



Mechanism for a transcriptional activator that works at the isomerization step

Citation

Dove, S. L., F. W. Huang, and A. Hochschild. 2000. "Mechanism for a Transcriptional Activator That Works at the Isomerization Step." *Proceedings of the National Academy of Sciences* 97 (24): 13215–20. <https://doi.org/10.1073/pnas.97.24.13215>.

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:41483173>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available. Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

Mechanism for a transcriptional activator that works at the isomerization step

Simon L. Dove, Franklin W. Huang, and Ann Hochschild*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

Edited by Mark Ptashne, Memorial Sloan-Kettering Cancer Center, New York, NY, and approved September 26, 2000 (received for review June 30, 2000)

Transcriptional activators in prokaryotes have been shown to stimulate different steps in the initiation process including the initial binding of RNA polymerase (RNAP) to the promoter and a postbinding step known as the isomerization step. Evidence suggests that activators that affect initial binding can work by a cooperative binding mechanism by making energetically favorable contacts with RNAP, but the mechanism by which activators affect the isomerization step is unclear. A well-studied example of an activator that normally exerts its effect exclusively on the isomerization step is the bacteriophage λ cI protein (λ cI), which has been shown genetically to interact with the C-terminal region of the σ^{70} subunit of RNAP. We show here that the interaction between λ cI and σ can stimulate transcription even when the relevant portion of σ is transplanted to another subunit of RNAP. This activation depends on the ability of λ cI to stabilize the binding of the transplanted σ moiety to an ectopic -35 element. Based on these and previous findings, we discuss a simple model that explains how an activator's ability to stabilize the binding of an RNAP subdomain to the DNA can account for its effect on either the initial binding of RNAP to a promoter or the isomerization step.

Many transcriptional activators in prokaryotes bind to specific sequences associated with the promoters they regulate and affect the initiation process through direct contacts with RNA polymerase (RNAP; subunit structure, $\alpha_2\beta\beta'\sigma$) (1–3). The process of transcription initiation in *Escherichia coli* can be described by a simplified two-step model (4). First, RNAP binds to fully duplex promoter DNA to form what is called the closed complex. Formation of this complex is reversible and is described by an equilibrium binding constant K_B . For transcription to initiate, the closed complex must then isomerize to form the transcriptionally active open complex in which the DNA is locally melted to expose the transcription start site. This isomerization step is usually irreversible and is described by a forward rate constant k_f . The cAMP receptor protein (CRP) is a well-characterized example of an activator that can exert its effect exclusively on K_B , whereas the bacteriophage λ cI protein is an activator that normally exerts its effect exclusively on k_f (4–6).

CRP activates transcription from the *lac* promoter by binding to a recognition site centered 61.5 bp upstream from the start point of transcription and contacting the α subunit of RNAP (7). The α subunit consists of two independently folded domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), separated by a flexible linker region (8–10). Whereas the α NTD mediates formation of the α dimer and serves as a scaffold for the assembly of RNAP, the α CTD is a DNA-binding domain that also serves as the target for many transcriptional activators (1, 3, 11). When bound at the *lac* promoter, CRP has been shown to stabilize the binding of the α CTD to the DNA in the region between the CRP recognition site and the promoter -35 element (7). Thus, CRP appears to work by a simple cooperative binding mechanism (1–3), stabilizing the closed complex at the *lac* promoter (4, 6).

In contrast, λ cI activates transcription from the λ promoter P_{RM} when bound to an operator site centered 42 bp upstream from the start point of transcription and is thought to contact the σ subunit of RNAP (reviewed in ref. 12). λ cI is a two-domain protein that binds as a dimer to its operator sites on the phage chromosome (13).

Its NTD contains a helix-turn-helix DNA-binding motif, and its CTD mediates dimer formation as well as cooperative binding to pairs of operator sites (14). At the right operator region (O_R), λ cI dimers bind cooperatively to sites O_{R1} and O_{R2} , and the dimer at O_{R2} activates transcription from promoter P_{RM} (see Fig. 1A) (13). The isolation of λ cI mutants specifically defective for activation (positive control mutants) led originally to the identification of a positive control surface located in the NTD of λ cI (15–17). The suggestion that λ cI uses this positive control surface to contact the σ subunit of RNAP is based on the isolation and analysis of σ mutants that affect λ cI-stimulated transcription (18–20).

The σ subunit of RNAP is responsible for recognition of specific promoter sequences; alternative σ factors combine with the enzymatic core ($\alpha_2\beta\beta'$) to form alternative holoenzyme species (21). Promoter P_{RM} is recognized by the σ^{70} form of RNAP ($E\sigma^{70}$), which directs transcription of the majority of *E. coli* genes. The σ^{70} subunit makes base-specific contacts with the promoter in both its -10 and -35 regions, using conserved regions 2 and 4, respectively, to do so (see ref. 21). λO_{R2} is centered just upstream of the -35 region of P_{RM} (at position -42), and residues in region 4 of σ^{70} , which contains a putative helix-turn-helix DNA-binding motif, have been implicated in the interaction with λ cI (18, 19). Nevertheless, there has been no direct demonstration of an interaction between λ cI and region 4 of σ^{70} .

Here, we design an *in vivo* assay that permits the detection of an energetically favorable interaction between λ cI and a σ fragment encompassing region 4. Specifically, we tether the relevant portion of σ to the α subunit of RNAP and then show that λ cI can activate transcription from a suitably designed test promoter by stabilizing the binding of the transplanted σ moiety to an ectopic -35 element (Fig. 1B). We present a model that explains how the ability of λ cI to stabilize the binding of region 4 of σ to the DNA can account for its effect on the rate of isomerization at P_{RM} .

