The bacteriophage λ Q antiterminator protein contacts the β -flap domain of RNA polymerase

Padraig Deighan[†], Cristina Montero Diez[†], Mark Leibman[†], Ann Hochschild^{†*}, and Bryce E. Nickels^{‡§}

[†]Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115; and [§]Waksman Institute and Department of Genetics, Rutgers, The State University of New Jersey, 190 Frelinghuysen Road, Piscataway, NJ 08854

Edited by E. Peter Geiduschek, University of California at San Diego, La Jolla, CA, and approved August 12, 2008 (received for review June 13, 2008)

The multisubunit RNA polymerase (RNAP) in bacteria consists of a catalytically active core enzyme ($\alpha_2\beta\beta'\omega$) complexed with a σ factor that is required for promoter-specific transcription initiation. During early elongation the stability of interactions between σ and core decreases, in part because of the nascent RNA-mediated destabilization of an interaction between region 4 of σ and the flap domain of the β -subunit (β -flap). The nascent RNA-mediated destabilization of the σ region 4/ β -flap interaction is required for the bacteriophage λ Q antiterminator protein (λ Q) to engage the RNAP holoenzyme. Here, we provide an explanation for this requirement by showing that λ Q establishes direct contact with the β -flap during the engagement process, thus competing with σ^{70} region 4 for access to the β -flap. We also show that λ Q's affinity for the β -flap is calibrated to ensure that λ Q activity is restricted to the λ late promoter P_R . Specifically, we find that strengthening the λ Q/ β -flap interaction allows λ Q to bypass the requirement for specific *cis*-acting sequence elements, a λ Q-DNA binding site and a RNAP pause-inducing element, that normally ensure λ Q is recruited exclusively to transcription complexes associated with P_R . Our findings demonstrate that the β -flap can serve as a direct target for regulators of elongation.

sigma | transcription elongation

The bacterial RNA polymerase (RNAP) holoenzyme consists of a catalytically active core enzyme ($\alpha_2\beta\beta'\omega$) in complex with a σ factor, which confers on the core enzyme the ability to initiate promoter-specific transcription (reviewed in ref. 1). In the context of the RNAP holoenzyme, σ^{70} (the primary σ factor in *Escherichia coli*) contacts the conserved -10 and -35 promoter elements. All primary σ factors share four regions of conserved sequence (regions 1–4) (2). Regions 2 and 4 contain DNA-binding domains responsible for recognition of the promoter -10 element and -35 element, respectively. Holoenzyme formation depends on an interaction between σ^{70} region 2 and a coiled-coil motif in the β' -subunit (the β' coiled coil) (3, 4); this interaction is also required for σ^{70} to make functional contact with the promoter -10 element (5). Interaction between σ^{70} region 4 and the flap domain of the β -subunit (the β -flap), although not required for holoenzyme formation, is required for contact with the promoter -35 element (6). In particular, the σ^{70} region 4/ β -flap interaction properly positions σ^{70} region 4 with respect to σ^{70} region 2, thus enabling σ^{70} region 4 and σ^{70} region 2 to simultaneously contact promoter elements separated by ≈ 17 bp (6).

During early elongation the stability of interactions between σ^{70} and core RNAP decreases (reviewed in ref. 7), in part because of the nascent RNA-mediated destabilization of the σ^{70} region 4/ β -flap interaction (8). Specifically, σ^{70} region 4, when bound to the β -flap, is positioned within the path of the nascent RNA as it first emerges from the RNA exit channel (the channel through which the nascent RNA is extruded during transcription elongation) (9, 10) at a nascent RNA length of ≈ 16 nt (8, 11–13). Thus, steric clash between the emerging nascent RNA and σ^{70} region 4 destabilizes the σ^{70} region 4/ β -flap interaction (8, 14). Prior work indicates that this nascent RNA-mediated destabili-

zation of the σ^{70} region 4/ β -flap interaction facilitates the process by which the bacteriophage λ Q antiterminator protein (λ Q) gains access to RNAP during early elongation (14, 15).

