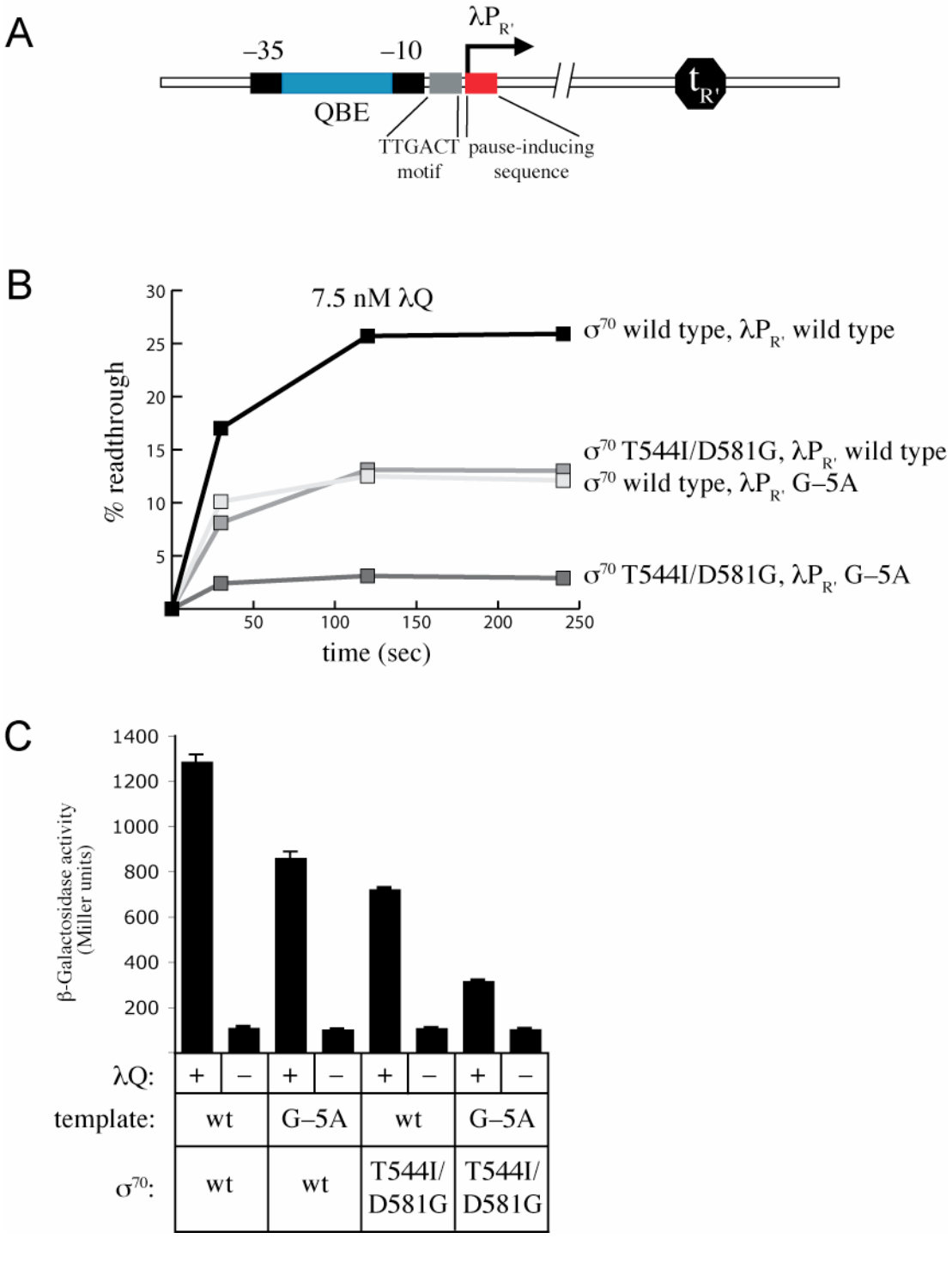
Figure S2.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λP R'
–35
–10
QBE
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TTGACT motif
pause-inducing sequence
B
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% readthrough
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σ70 wild type, λP R' wild type
σ70 T544I/D581G, λP R' wild type
σ70 wild type, λP R' G–5A
σ70 T544I/D581G, λP R' G–5A
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T544I/ D581G

Figure S3.