Materials and Methods

Plasmids and Strains. Plasmid pAC λ cI harbors the wild-type *cI* gene under the control of the *lacUV5* promoter (22). pAC λ cI(Sa109) is a derivative of pAC λ cI and encodes λ cI(S35L, D38Y, K39N). pAC λ cI(Sa104) is a derivative of pAC λ cI and encodes λ cI(S35L, D38Y, K39E). Plasmids pAC λ cI(Sa109) and pAC λ cI(Sa104) were constructed by cloning the appropriate *NdeI*–*NsiI* cut PCR products [made using plasmids pFB109 and pFB104 (see ref. 17) as templates] into an *NdeI*–*NsiI* cut derivative of pAC λ cI that contains an *NdeI* site at the start of the *cI* gene. pAC λ cI(Sa109-Y38N) is a derivative of pAC λ cI(Sa109) in which the Y38N change was introduced by the PCR. pAC λ cI(D38N) is a derivative of pAC λ cI that was made by cloning an *NdeI*–*NsiI* cut PCR product [made using plasmid p16 (see ref. 16) as template] into the *NdeI*–*NsiI* cut

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: O_R , operator region; RNAP, RNA polymerase; CRP, cAMP receptor protein; NTD, N-terminal domain; CTD, C-terminal domain.

*To whom reprint requests should be addressed. E-mail: ahochschild@hms.harvard.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

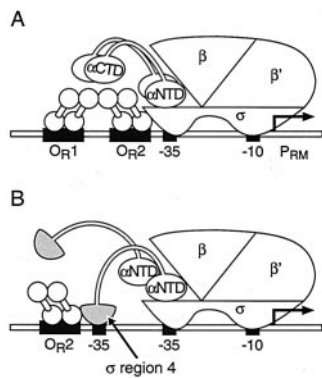


Fig. 1. (A) λ cl binds cooperatively to operator sites O_{R1} and O_{R2} to activate transcription from P_{RM} . Activation is mediated by the λ cl dimer bound at O_{R2} , which likely contacts the σ^{70} subunit of RNAP. (B) Genetic strategy for detecting the interaction between λ cl and region 4 of σ^{70} . Replacement of the RNAP α CTD with region 4 of σ^{70} permits interaction between the transplanted region of σ^{70} and a λ cl dimer bound adjacent to an auxiliary -35 element. The artificial promoter derivative *plac* $O_{R2-55}/Cons-35$ is shown; this bears the auxiliary -35 element (TTGACA) and the λ operator O_{R2} , centered 45.5 bp and 55 bp, respectively, upstream of the transcriptional start site of the *lac* promoter.

derivative of pAC λ cl. pAC Δ cl and pBR α have been described previously (22).

Plasmid pBR α - σ^{70} encodes residues 1–248 of the α subunit of *E. coli* RNAP fused to residues 528–613 of the σ^{70} subunit of *E. coli* RNAP under the control of tandem *lpp* and *lacUV5* promoters. The hybrid α - σ^{70} gene was created using overlap PCR and cloned into *EcoRI*–*Bam*HI-digested pBR α to make pBR α - σ^{70} . Plasmid pBR α - σ^{70} (R596H) was made in a similar manner and is identical to pBR α - σ^{70} , except the σ moiety of the encoded chimera contains the R596H substitution. pBR α - σ^{70} (R588H) is a derivative of pBR α - σ^{70} in which the R588H change in the σ moiety of the chimera was introduced by the PCR. Plasmid pBR α - 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 *lacUV5* promoters. pBR α - σ^{38} was made essentially the same way as pBR α - σ^{70} .

Plasmid pFW11- $O_{R2-55}/Cons-35$ was constructed by cloning an *EcoRI*–*Hind*III cut PCR product containing the *lac* promoter derivative *plac* $O_{R2-55}/Cons-35$ into pFW11 (23) cut with *EcoRI*–*Hind*III. pFW11- $O_{R2-55}/Cons-35$ was then transformed into strain CSH100, and the promoter-*lacZ* fusion was recombined onto an F' episome and mated into strain FW102 to create reporter strain SF1 (see ref. 23). Plasmid pFW11- $O_{R2-55}/TTAACA$ was similarly constructed, and reporter strain SF2, which is identical to strain SF1 except for the sequence of the auxiliary -35 element of the test promoter (TTAACA instead of TTGACA), was made in the same manner as strain SF1. The PCR-amplified regions of all constructs were sequenced to confirm that no errors had been introduced as a result of the PCR process.

Experimental Procedures

For all experiments, cells were grown in LB supplemented with carbenicillin (50 μ g ml⁻¹), chloramphenicol (25 μ g ml⁻¹), and kanamycin (50 μ g ml⁻¹) together with isopropyl- β -D-thiogalactoside (IPTG) at the concentration indicated. SDS-CHCl₃ permeabilized cells were assayed for β -galactosidase activity essentially as described (24). Assays were done at least three times in duplicate on separate occasions, with similar results. Values are the averages from one experiment; duplicate measurements differed by <10%. For all primer extension analyses, IPTG was added to the growth medium to a final concentration of 50 μ M. RNA isolation, primer labeling, and primer extension assays were essentially as described previously (22).