An operon-specific elongation factor that controls the expression of the phage λ late genes, λ Q has served as an important model for achieving a mechanistic understanding of the regulation of RNAP function during transcription elongation (reviewed in ref. 16). Through its association with RNAP, λ Q renders RNAP resistant to transcription pausing and insensitive to downstream termination signals (reviewed in ref. 16). Although these effects occur in the context of a highly stable λ Q-containing elongation complex (17–19), λ Q requires two specialized *cis*-acting sequences associated with the λ late promoter λP_R to gain access to the transcription complex: a Q binding element (QBE) and a pause-inducing element (reviewed in ref. 16) (Fig. 1A). The pause-inducing element resembles a promoter -10 element and pausing of RNAP (which occurs at a nascent RNA length of 16 or 17 nt) is mediated by interaction between σ^{70} region 2 and this -10 -like element (20) (Fig. 1B). Interactions that occur between λ Q bound to the QBE and the paused transcription complex facilitate λ Q's stable association with the elongation complex (21). Among these is an interaction between λ Q and σ^{70} region 4, which stabilizes a conformation of the RNAP holoenzyme in which σ^{70} region 4 is displaced from the β -flap (15, 22) (Fig. 1C).

Here, we demonstrate that during its initial engagement with the RNAP holoenzyme λ Q establishes direct contact with the β -flap. Thus, our findings provide an explanation for the mechanistic requirement that σ^{70} region 4 be displaced from the β -flap during the λ Q engagement process (14, 15, 22). In addition, we demonstrate that strengthening the λ Q/ β -flap interaction allows λ Q to bypass the requirements for the QBE and the pause-inducing element in the engagement process. This finding indicates that λ Q's affinity for the β -flap is calibrated to help ensure that λ Q acts exclusively on transcription complexes associated with the λ late promoter P_R .

Results

λ Q Interacts with the β -Flap. The finding that λ Q's initial engagement with the holoenzyme requires the displacement of σ^{70} region 4 from the β -flap raised the possibility that λ Q competes with σ^{70} region 4 for access to the β -flap. As an initial test of this hypothesis we used a bacterial two-hybrid assay (23) (Fig. 2A) to determine whether λ Q directly contacts the β -flap (residues 831–1057 of β). Using this assay, we detected a weak, but

Author contributions: P.D., A.H., and B.E.N. designed research; P.D., C.M.D., M.L., and B.E.N. performed research; P.D., A.H., and B.E.N. analyzed data; and P.D., A.H., and B.E.N. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

*To whom correspondence may be addressed. E-mail: ahochschild@hms.harvard.edu or bnickels@waksman.rutgers.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0805757105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

Fig. 1. Sequence of events at λP_R . (A) Presence of λQ (shown in blue) allows RNAP that has initiated transcription from λP_R to read-through terminator t_R . Blow-up shows the functionally important elements at λP_R : the promoter -10 and -35 elements, the QBE, the pause-inducing -10-like element, and a -35-like element (TTGACT motif) positioned between the QBE and the pause-inducing element. Note that the TTGACT motif is separated by one base pair from the pause-inducing -10-like element. (B) Sequence of events during initiation and early elongation at λP_R . Shown first is the initiation complex; σ^{70} region 4 (σ_4) is bound to the β -flap (blue triangle). σ^{70} region 3.2 ($\sigma_{3.2}$) is a flexible linker region that is located within the RNA exit channel (9, 10). Shown just below the initiation complex is the paused early elongation complex at λP_R with σ^{70} region 2 (σ_2) bound to the pause-inducing -10-like element (shown in red). The nascent RNA (not shown) has displaced $\sigma_{3.2}$ from the RNA exit channel (22) and has destabilized the σ^{70} region 4/ β -flap interaction (8). Accordingly, σ_4 (dotted outline) is shown oscillating between β -flap-bound and unbound states. (C) λQ (shown as a dimer) binds to the QBE and engages the paused elongation complex. λQ contacts σ^{70} region 4 and stabilizes its binding to the TTGACT motif (gray rectangle), causing RNAP holoenzyme to adopt a conformation in which σ_4 and σ_2 are simultaneously bound to DNA elements that are separated by 1 bp (15). Formation of this RNAP holoenzyme–DNA complex requires σ_4 be displaced from the β -flap. λQ is depicted as contacting the β -flap.

significant, interaction between λQ and the β -flap (Fig. 2B). We next tested the Q proteins of several other lambdoid phage (21Q, 80Q and 82Q), all of which share a conserved mechanism of

action with λQ (reviewed in ref. 16). Each of these Q proteins also interacted with the β -flap, and the magnitude of the interaction observed was higher in each case than that observed with λQ (Fig. 2B). Taken together, these data suggest that λQ , 21Q, 80Q, and 82Q interact with the β -flap.

We next tested whether the Q proteins and σ^{70} region 4 contact the same surface of the β -flap. To do this, we tested the effect of removing the flap-tip helix (β residues 900–909), the primary target of σ^{70} region 4 (24). Removal of the flap-tip helix abolished the interaction between the β -flap and each of the Q proteins (Fig. 2B) and, as expected, abolished the interaction between the β -flap and σ^{70} region 4 [which can also be detected in the bacterial two-hybrid assay (6, 8)] [supporting information (SI) Fig. S1]. These results imply that σ^{70} region 4 and the Q proteins contact the same surface of the β -flap (i.e., participate in mutually exclusive interactions with the β -flap).