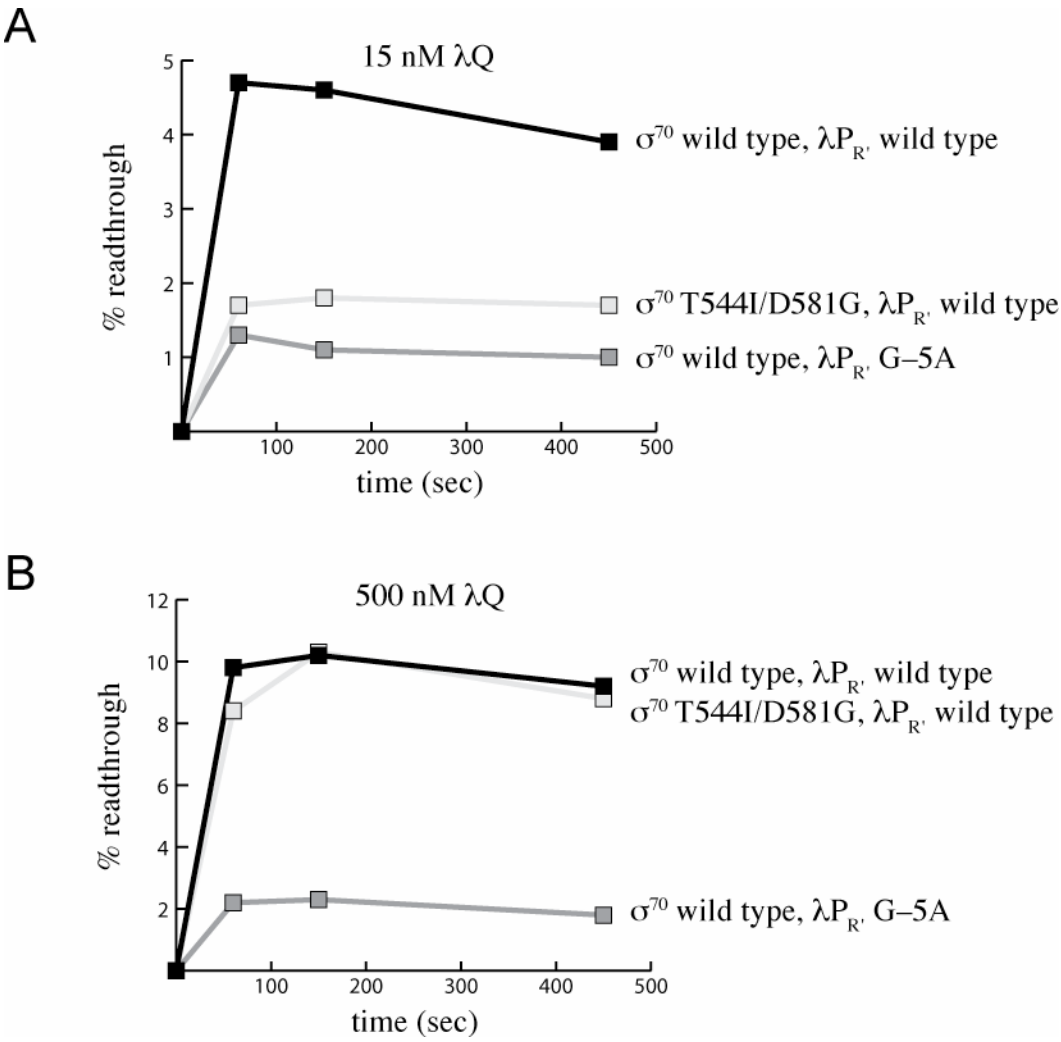
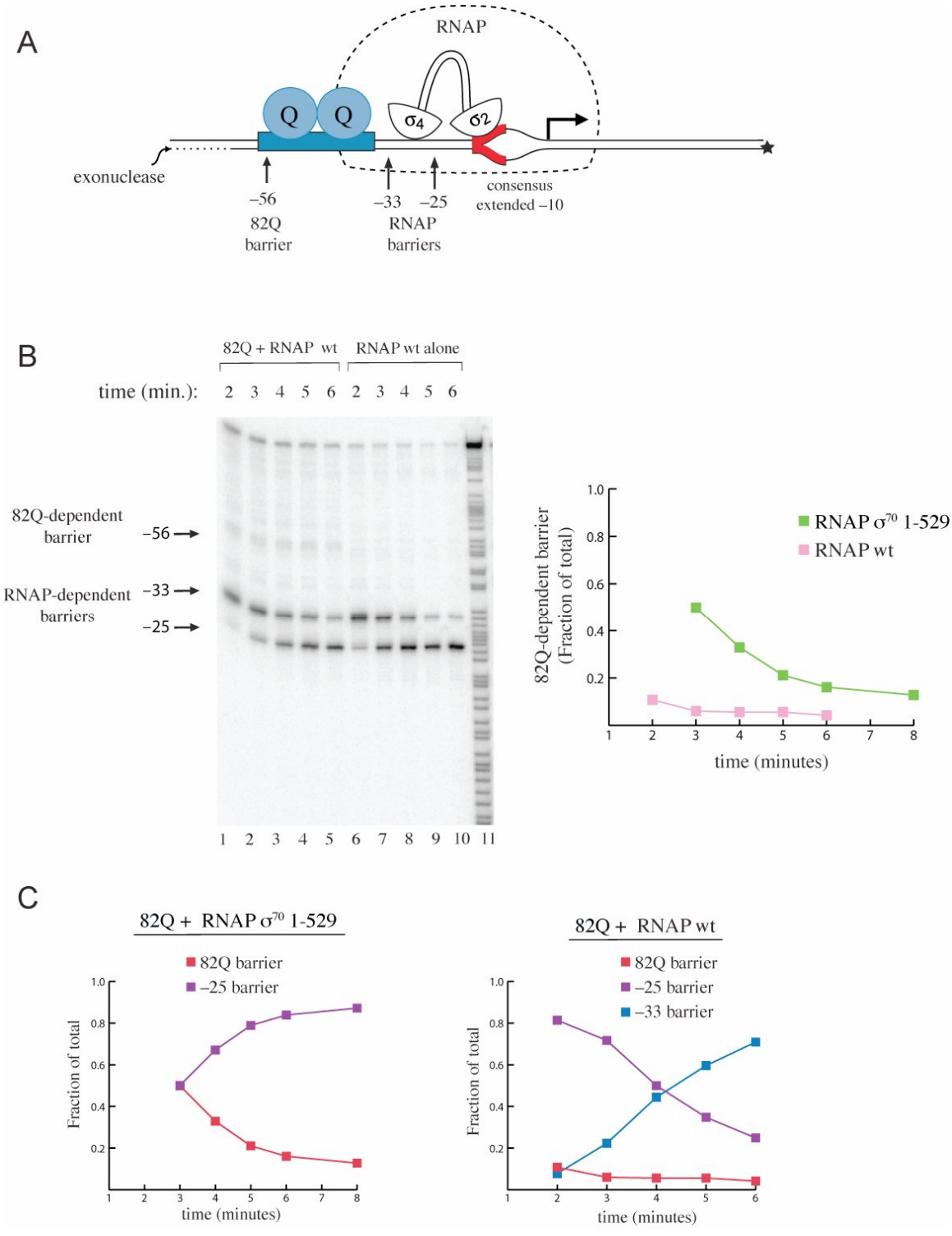

A
15 nM λQ
% readthrough
time (sec)
σ70 wild type, λP R' wild type
σ70 T544I/D581G, λP R' wild type
σ70 wild type, λP R' G–5A
B
500 nM λQ
% readthrough
time (sec)
σ70 wild type, λP R' wild type
σ70 T544I/D581G, λP R' wild type
σ70 wild type, λP R' G–5A

Figure S4.


A
RNAP
Q
Q
σ4
σ2
exonuclease
–56
82Q
barrier
–33
–25
RNAP
barriers
consensus
extended –10
B
82Q + RNAP wt
RNAP wt alone
time (min.): 2 3 4 5 6 2 3 4 5 6
82Q-dependent
barrier
–56
RNAP-dependent
barriers
–33
–25
1 2 3 4 5 6 7 8 9 10 11
82Q-dependent barrier
(Fraction of total)
RNAP σ70 1-529
RNAP wt
time (minutes)
C
82Q + RNAP σ70 1-529
82Q barrier
–25 barrier
Fraction of total
time (minutes)
82Q + RNAP wt
82Q barrier
–25 barrier
–33 barrier
Fraction of total
time (minutes)