Results

Design of the Experiment. We devised a strategy for assaying the ability of λ cl to interact with region 4 of σ *in vivo*. This strategy was based on our previous demonstration that transcription can be activated by any sufficiently strong contact between a DNA-bound protein and a protein domain fused to RNAP (refs. 22 and 24; see also refs. 2 and 25). In particular, we showed that protein domains fused to the α subunit of RNAP in place of the α CTD can mediate transcriptional activation by serving as artificial activation targets for DNA-bound proteins (22, 24). We also showed that transcription can be activated by a sufficiently strong protein–DNA interaction between a DNA-binding domain tethered to RNAP and a cognate recognition site positioned upstream of a test promoter (24). Accordingly, we reasoned that region 4 of σ^{70} might be able to activate transcription from a suitably designed test promoter (bearing an auxiliary -35 element) when tethered to the α NTD. We anticipated further that such a system should allow us to detect energetically favorable protein–protein interactions between the tethered σ moiety and adjacently bound proteins because such interactions would stabilize the binding of the σ moiety to the DNA and hence increase the magnitude of the activation.

Following this strategy, we replaced the α CTD with a C-terminal fragment of σ^{70} encompassing region 4 and constructed a test promoter bearing an auxiliary -35 element in the upstream region together with a flanking λ operator. This experimental setup enabled us to ask whether a DNA-bound λ cl dimer would activate transcription from the test promoter by stabilizing the binding of the tethered σ moiety to the auxiliary -35 element (see Fig. 1B). The hybrid α - σ^{70} gene consisted of codons 1–248 of α fused to codons 528–613 of σ^{70} (the final 86 codons). The test promoter *plac* $O_{R2-55}/Cons-35$ consisted of the *lac* core promoter, a second -35 hexamer centered at position -45.5 , and the flanking λ operator (O_{R2}) centered at position -55 . The position of O_{R2} relative to the auxiliary -35 element is the same as at the λ P_{RM} promoter.

λ cl Proteins Activate Transcription from the Test Promoter in the Presence of the α - σ Chimera. The test promoter (*plac* $O_{R2-55}/Cons-35$) was fused to the *lacZ* gene and introduced into *E. coli* strain FW102 (23) in single copy on an F' episome to create reporter strain SF1. We assayed the abilities of wild-type λ cl and two superactivating variants (17) to activate transcription from the test promoter in the presence or absence of the α - σ^{70} chimera. Superactivators 104 and 109 activated transcription a maximum of \approx 6-fold in the presence of the α - σ^{70} chimera, and wild-type λ cl activated transcription weakly (<2-fold) (Fig. 2A). This difference in the stimulatory activities of wild-type λ cl and the superactivators mirrors that previously observed when the proteins were assayed for their abilities to activate transcription from λ P_{RM} (17). The λ cl proteins only activated transcription from the test promoter in the presence of the α - σ^{70} chimera; no stimulation was detected in the presence of excess wild-type α (Fig. 2A) or excess wild-type σ^{70} (data not shown). Furthermore, the α - σ^{70} chimera did not mediate any stimulatory effect in the absence of λ cl (data not shown). Primer extension analysis confirmed that the three λ cl proteins stimulated the production of correctly initiated transcripts (Fig. 2B).

Transcriptional Activation by λ cl Proteins at the Test Promoter Depends on the Activating Region of λ cl and on the Ability of the Tethered σ Moiety to Interact with the Auxiliary -35 Element. The results shown in Fig. 2 suggest that λ cl and the two superactivators are interacting with the tethered σ moiety and stabilizing its binding to the auxiliary -35 element. To test the hypothesis that the observed activation depends on the positive control surface of λ cl, we introduced a positive control mutation into the

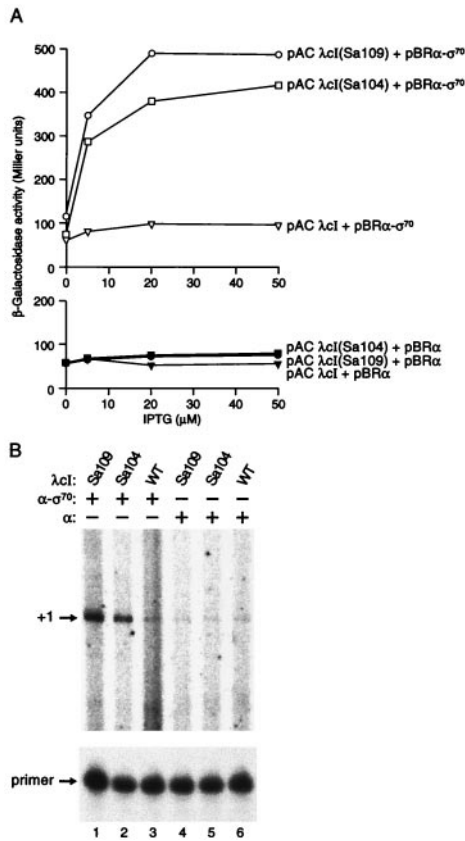


Fig. 2. Effects of wild-type λ cl and λ cl superactivators on transcription in the presence of the α - σ^{70} chimera. (A) SF1 cells harboring the indicated plasmids were assayed for β -galactosidase activity. pACYC-derived plasmids encoded λ cl (pAC λ cl), λ clSa109 [pAC λ cl(Sa109)], or λ clSa104 [pAC λ cl(Sa104)]; pBR322-derived plasmids encoded either the α - σ^{70} chimera (pBR α - σ^{70}) or wild-type α (pBR α). (B) Primer extension analysis of transcripts produced from *plac* O_R2-55/Cons-35 with wild-type λ cl or λ cl superactivators in the presence of the α - σ^{70} chimera. Total RNA was isolated from SF1 cells harboring plasmids encoding the indicated proteins, and the primer extension analysis was done by using a primer complementary to the *lacZ* transcript produced by the *plac* O_R2-55/Cons-35 promoter. Primer extension products produced by correctly initiated transcripts are indicated by +1. Excess unincorporated primer is shown in the lower panel.

genes encoding λ clSa109 and λ clSa104. The resulting λ cl variants (bearing amino acid substitution Y38N) manifested a substantially decreased ability to activate transcription from the test promoter (Fig. 3B and data not shown). We confirmed that these activation defects were not attributable to DNA-binding defects of the altered superactivators by performing an *in vivo* repression assay using a test promoter bearing a single λ operator between its -10 and -35 regions (data not shown).