Isolation of Amino Acid Substitutions That Strengthen or Weaken the λQ / β -Flap Interaction. We next sought to identify amino acid substitutions that alter the strength of the λQ / β -flap interaction. First, we performed a screen to identify substitutions in λQ that strengthen its interaction with the β -flap (see *SI Text*). From this screen we identified three individual substitutions (T101I, A160V, and T165A) that modestly increased the strength of the λQ / β -flap interaction (Fig. 2C). We next combined these three substitutions to generate a protein we call λQ^{***} , which interacted significantly more strongly with the β -flap than did WT λQ (Fig. 2C).

Next, we sought to identify substitutions in the β -flap that weaken its interaction with λQ . To facilitate the identification of such substitutions, we carried out the screen by using λQ^{***} instead of WT λQ . Because we found that removing the flap-tip helix eliminated the λQ / β -flap interaction (Fig. 2B), we reasoned that we would be able to identify substitutions in the flap-tip helix that weaken the λQ / β -flap interaction.

We showed previously that disrupting the σ^{70} region 4/ β -flap interaction facilitates the ability of λQ to engage RNAP at λP_R (14). In principle, therefore, interpreting the effects of substitutions that disrupt both the λQ / β -flap interaction and the σ^{70} region 4/ β -flap interaction might be complicated (i.e., any possible inhibitory effect of disrupting the λQ / β -flap interaction might be masked by a compensatory effect of disrupting the σ^{70} region 4/ β -flap interaction). For this reason, we sought to identify at least one substitution that disrupted the λQ / β -flap interaction without affecting the σ^{70} region 4/ β -flap interaction.

We introduced alanine substitutions at all positions between 900–909 (except 904, which is already an alanine) and assayed the effects of these substitutions on the λQ / β -flap interaction (Fig. 2D and data not shown) and the σ^{70} region 4/ β -flap interaction (Fig. S1 and data not shown). From this analysis, we identified two substitutions, I905A and F906A, that strongly disrupted both the λQ / β -flap interaction (Fig. 2D) and σ^{70} region 4/ β -flap interaction (Fig. S1) and one substitution G907A that weakened the λQ / β -flap interaction (Fig. 2D) and did not affect the σ^{70} region 4/ β -flap interaction (Fig. S1). We next randomized the codon for residue 907 and found that substitution G907K had the largest effect on the λQ / β -flap interaction without significantly affecting the σ^{70} region 4/ β -flap interaction (Fig. 2D and Fig. S1). For our subsequent analysis, we used the G907K substitution (for its preferential effect on the λQ / β -flap interaction) and the pair of disruptive substitutions I905A and F906A [which, when combined, essentially abolished both the λQ / β -flap and the σ^{70} region 4/ β -flap interactions, as detected in the two-hybrid assay (Fig. 2D and Fig. S1)].

Effects of Weakening the λQ / β -Flap Interaction *in Vitro*. We next tested the effect of weakening the λQ / β -flap interaction on λQ antitermination function *in vitro*. To do this, we purified wild-

Fig. 2. Q interacts with the β -flap. (A) Bacterial two-hybrid assay used to test whether the Q proteins from various bacteriophage interact with the β -flap. Cartoon depicts how the interaction between Q, fused to the α -N-terminal domain (α -NTD), and the β -flap, fused to the bacteriophage λ CI protein, activates transcription from test promoter p_{lacO_2-62} , which bears the λ operator O_{L2} centered 62 bp upstream of the start site of the lac core promoter. In reporter strain FW102 O_{L2-62} , test promoter p_{lacO_2-62} is located on an F' episome and drives the expression of a linked $lacZ$ gene. (B) Results of β -galactosidase assays performed with FW102 O_{L2-62} cells that contained two compatible plasmids: one that encoded the indicated α -Q fusion protein or WT α , and another that encoded λ CI, the λ CI- β -flap fusion protein, or a derivative of the λ CI- β -flap fusion protein lacking the flap-tip helix (Δ FTH). The plasmids directed the synthesis of the fusion proteins under the control of isopropyl β -D-thiogalactoside (IPTG)-inducible promoters, and the cells were grown in the presence of 50 μ M IPTG, except for those containing the α -82Q fusion protein, which were grown in the presence of 20 μ M IPTG (because of the toxicity of this fusion protein at higher IPTG concentrations). The bar graph shows the averages of four independent measurements and standard deviations. We note that Western blot analysis to assess the intracellular levels of CI- β -flap fusion proteins used here and in D ruled out the possibility that the failure of the mutant CI- β -flap fusion proteins to detectably interact with each of the Q proteins was caused by protein instability (Fig. S2). (C) Amino acid substitutions in λ Q that strengthen the λ Q/ β -flap interaction. Results of β -galactosidase assays performed with FW102 O_{L2-62} cells that

Fig. 3. Effects of weakening the λ Q/ β -flap interaction *in vitro*. (A) Diagram of the DNA template used for *in vitro* transcription assays. The template contains $\lambda P_{R'}$ sequence extending from -109 to +232 that includes the natural terminator $t_{R'}$. The QBE bears a single base pair substitution (G-25C) that weakens the binding of λ Q to this DNA element (26). Indicated are the RNA products (wavy lines, labeled T and RT) that result, respectively, when RNAP terminates at $t_{R'}$ and when RNAP reads through the terminator to the end of the template. (B) Single-round *in vitro* transcription assays (see SI Text). Assays were done in either the absence of λ Q or the presence of increasing concentrations of λ Q (12.5, 25, 50, and 200 nM) using either WT RNAP or mutant RNAPs carrying substitutions in the β -flap that weaken the λ Q/ β -flap interaction (β I905A/F906A or β G907K). Transcripts were end-labeled with [γ - 32 P]ATP. The 192-nt terminated transcript (T) and the 330-nt read-through (run-off) transcript (RT) are indicated. For each reaction, the percentage of transcripts derived from terminator read-through, %RT (read-through/[read-through + terminated]) $\times$ 100, is shown. Assays were repeated four times with similar results.

type RNAP and two mutants: β I905A/F906A RNAP and β G907K RNAP. Because the β -flap has been implicated in the process of intrinsic termination (25), we first tested whether the substitutions in the flap-tip helix inhibited the ability of RNAP to respond to an intrinsic terminator. Using these RNAPs and a $\lambda P_{R'}$ template bearing the natural λ late terminator $t_{R'}$, we performed single-round *in vitro* transcription assays. We found that for WT RNAP and both of the mutant RNAPs (β I905A/F906A RNAP and β G907K RNAP) essentially all of the RNAP molecules terminated at $t_{R'}$ (Fig. 3 and data not shown). Next, we performed reactions in the presence of a range of λ Q concentrations and measured λ Q antitermination function by calculating the percentage of full-length transcripts (percentage read-through) emanating from $\lambda P_{R'}$. We observed no difference

contained two compatible plasmids: one that encoded the indicated α - λ Q fusion protein and another that encoded either λ CI or the λ CI- β -flap fusion protein. λ Q*** contains substitutions T101I, A160V, and T165A in combination. The plasmids directed the synthesis of the fusion proteins under the control of IPTG-inducible promoters, and the cells were grown in the presence of 20 μ M IPTG. The bar graph shows the averages of four independent measurements and standard deviations. (D) Amino acid substitutions in the β -flap that weaken the λ Q/ β -flap interaction. Results of β -galactosidase assays performed with FW102 O_{L2-62} cells containing one plasmid that encoded the α - λ Q*** fusion protein and a compatible plasmid that encoded either λ CI or the indicated λ CI- β -flap variant. The plasmids directed the synthesis of the fusion proteins under the control of IPTG-inducible promoters, and the cells were grown in the presence of 100 μ M IPTG. The bar graph shows the averages of four independent measurements and standard deviations.

Fig. 4. Effects of strengthening the λQ/β-flap interaction *in vivo*. (A) Results of β-galactosidase assays performed with reporter strain cells (BN147, PD105, PD104, or BN617) harboring either a WT λP_{R'}-lacZ reporter or the indicated mutant λP_{R'}-lacZ reporter (schematic on top). Cells contained a plasmid that directed the synthesis of WT λQ, λQ***, or no λQ (empty), under the control of an IPTG-inducible promoter. The cells were grown in the presence of 20 μM IPTG. The bar graph shows the averages of four independent measurements and standard deviations. (B) Results of β-galactosidase assays performed with reporter strain cells (ML101) harboring a placUV5-t_{R'}/t_R-lacZ reporter (schematic on top; see *SI Text* for details) and containing a plasmid that directed the synthesis of WT λQ, λQ***, or no λQ (empty). The cells were grown in the presence of 100 μM IPTG. The bar graph shows the averages of four independent measurements and standard deviations.

in the amount of λQ-dependent read-through in reactions performed with WT RNAP, β I905A/F906A RNAP, or β G907K RNAP (data not shown).