We then tested the hypothesis that the observed activation depends on the ability of the tethered σ moiety to interact with the auxiliary -35 element positioned adjacent to the λ operator. To do this, we introduced mutations predicted to disrupt this interaction into either the -35 element (see Fig. 3A) or the gene encoding the α - σ^{70} chimera. When we weakened the auxiliary -35 element present on the reporter template by introducing a G to A substitution at the third position, λ clSa109 failed to activate transcription from the test promoter (Fig. 3B). Similarly, λ clSa109 failed to activate transcription in the presence of a mutant α - σ^{70} chimera bearing an amino acid substitution in the σ moiety (R588H) predicted to disrupt DNA binding (26) (Fig. 3B).

Activation with a Mutant-Suppressor Pair. Our results demonstrate that wild-type λ cl and two superactivating variants can stabilize the

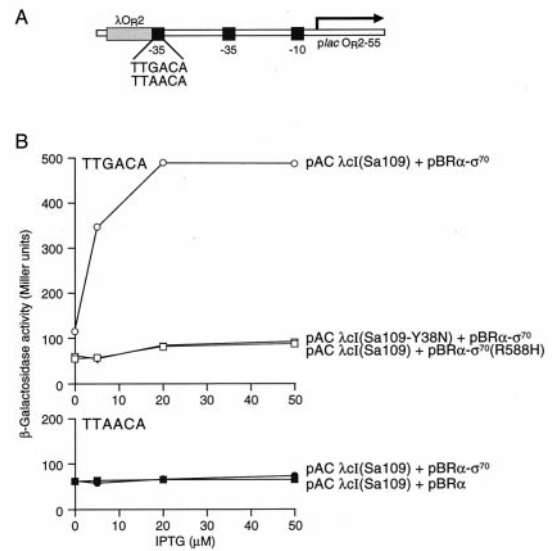


Fig. 3. Effects of mutations in the α - σ^{70} chimera or the additional -35 element on activation by λ cl superactivator. (A) Schematic of test promoters used to determine the effect of mutating the additional -35 element on transcriptional activation by Sa109 in the presence of the α - σ^{70} chimera. The sequences of the additional -35 elements from the two test promoters are indicated (consensus = TTGACA). (B) SF1 and SF2 cells harboring the indicated plasmids were assayed for β -galactosidase activity. In each panel, the sequence of the additional -35 element from the relevant test promoter is given. pACYC-derived plasmids encoded either λ clSa109 [pAC λ cl(Sa109)] or λ clSa109-Y38N [pAC λ cl(Sa109-Y38N)]; pBR322-derived plasmids encoded the α - σ^{70} chimera (pBR α - σ^{70}), the α - σ^{70} (R588H) chimera [pBR α - σ^{70} (R588H)], or wild-type α (pBR α).

sequence-specific binding of a C-terminal fragment of σ^{70} to DNA. For wild-type λ cl, this effect is close to the threshold of detection in our *in vivo* assay, and for the superactivating variants the effects are larger, as predicted based on their activities at P_{RM} (17). Because these superactivating variants of λ cl have not been subjected to kinetic analysis at P_{RM}, we examined the effect of another λ cl mutant that has been analyzed kinetically and was found to stimulate the rate of isomerization more efficiently than wild-type λ cl. Mutant λ cl-D38N is a positive control mutant (16), the activation defect of which can be suppressed by a mutant form of σ bearing the substitution R596H (σ -R596H) (18). *In vitro* experiments done with reconstituted mutant RNAP (E σ -R596H) revealed that λ cl-D38N exerts its effect exclusively on the isomerization step, producing a 27-fold increase in the isomerization rate constant (k_i) as compared with a 17-fold increase produced by wild-type λ cl working on wild-type RNAP (20).

To test whether our artificial system could detect an interaction between λ cl-D38N and σ -R596H, we compared the abilities of λ cl-D38N to activate transcription from the artificial promoter in the presence of the α - σ^{70} chimera with or without the R596H substitution in the σ moiety. λ cl-D38N failed to activate transcription in the presence of the unmodified form of the α - σ^{70} chimera but activated transcription ≈ 4 -fold when the σ moiety of the chimera bore the R596H substitution (Fig. 4). Primer extension analysis confirmed that this activation reflected an increase in correctly initiated transcripts (data not shown). Like the unmodified form of the α - σ^{70} chimera, the α - σ^{70} (R596H) variant did not activate transcription from the test promoter in the absence of λ cl-D38N (data not shown).