Several lines of evidence suggest that multiple interactions stabilize the initial complex that forms when λQ binds to the QBE and engages RNAP (15, 21). Thus we reasoned that it might be necessary to perform the assays under conditions that destabilize the λQ-engaged transcription complex to reveal the effects of substitutions that weaken the λQ/β-flap interaction. Therefore, we performed assays using a λP_{R'} template that contained a single base pair substitution in the QBE (G-25C) that weakens the interaction between λQ and the QBE (26) (Fig. 3A). When reactions were performed with this template, weakening the λQ/β-flap interaction resulted in a reduction in λQ-dependent antitermination (Fig. 3B). In particular, when reactions were performed with β G907K RNAP, λQ-dependent read-through was slightly reduced compared with reactions performed with WT RNAP, and when reactions were performed with β I905A/F906A RNAP, λQ-dependent read-through was significantly reduced (Fig. 3B).

Effects of Strengthening the λQ/β-Flap Interaction *in Vivo*. We next tested the effect of strengthening the λQ/β-flap interaction on λQ-dependent antitermination *in vivo*. To do this, we used a reporter strain carrying a λP_{R'}-t_{R'}-lacZ fusion (Fig. 4A) and assayed the ability of WT λQ or λQ*** to induce the expression of lacZ (which provides a measure of λQ's antitermination activity). We found that λQ*** exhibited slightly higher antitermination activity than WT λQ (Fig. 4A). Because we had found that it was necessary to measure λQ-dependent antitermination under suboptimal conditions to reveal the effects of weakening the λQ/β-flap interaction, we tested what effect strengthening the λQ/β-flap interaction had under suboptimal conditions.

We first used the mutant λP_{R'}-lacZ reporter construct containing the G-25C base pair substitution in the QBE. On this mutant λP_{R'}-lacZ reporter, λQ*** was ≈2-fold more active than

WT λQ in mediating terminator read-through (data not shown). Next, we used a λP_{R'}-lacZ reporter construct containing two base pair substitutions in the QBE (G-25C, T-22G) that, together, nearly eliminate the binding of λQ to the QBE (18, 26). On this mutant λP_{R'} template (ΔQBE), λQ*** was ≈6-fold more active than WT λQ in mediating terminator read-through (i.e., WT λQ and λQ*** caused 2.5- and 14.6-fold increases in lacZ expression, respectively) (Fig. 4A). Western blot analysis indicated that WT λQ and λQ*** were present at comparable levels in the cell (data not shown). Thus, strengthening the λQ/β-flap interaction suppresses the effect of substitutions in the QBE that disrupt λQ binding.

We next tested whether strengthening the λQ/β-flap interaction might also enable λQ to bypass the requirement for a pause-inducing element. To this end, we tested a λP_{R'}-lacZ reporter construct that contained base pair substitutions in the pause-inducing element (A+2G, T+6G) that eliminate σ⁷⁰-dependent pausing (20, 21, 27). On this mutant λP_{R'}-lacZ template (Δpause) we found that λQ*** was ≈3-fold more active than WT λQ in mediating terminator read-through (Fig. 4A). Finally, on a λP_{R'}-lacZ reporter construct containing base pair substitutions in both the QBE and pause-inducing element (ΔQBE Δpause), we found that λQ*** was ≈5-fold more active than WT λQ (Fig. 4A). These results suggest that strengthening the λQ/β-flap interaction enables λQ to efficiently engage RNAP in a manner that requires no specific *cis*-acting sequence elements *in vivo*.

To confirm the ability of λQ*** to engage RNAP without the requirement for specific *cis*-acting sequence elements, we constructed a reporter in which a heterologous promoter, placUV5, was placed upstream of tandem t_{R'} terminators with a downstream lacZ gene (Fig. 4B). Once again, λQ*** was significantly more active (≈7-fold) than WT λQ in mediating terminator read-through (Fig. 4B). [The use of tandem terminators significantly enhances the sensitivity of the assay, revealing that even WT λQ can functionally engage RNAP independently of the

Fig. 5. Effects of strengthening the $\lambda Q/\beta$ -flap interaction *in vitro*. Single-round *in vitro* transcription assays (see *SI Text*) were performed by using a linear template containing sequence extending from -109 to $+232$ of $\lambda P_{R'}$ that includes the natural terminator $t_{R'}$. Assays were performed in the absence of λQ or the presence of 500 nM WT λQ or λQ^{***} , using a WT template (WT $P_{R'}$) or a template carrying base pair substitutions in the QBE and pause-inducing sequence ($P_{R'} \Delta QBE \Delta pause$). The transcripts were end-labeled with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The terminated transcript (T) and read-through transcript (RT) are indicated. For each reaction, the percentage of transcripts derived from terminator read-through, %RT (read-through/read-through + terminated) $\times 100$, is shown. Assays were repeated three times with similar results.

QBE and pause-inducing element, although not nearly as efficiently as λQ^{***} (Fig. 4B).]

Effects of Strengthening the $\lambda Q/\beta$ -Flap Interaction *in Vitro*. We next tested the effect of strengthening the $\lambda Q/\beta$ -flap interaction on λQ -dependent antitermination *in vitro*. When single-round *in vitro* transcription assays were performed with the WT $\lambda P_{R'}$ template at high concentrations of λQ or λQ^{***} , we observed similar amounts of λQ -dependent readthrough ($\approx 55\%$; Fig. 5). In contrast, when the assays were performed by using the $\lambda P_{R'} \Delta QBE \Delta pause$ template, WT λQ did not increase terminator read-through compared with reactions performed in the absence of λQ ($\approx 1\%$ read-through was observed in each case; Fig. 5), whereas λQ^{***} resulted in $\approx 10\%$ read-through (Fig. 5). Thus, strengthening the $\lambda Q/\beta$ -flap interaction allows λQ to efficiently engage RNAP in a manner that requires no specific *cis*-acting sequence elements *in vitro*.

Discussion

Here, we demonstrate that λQ establishes direct contact with the β -flap as it engages the RNAP holoenzyme during early elongation at $\lambda P_{R'}$. Our findings imply that λQ competes with σ^{70} region 4 for access to the β -flap, thus explaining why the nascent RNA-mediated destabilization of the σ^{70} region 4/ β -flap interaction is required for λQ to engage the transcription complex. We also show that λQ 's affinity for the β -flap is an important determinant that controls λQ 's access to the transcription complex. Specifically, we find that strengthening the $\lambda Q/\beta$ -flap interaction allows λQ to bypass requirements for the QBE and the pause-inducing element in the engagement process.

The Role of the $\lambda Q/\beta$ -Flap Interaction. Because we found that it was necessary to destabilize the binding of λQ to the QBE (by introduction of the G-25C mutation) to detect the destabilizing effect of weakening the $\lambda Q/\beta$ -flap interaction (Fig. 3B), we infer that destabilizing the $\lambda Q/\beta$ -flap interaction compromises events that occur when λQ is still bound to the QBE, i.e., the process by which λQ engages RNAP at $P_{R'}$. Consistent with the idea that contact between λQ and the β -flap first occurs while λQ is bound to the QBE, we were able to detect an interaction between DNA-bound 21Q (the Q that interacts most strongly with the β -flap in the context of the two-hybrid assay; Fig. 2B) and the β -flap by using a one-hybrid assay (Fig. S3).

Conversely, because we did not detect an effect of destabilizing the $\lambda Q/\beta$ -flap interaction when we used a WT $\lambda P_{R'}$ template *in vitro* (data not shown), we infer that, under the conditions of our *in vitro* transcription assays, the substitutions tested did not have a disruptive effect on antitermination *per se*. Nevertheless, we suspect that interactions between λQ and the β -flap, once established during λQ 's initial engagement with RNAP, are maintained during elongation. In support of this idea, experiments performed with static elongation complexes indicate that 82Q (along with the cofactor NusA) form a "protective barrier" around the nascent RNA transcript as the RNA emerges from the RNA exit channel (19). These findings suggest that 82Q is positioned near the exterior opening of the RNA exit channel in the context of the elongation complex. Because the β -flap largely defines the RNA exit channel and the nascent RNA actually emerges from underneath the β -flap (13), these results suggest that 82Q (and, by analogy, λQ) is positioned near the β -flap in the context of the elongation complex (19).

We propose that our inability to detect any effects of destabilizing the $\lambda Q/\beta$ -flap interaction on the efficiency of λQ -mediated antitermination *per se* may reflect the stability of λQ 's association with the elongation complex (18). Thus, λQ may form an extended network of interactions with the β -flap and possibly with surrounding surfaces and/or the nascent RNA. Consequently, our *in vitro* transcription assays may not have been sensitive enough to reveal effects of the amino acid substitutions used in this work.