Superactivating Variants of λ cl Activate Transcription from the Test Promoter in the Presence of an α - σ^{38} Chimera. The stationary phase σ factor, σ^{38} , is very similar to σ^{70} in the DNA-binding regions, particularly throughout region 4.2, and recognizes the same -35

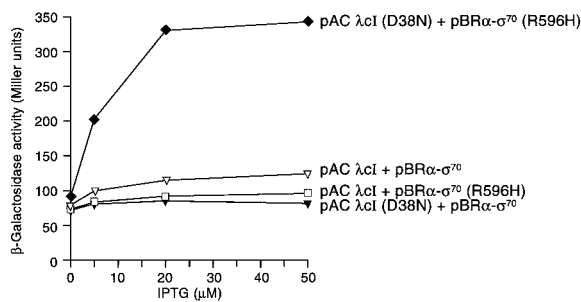


Fig. 4. Effects of wild-type and mutant λ clI on transcription in the presence of α - σ^{70} chimeras. SF1 cells harboring the indicated plasmids were assayed for β -galactosidase activity. pACYC-derived plasmids encoded either λ clI (pAC λ clI) or λ clI(D38N) [pAC λ clI(D38N)]; pBR322-derived plasmids encoded either the α - σ^{70} chimera (pBR α - σ^{70}) or the α - σ^{70} (R596H) chimera [pBR α - σ^{70} (R596H)].

consensus sequence as does σ^{70} (refs. 27 and 28; T. Gaal & R. L. Gourse, personal communication). We replaced the σ^{70} moiety of the α - σ^{70} chimera with the corresponding region of σ^{38} (residues 243 to 330) and tested whether the resulting α - σ^{38} chimera could mediate transcriptional activation from our artificial test promoter. The experiment of Fig. 5A shows that both λ clSa109 and λ clSa104 stimulated transcription efficiently in the presence of the α - σ^{38} chimera.

Wild-type λ clI appeared to exert a slight stimulatory effect on

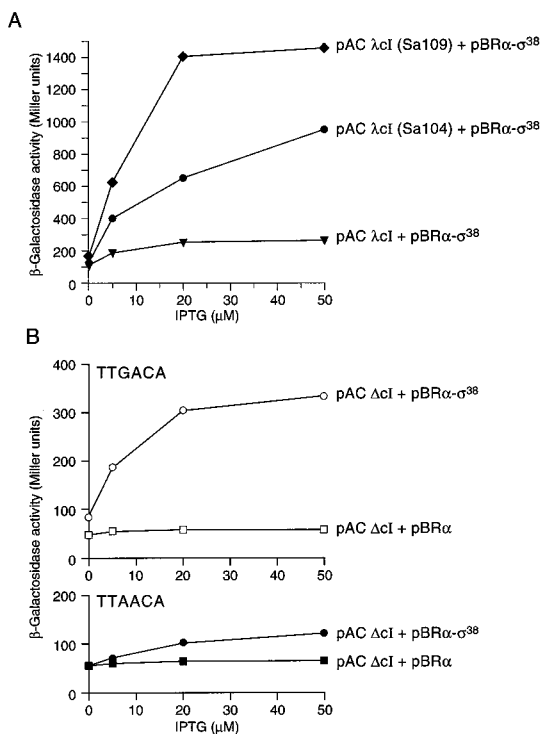


Fig. 5. (A) Effects of wild-type λ clI and λ clI superactivators on transcription in the presence of the α - σ^{38} chimera. SF1 cells harboring the indicated plasmids were assayed for β -galactosidase activity. pACYC-derived plasmids encoded λ clI (pAC λ clI), λ clSa109 [pAC λ clI(Sa109)], or λ clSa104 [pAC λ clI(Sa104)]; the pBR322-derived plasmid encoded the α - σ^{38} chimera (pBR α - σ^{38}). (B) Interaction between σ^{38} region 4 and the DNA mediates transcriptional activation. SF1 and SF2 cells harboring the indicated plasmids were assayed for β -galactosidase activity. In each panel, the sequence of the additional -35 element from the relevant test promoter is given. The pACYC-derived plasmid encoded no λ clI (pAC Δ clI); pBR322-derived plasmids encoded either the α - σ^{38} chimera (pBR α - σ^{38}) or wild-type α (pBR α).

transcription in the presence of the α - σ^{38} chimera (Fig. 5A); however, the interpretation of this effect is complicated by the results obtained in the absence of λ clI (see Discussion). Fig. 5B shows that in the absence of any form of λ clI, the α - σ^{38} chimera activated transcription from the test promoter bearing the consensus (TTGACA) ectopic -35 element ≈ 6 -fold. This activation evidently depends on the ability of the tethered σ^{38} moiety to bind to the ectopic -35 element since replacement of the consensus element with a mutated element (TTAACA) significantly reduced the magnitude of the activation (Fig. 5B). Primer extension analysis confirmed that the activation mediated by the α - σ^{38} chimera either in the absence or the presence of a λ clI variant reflected an increase in correctly initiated transcripts (data not shown).

Discussion

Genetic Evidence That the Interaction Between λ clI and the Tethered σ Moiety Is the Same Interaction That Activates Transcription at P_{RM} .

Our demonstration that λ clI can stabilize the binding of region 4 of σ^{70} to a -35 element provides strong support for the idea that there is an energetically favorable interaction between the activating region of λ clI and a complementary surface of σ . Importantly, we observed a close correlation between the effects of amino acid substitutions in both λ clI and region 4 of σ^{70} on transcriptional activation at P_{RM} (16–18) and at our artificial promoter. First, we showed that two superactivating variants of λ clI (Sa104 and Sa109), so designated based on their behavior at P_{RM} (17), also activated transcription more strongly than wild-type λ clI at the artificial promoter. Second, we showed that activation by wild-type λ clI and the superactivating variants at the artificial promoter was dependent on their having functional activating regions as defined by their abilities to activate transcription from P_{RM} . Finally, we tested the effect of introducing into the σ moiety of the α - σ chimera an amino acid substitution (R596H) that suppresses the activation defect of a λ clI positive control mutant (λ clI-D38N) at P_{RM} (18); in the context of the α - σ chimera, this amino acid substitution specifically enhanced the ability of λ clI-D38N to activate transcription from the artificial promoter.

Mechanism by Which λ clI Influences the Isomerization Step at P_{RM} .