The β -flap is well positioned to influence transcription pausing and termination by interacting directly with the nascent RNA (12, 13). Consistent with this idea, cross-linking experiments suggest that the hairpin structure that forms in the nascent RNA during hairpin-dependent pausing interacts with the β -flap (28, 29). Furthermore, removal of the β -flap can impair the ability of RNAP to respond to intrinsic terminators (25, 30). These considerations have led to the proposal that the β -flap may be targeted by regulatory factors, like Q, that affect the termination properties of RNAP (29). When bound to the β -flap, Q might inhibit termination by a passive mechanism, creating an obstruction or barrier that prevents either the nascent RNA or a domain of RNAP from adopting a termination competent conformation (19). Alternatively, Q might inhibit termination by actively restructuring the incipient termination complex. Because the β -flap is a conserved feature of all multisubunit RNAPs, it is likely to serve as a contact site for regulators that modify the elongation properties of RNAP in other organisms, as well.

Conversion of λQ to a Global Regulator. We found that strengthening the $\lambda Q/\beta$ -flap interaction enables λQ to access the transcription complex in a manner that requires no specific *cis*-acting sequences (the QBE and the pause-inducing element) (Figs. 4 and 5). The requirements for the QBE and the pause-inducing element ensure that λQ is recruited exclusively to transcription complexes associated with $\lambda P_{R'}$. Thus strengthening the interaction between λQ and the β -flap allows λQ to efficiently associate with transcription complexes that have initiated transcription from promoters other than $\lambda P_{R'}$ (Fig. 4B). These findings suggest that the strength of the $\lambda Q/\beta$ -flap interaction is calibrated to ensure that λQ activity is restricted to $\lambda P_{R'}$.

Methods

Proteins. For the experiment of Fig. 3, WT RNAP core enzyme and mutant RNAP core enzymes (β I905A/F906A and β G907K) were purified as described (31) from BL21(DE3) cells after induction of plasmid pIA679 *rpoB*-His₆ (gift from I. Artsmovitsch, Ohio State University; directs the synthesis of high levels of α , β -His₆, β' , and ω) or its *rpoB*-His₆ mutant derivatives, in Overnight Express Instant TB medium (Novagen). For the experiment in Fig. 5, *E. coli* RNAP core was obtained from Epicentre. Holoenzyme was formed by incubating RNAP core enzymes with a 5-fold molar excess of His-tagged WT σ^{70} [purified as

described (32)]. WT λ Q and λ Q*** were purified as described (21). NusA was a gift from J. Roberts, Cornell University.

Strains and Plasmids. A complete list of strains and plasmids is provided in Table S1.

β -Galactosidase Assays. For the assays shown in Fig. 2, FW102 O₁2-62 reporter strains cells were cotransformed with the indicated pAC- and pBR-derived plasmids. For the assays shown in Fig. 4, reporter strain cells harboring the

indicated λ P_R-lacZ fusion or the p_{lac}UV5-t_R/t_R-lacZ fusion were transformed with pAC λ Q, pAC λ Q***, or pAC Δ Q as indicated. β -Galactosidase assays were performed as described (33) by using microtiter plates and a microtiter plate reader. Miller units were calculated as described (33).

ACKNOWLEDGMENTS. We thank Sean Garrity for technical expertise and helpful discussion, Jeff Roberts for advice and reagents, and Irina Artsimovitch for plasmid pIA679 *rpoB*-His₆. This work was supported by National Institutes of Health Grant GM44025 (to A.H.).