We have shown that an activator (either wild-type λ clI or λ clI-D38N), which is known to function by accelerating the rate of isomerization at P_{RM} , can activate transcription from our artificial promoter by stabilizing the binding of a tethered σ moiety encompassing region 4 to an ectopic -35 element. The simplest interpretation of these findings is that both wild-type λ clI and λ clI-D38N similarly stabilize the binding of intact σ^{70} to the -35 element when they activate transcription from P_{RM} . How can this proposal be reconciled with the observed kinetics of the activation process? Any detailed mechanistic model for the action of λ clI at P_{RM} must account both for its stimulatory effect on k_f and for its lack of an effect on the initial binding step (described by an equilibrium constant K_B for the formation of the closed complex). We suggest that when $E\sigma^{70}$ (or $E\sigma^{70}$ -R596H) forms a closed complex at P_{RM} , the activating region of λ clI (or λ clI-D38N) and its target surface on σ are improperly aligned so that no energetically significant interaction can occur (Fig. 6A). We propose further that during the transition from the closed to the transcriptionally active open (melted) complex, the activating region of λ clI and its target on σ come into alignment, thus permitting an energetically significant interaction to occur (Fig. 6A). To explain the stimulatory effect of λ clI on the rate of isomerization, we postulate that λ clI stabilizes an intermediate along the pathway from the closed to the open complex, the formation of which limits the rate of initiation. More particularly, we suggest that during the isomerization process, there may be a tendency for region 4 of σ to disengage from the -35 element, which limits open complex formation (Fig. 6B). DNA-bound λ clI would function to counteract this tendency, thus

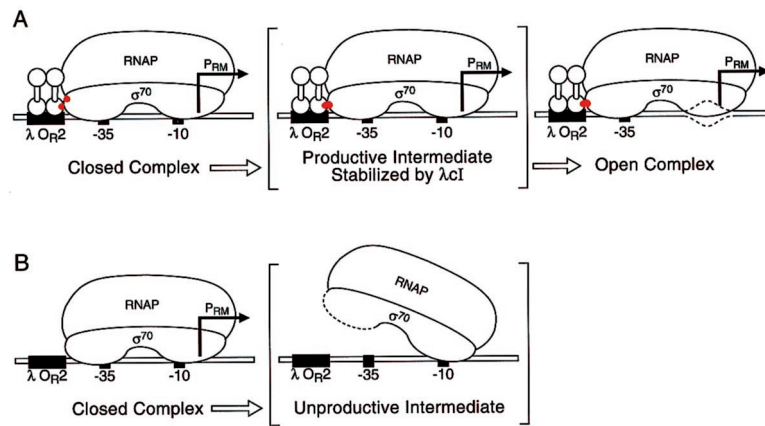


Fig. 6. Model for mechanism of action of λcI at P_{RM} . (A) Activating region and its target (red patches) are misaligned in the closed complex but come into alignment subsequently during the process of open complex formation. Depicted in brackets is a hypothetical productive intermediate that is stabilized by λcI . (B) In the absence of λcI , formation of an unproductive intermediate limits open complex formation at P_{RM} .

stabilizing a productive intermediate along the pathway to open complex formation (Fig. 6A). The ability of λcI to stabilize the binding of the tethered σ moiety to the DNA at our artificial promoter but not when RNAP forms a closed complex at P_{RM} suggests that region 4 of σ may be more constrained in its natural context in the holoenzyme than when it is tethered to the α NTD by a flexible linker region.

We think it likely that the interaction between λcI and the σ moiety of the α - σ chimera functions to stabilize the closed complex (affects K_B) at our artificial test promoter. Using the same core promoter, we have previously shown that transcription can be activated by any sufficiently strong contact between a DNA-bound protein and a protein domain tethered to a subunit of RNAP or between a DNA-binding domain tethered to RNAP and a cognate recognition site positioned upstream of the core promoter (22, 24). Furthermore, our results established a correlation between the strength of the protein–protein (or the protein–DNA) interaction and the magnitude of the activation (ref. 22; S.L.D. & A.H., unpublished results). The simplest interpretation of our findings is that these arbitrarily selected protein–protein or protein–DNA interactions stabilize the binding of RNAP to the promoter. In the experimental setup used here, transcriptional activation results from the combined effects of a relatively weak protein–protein interaction (between λcI and the tethered σ moiety) and a relatively weak protein–DNA interaction (between the tethered σ moiety and the auxiliary -35 element). We note that the role of λcI at this artificial promoter is formally analogous to the role of CRP at the natural *lac* promoter; that is, CRP interacts with the α CTD and stabilizes its association with the DNA in the region between the CRP recognition site and the promoter -35 element (1, 7). In this case, CRP has been shown to exert its effect exclusively on K_B (6).

Regardless of the kinetic effect of λcI on transcription at our artificial test promoter, two principal findings, namely that λcI can (i) interact productively with its target when that target is transplanted from the σ subunit to the α subunit and (ii) stabilize the binding of the transplanted σ fragment to an ectopic -35 element, suggest that the functional significance of the λcI - σ interaction is simply that contacts between σ region 4 and the -35 element of P_{RM} are stabilized.

A Common Mechanism for the Effects of Activators That Work at Different Steps in the Initiation Process. An implication of our results is that there need not be any fundamental difference between an activator that affects K_B and an activator that affects

k_f , both merely requiring a surface that can interact with an accessible complementary surface on RNAP (2, 3, 29). The kinetic effect of a particular activator working at a particular promoter may instead depend on when during the initiation process the appropriate surfaces can interact (29). If the interaction can take place while RNAP is in the closed complex, an effect on K_B would be expected, whereas if the interaction can take place only after the closed to open transition has begun, then an effect on k_f would be expected. Thus, the same protein–protein interaction between an activator and RNAP might, in principle, produce an effect on K_B , k_f , or both, depending on spatial constraints imposed by the promoter itself and on the arrangement of the activator-binding site(s).