- Gross CA, et al. (1998) The functional and regulatory roles of sigma factors in transcription. *Cold Spring Harbor Symp Quant Biol* 63:141–155.
- Lonetto M, Gribskov M, Gross CA (1992) The σ^{70} family: Sequence conservation and evolutionary relationships. *J Bacteriol* 174:3843–3849.
- Arthur TM, Burgess RR (1998) Localization of a σ^{70} binding site on the N terminus of the *Escherichia coli* RNA polymerase β' subunit. *J Biol Chem* 273:31381–31387.
- Young BA, et al. (2001) A coiled-coil from the RNA polymerase β' subunit allosterically induces selective nontemplate strand binding by σ^{70} . *Cell* 105:935–944.
- Young BA, Gruber TM, Gross CA (2004) Minimal machinery of RNA polymerase holoenzyme sufficient for promoter melting. *Science* 303:1382–1384.
- Kuznedelov K, et al. (2002) A role for interaction of the RNA polymerase flap domain with the sigma subunit in promoter recognition. *Science* 295:855–857.
- Mooney RA, Darst SA, Landick R (2005) Sigma and RNA polymerase: An on-again, off-again relationship? *Mol Cell* 20:335–345.
- Nickels BE, et al. (2005) The interaction between σ^{70} and the β -flap of *Escherichia coli* RNA polymerase inhibits extension of nascent RNA during early elongation. *Proc Natl Acad Sci USA* 102:4488–4493.
- Murakami KS, Masuda S, Darst SA (2002) Structural basis of transcription initiation: RNA polymerase holoenzyme at 4-Å resolution. *Science* 296:1280–1284.
- Vassilyev DG, et al. (2002) Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6-Å resolution. *Nature* 417:712–719.
- Komissarova N, Kashlev M (1998) Functional topography of nascent RNA in elongation intermediates of RNA polymerase. *Proc Natl Acad Sci USA* 95:14699–14704.
- Korzheva N, et al. (2000) A structural model of transcription elongation. *Science* 289:619–625.
- Vassilyev DG, Vassilyeva MN, Perederina A, Tahirou TH, Artsimovitch I (2007) Structural basis for transcription elongation by bacterial RNA polymerase. *Nature* 448:157–162.
- Nickels BE, Roberts CW, Roberts JW, Hochschild A (2006) RNA-mediated destabilization of the σ^{70} region 4/ β -flap interaction facilitates engagement of RNA polymerase by the Q antiterminator. *Mol Cell* 24:457–468.
- Nickels BE, Roberts CW, Sun H, Roberts JW, Hochschild A (2002) The σ^{70} subunit of RNA polymerase is contacted by the λ Q antiterminator during early elongation. *Mol Cell* 10:611–622.
- Roberts JW, et al. (1998) Antitermination by bacteriophage lambda Q protein. *Cold Spring Harbor Symp Quant Biol* 63:319–325.
- Yarnell WS, Roberts JW (1999) Mechanism of intrinsic transcription termination and antitermination. *Science* 284:611–615.
- Deighan P, Hochschild A (2007) The bacteriophage λ Q antiterminator protein regulates late gene expression as a stable component of the transcription elongation complex. *Mol Microbiol* 63:911–920.
- Shankar S, Hatoum A, Roberts JW (2007) A transcription antiterminator constructs a NusA-dependent shield to the emerging transcript. *Mol Cell* 27:914–927.
- Ring BZ, Yarnell WS, Roberts JW (1996) Function of *E. coli* RNA polymerase sigma factor σ^{70} in promoter-proximal pausing. *Cell* 86:485–493.
- Yarnell WS, Roberts JW (1992) The phage lambda gene Q transcription antiterminator binds DNA in the late gene promoter as it modifies RNA polymerase. *Cell* 69:1181–1189.
- Marr MT, Datwyler SA, Meares CF, Roberts JW (2001) Restructuring of an RNA polymerase holoenzyme elongation complex by lambdoid phage Q proteins. *Proc Natl Acad Sci USA* 98:8972–8978.
- Dove SL, Joung JK, Hochschild A (1997) Activation of prokaryotic transcription through arbitrary protein-protein contacts. *Nature* 386:627–630.
- Geszvain K, Gruber TM, Mooney RA, Gross CA, Landick R (2004) A hydrophobic patch on the flap-tip helix of *E. coli* RNA polymerase mediates σ^{70} region 4 function. *J Mol Biol* 343:569–587.
- Touloukhonov I, Landick R (2003) The flap domain is required for pause RNA hairpin inhibition of catalysis by RNA polymerase and can modulate intrinsic termination. *Mol Cell* 12:1125–1136.
- Guo J, Roberts JW (2004) DNA binding regions of Q proteins of phages lambda and phi80. *J Bacteriol* 186:3599–3608.
- Ring BZ, Roberts JW (1994) Function of a nontranscribed DNA strand site in transcription elongation. *Cell* 78:317–324.
- Wang D, Severinov K, Landick R (1997) Preferential interaction of the his pause RNA hairpin with RNA polymerase β subunit residues 904–950 correlates with strong transcriptional pausing. *Proc Natl Acad Sci USA* 94:8433–8438.
- Touloukhonov I, Artsimovitch I, Landick R (2001) Allosteric control of RNA polymerase by a site that contacts nascent RNA hairpins. *Science* 292:730–733.
- Kuznedelov KD, Komissarova NV, Severinov KV (2006) The role of the bacterial RNA polymerase β subunit flexible flap domain in transcription termination. *Dokl Biochem Biophys* 410:263–266.
- Vrentas CE, Gaal T, Ross W, Ebright RH, Gourse RL (2005) Response of RNA polymerase to ppGpp: Requirement for the omega subunit and relief of this requirement by DksA. *Genes Dev* 19:2378–2387.
- Panaghie G, Aiyar SE, Bobb KL, Hayward RS, de Haseth PL (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.
- Thibodeau SA, Fang R, Joung JK (2004) High-throughput β -galactosidase assay for bacterial cell-based reporter systems. *BioTechniques* 36:410–415.