This picture of the activation process provides an explanation for an unexpected effect uncovered by the kinetic analysis of the λcI -D38N/ $E\sigma^{70}$ -R596H mutant/suppressor pair, namely that wild-type λcI at P_{RM} stimulates closed complex formation (i.e., exerts its effect predominantly on K_B) when assayed with $E\sigma^{70}$ -R596H (20). We suggest that this apparent change in activation mechanism may simply reflect a subtle change in the geometry of the interaction so that λcI can interact productively with region 4 of σ^{70} when $E\sigma^{70}$ -R596H forms a closed complex (29).

It is possible that other σ^{70} -dependent activators that exert their effects on the rate of isomerization may in some cases do so by stabilizing the interaction of region 4 of σ with the -35 element. In the case we have described, this stabilization apparently occurs by a simple cooperative binding mechanism: the activator contacts region 4 of σ directly. In principle, however, direct contact with this DNA-binding domain of σ would not necessarily be required. Instead, contact with another region or subunit of RNAP might function indirectly to stabilize the interaction between σ region 4 and the -35 element. Several σ^{70} -dependent activators have been shown to affect the rate of isomerization (5, 20, 30, 31). A particularly well-characterized example is provided by CRP, which possesses at least two distinct activating regions (AR1 and AR2). When bound at a so-called class II promoter, which bears a CRP recognition site that overlaps the promoter -35 region, CRP uses AR1 to contact the α CTD and AR2 to contact the α NTD, the former contact mediating an effect on K_B and the latter an effect on k_f (31). Moreover, it has been shown that AR2 of CRP participates in an energetically favorable interaction with RNAP (31).

Activators of another class have been shown to exert their effects on the isomerization step; these activators work on promoters recognized by the alternative σ factor, σ^{54} , which appears to be unrelated to the σ^{70} class of σ factors (27). Unlike

most σ^{70} -dependent activators, the σ^{54} -dependent activators generally bind well upstream of their target promoters and interact with RNAP with concomitant formation of a DNA loop (see ref. 32); furthermore, ATP hydrolysis is required for this activation. Although the mechanism of action of the σ^{54} -dependent activators is likely to be complex, it is possible that stabilization of appropriate σ^{54} -DNA contacts is a component of the activation process. It should be noted, however, that any such stabilization evidently does not occur by a tethering mechanism because σ^{54} -dependent activators can, when present at high concentrations, work directly from solution (33). In eukaryotes, as well, transcriptional activators have been implicated in post-binding steps in the initiation process (34–37), and our findings could be relevant to the understanding of how eukaryotic activators can exert these effects.

A General Assay for the Interaction of DNA-Bound Regulators with σ Factors. Many prokaryotic activators that bind to the DNA upstream of the promoters they regulate have binding sites that are centered roughly 40 bp upstream from the transcription start site, and some of these have been shown genetically to interact with region 4 of σ^{70} (38–43). Our *in vivo* cooperative binding assay should be useful in determining whether any of these activators can stabilize the binding of region 4 to a –35 element. For those that can, our assay should also facilitate the genetic dissection of the protein–protein interaction between the activator and the tethered σ moiety. A potential benefit of our system is that it permits the isolation of amino acid substitutions in the σ moiety of an inessential α - σ chimera, the transcriptional effects of which should be limited to the test promoter. Thus, mutations affecting an essential σ factor that might otherwise be pleiotropic or even lethal can be isolated and studied.

We have used our experimental system to detect interactions of both σ^{70} and σ^{38} . Interestingly, the α - σ^{38} chimera, unlike the α - σ^{70} chimera, activated transcription on its own from our artificial test promoter (i.e., in the absence of an adjacently bound λ CI molecule). The reason for this difference is, as yet, unknown. We note that this activation-based assay may provide

an especially convenient system for carrying out a genetic analysis of the protein–DNA interaction between region 4 of σ^{38} and the –35 element. Since our *in vivo* assays were performed with cells that contain both plasmid-encoded α - σ chimera and chromosomally encoded wild-type α , we do not know whether the activation mediated by the α - σ^{38} chimera in the absence of λ CI results from homodimeric or heterodimeric RNAP complexes. One σ^{38} moiety is evidently bound to the auxiliary –35 element, and either a second σ^{38} moiety or the α CTD may be bound nonspecifically to the DNA upstream of this –35 element (within the λ operator). This hypothesis could account for the inhibitory effect of wild-type λ CI on α - σ^{38} -dependent activation, as λ CI might displace either a second, nonspecifically bound σ^{38} moiety or the α CTD from the DNA, thereby reducing the magnitude of the activation. Further experiments will be required to distinguish between these and other possibilities.

Conclusions

In summary, our findings with the α - σ^{70} chimera define a minimal target (86 amino acids) of σ^{70} that can interact with the activating region of λ CI. Furthermore, we have shown that λ CI can stabilize the binding of this region of σ^{70} to a –35 element. We propose that the ability of λ CI to stabilize the binding of region 4 of σ to a –35 element can account for its stimulatory effect on transcription from P_{RM} , and we discuss a model that can reconcile this finding with the apparently paradoxical observation that λ CI does not stabilize the initial binding of RNAP to P_{RM} but rather stimulates the isomerization step. Finally, our findings validate the use of a novel genetic system that should facilitate the detection and analysis of interactions between other transcriptional regulatory proteins and various σ factors from *E. coli* or other bacteria.

We thank M. Ptashne and A. Gann for discussion, R. Kolter for plasmid pDEB2 encoding σ^{38} , and Yan Ye Xia for excellent technical assistance. This work was supported by National Institutes of Health Grant GM44025, an established investigatorship from the American Heart Association (to A.H.), and a Charles A. King Trust postdoctoral fellowship (to S.L.D.).

- Busby, S. & Ebright, R. H. (1994) *Cell* **79**, 743–746.
- Ptashne, M. & Gann, A. (1997) *Nature (London)* **386**, 569–577.
- Hochschild, A. & Dove, S. L. (1998) *Cell* **92**, 597–600.
- McClure, W. R. (1985) *Annu. Rev. Biochem.* **54**, 171–204.
- Hawley, D. K. & McClure, W. R. (1982) *J. Mol. Biol.* **157**, 493–525.
- Malan, T. P., Kolb, A., Buc, H. & McClure, W. R. (1984) *J. Mol. Biol.* **180**, 881–909.
- Busby, S. & Ebright, R. H. (1999) *J. Mol. Biol.* **293**, 199–213.
- Blatter, E., Ross, W., Tang, H., Gourse, R. L. & Ebright, R. H. (1994) *Cell* **78**, 889–896.
- Negishi, T., Fujita, N. & Ishihama, A. (1995) *J. Mol. Biol.* **248**, 723–728.
- Jeon, Y. H., Yamazaki, T., Otomo, T., Ishihama, A. & Kyogoku, Y. (1997) *J. Mol. Biol.* **267**, 953–962.
- Ross, W., Gosink, K. K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K. & Gourse, R. L. (1993) *Science* **262**, 1407–1413.
- Hochschild, A. (1994) *Curr. Biol.* **4**, 440–442.
- Ptashne, M. (1992) *A Genetic Switch: Phage λ and Higher Organisms*, 2nd Ed. (Blackwell, Cambridge, MA).
- Sauer, R. T., Jordan, S. R. & Pabo, C. O. (1990) *Adv. Protein Chem.* **40**, 1–61.
- Guarente, L., Nye, J. S., Hochschild, A. & Ptashne, M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2236–2239.
- Hochschild, A., Irwin, N. & Ptashne, M. (1983) *Cell* **32**, 319–325.
- Bushman, F. D., Shang, C. & Ptashne, M. (1989) *Cell* **58**, 1163–1171.
- Li, M., Moyle, H. & Susskind, M. M. (1994) *Science* **263**, 75–77.
- Kuldell, N. & Hochschild, A. (1994) *J. Bacteriol.* **176**, 2991–2998.
- Li, M., McClure, W. R. & Susskind, M. M. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 3691–3696.
- Gross, C. A., Chan, C., Dombroski, A., Gruber, T., Sharp, M., Tupy, J. & Young, B. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63**, 141–155.
- Dove, S. L., Joung, J. K. & Hochschild, A. (1997) *Nature (London)* **386**, 627–630.
- Whipple, F. W. (1998) *Nucleic Acids Res.* **26**, 3700–3706.
- Dove, S. L. & Hochschild, A. (1998) *Genes & Dev.* **12**, 745–754.
- Farrell, S., Simkovich, N., Wu, Y., Barberis, A. & Ptashne, M. (1996) *Genes & Dev.* **10**, 2359–2367.
- Gardella, T., Moyle, H. & Susskind, M. M. (1989) *J. Mol. Biol.* **206**, 579–590.
- Lonetto, M., Gribskov, M. & Gross, C. A. (1992) *J. Bacteriol.* **174**, 3843–3849.
- Nguyen, L. H. & Burgess, R. R. (1997) *Biochemistry* **36**, 1748–1754.
- Roy, S., Garges, S. & Adhya, S. (1998) *J. Biol. Chem.* **273**, 14059–14062.
- Shih, M.-C. & Gussin, G. N. (1984) *J. Mol. Biol.* **172**, 489–506.
- Niu, W., Kim, Y., Tau, G., Heyduk, T. & Ebright, R. H. (1996) *Cell* **87**, 1123–1134.
- Rombel, I., North, A., Hwang, I., Wyman, C. & Kustu, S. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63**, 157–166.
- North, A. K. & Kustu, S. (1997) *J. Mol. Biol.* **267**, 17–36.
- Kingston, R. E. & Green, M. R. (1994) *Curr. Biol.* **4**, 325–332.
- Chi, T. & Carey, M. (1996) *Genes & Dev.* **10**, 2540–2550.
- Holstege, F. C., Fiedler, U. & Timmers, H. T. (1997) *EMBO J.* **16**, 7468–7480.
- Kassavetis, G. A., Kumar, A., Letts, G. A. & Geiduschek, E. P. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 9196–9201.
- Artsimovitch, I., Murakami, K., Ishihama, A. and Howe, M. M. (1996) *J. Biol. Chem.* **271**, 32343–32348.
- Lonetto, M. A., Rhodius, V., Lamberg, K., Kiley, P., Busby, S. & Gross, C. (1998) *J. Mol. Biol.* **284**, 1353–1365.
- Landini, P. & Busby, S. J. (1999) *J. Bacteriol.* **181**, 1524–1529.
- Rhodius, V. A. & Busby, S. J. (2000) *J. Mol. Biol.* **299**, 311–324.
- Kim, S. K., Makino, K., Amemura, M., Nakata, A. & Shinagawa, H. (1995) *Mol. Gen. Genet.* **248**, 1–8.
- Hu, J. C. & Gross, C. A. (1985) *Mol. Gen. Genet.* **199**, 7–13.