DNA-based Positive Control Mutants in the Binding Site Sequence of 434 Repressor*

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As detected by chemical nuclease treatments, the conformation of the 434 repressor-DNA complex depends on the sequence of the bound DNA (Bell, A. C., and Koudelka, G. B. (1993) J. Mol. Biol. 234, 542–553). We show here that these DNA sequence-dependent conformational changes alter the efficiency with which the repressor activates transcription from 434 PRM. Several lines of evidence suggest that binding site sequence affects the repressor's ability to activate transcription by altering the accessibility of the activation surface on the repressor to RNA polymerase. The results presented here show that in addition to affecting transcription by altering the overall binding affinity of protein for DNA, DNA sequence may also modulate the activity of the DNA-bound protein.

Many prokaryotic transcriptional activators bind to a specific DNA sequence at or near the promoter and affect transcription initiation by making direct contacts with RNA polymerase. Several studies have shown that changing the juxtaposition of the activator binding site with respect to the promoter can alter the effectiveness of the activator protein (2–5). Moreover, the ability of certain proteins to activate or repress transcription initiation depends on the sequence to which they bind (6, 7). These observations indicate that proper alignment of the activator surface and the RNA polymerase is critical for functional catalysis of transcription initiation by the activator protein. Hence, alterations in the geometry of the ternary complex formed between RNA polymerase, the activator protein, and DNA may be expected to influence the efficiency of transcription initiation.

A variety of sequence-dependent DNA structure effects, such as twist and twisting deformability of the DNA, the width of the major and/or minor grooves, the ability of the DNA to bend or sequence-directed intrinsic bends within the DNA, have been shown to influence protein-DNA complex stability (8–12). Given that the activation surfaces of the transcriptional activator proteins must be precisely aligned with their specific targets, DNA sequence-dependent differences in the conformation of the activator protein-DNA complexes may alter their efficiency of transcriptional activation. Despite this realization, very little data exist concerning the role of DNA sequence-dependent structure on transcriptional activator function. This is in part the result of the lack of knowledge concerning the effects of variations in binding site sequence on the conformation of protein-DNA complexes. In this paper, we explore the effects of sequence-dependent differences in the conformation of a bacteriophage 434 repressor-DNA complex on its transcriptional activation function.

The repressor protein of bacteriophage 434 is a helix-turn-helix-containing DNA-binding protein encoded by the cI gene. This protein controls the developmental fate of the phage by acting as a transcriptional regulator. The bacteriophage chromosome contains two operator regions OR and OL, each of which is divided into three repressor binding sites. The repressor binds to each of six binding sites or operators in the bacteriophage chromosome with differing affinities. This binding site discrimination is critical for the phage's choice between lytic and lysogenic development. For example, in OR, the repressor binds with highest affinity to two sites, OR1 and OR2. In this configuration, the repressor binds at OR2 activates transcription at the PRM promoter, presumably by contacting RNA polymerase, leading to expression of the genes that are responsible for maintenance of the lysogenic state (13, 14). This binding configuration also permits the repressor to concurrently inhibit transcription of genes needed for lytic phage growth by repressing transcription from the PR promoter. Higher concentrations of the repressor result in its binding to OR3, causing repression of PRM transcription.

The repressor binds its site as a dimer of identical subunits. The sequences of these sites display incomplete rotational symmetry (for examples, see Fig. 1). The 2-fold related "recognition" α-helices, one from each protein monomer, lie in successive major grooves on one face of the DNA. Each recognition helix is positioned in the major groove so that its side chains can make base-specific contacts with the outermost 4 or 5 base pairs in each half of the operator sequence. The repressor makes no base-specific contacts to the central 4 bases of the site.

Earlier work established that sequence-specific differences in DNA structure and/or flexibility of the noncontacted bases at position 6–9 at the center of the 434 operator affect both the affinity of the 434 repressor for operator and modulates the repressor's ability to recognize the base pairs at specific operator positions (1, 8, 15, 16). Noncontacted bases affect the 434 repressor's affinity and specificity for operator through two related structural phenomena. First, sequence-dependent differences in the structure of the unbound DNA and the varying abilities of these structures to be distorted affect overall affinity of operator for the repressor (8). Second, the sequence of the noncontacted bases affects specificity and affinity by preventing optimal interaction between the repressor's DNA contacting residues and the contacted bases (1, 15). These conformational changes are manifested in an increased nuclease sensitivity of particular DNA sequences in complex with the repressor. The effects of noncontacted bases sequence on operator affinity for the repressor are important in determining the life cycle choices of the phage; the differences between the noncontacted base sequences of OR1, OR2, and OR3 prevent the repressor from recognizing the position 4 base in OR3, but allow...
recognition of the base at this position in O$_{91}$ and O$_{92}$ (15).

The binding of the 434 repressor to O$_R$ is necessary and sufficient for activation of P$_{RM}$ transcription (14). When bound at O$_{92}$, the repressor closely approaches RNA polymerase at P$_{RM}$ (13). Since the conformation of the 434 repressor-DNA complexes vary with DNA sequence, it follows that the precise alignment of the activation surface on the repressor may vary with binding site sequence. We present data supporting the assertion that the conformation of the repressor-operator complex, as modified by sequence-dependent differences in DNA structure, critically affects the ability of the 434 repressor to activate transcription. We also discuss the structural alteration that may be related to sequence-dependent differences in repressor transactivation.

**EXPERIMENTAL PROCEDURES**

**Enzyme and Reagents**—Wild-type and mutant 434 repressors were prepared as described by Wharton (43). Sigma-saturated wild-type E. coli RNA polymerase was obtained from Epicentre Technologies or prepared as described (17). Holo-RNA polymerase bearing a deletion of the COOH-terminal domain of the $\alpha$-subunit (residues 201–310) was reconstituted from subunits by the method described (17). [a-$^32$P]UTP and [a-$^32$P]dATP (3000 Ci/mmol) were obtained from NEN Life Science Products. Unlabeled nucleoside triphosphates were purchased from Boehringer Mannheim.

**DNA Templates**—Transcription reactions were programmed with DNA fragment isolated from variants of the plasmid pJX. pJX was constructed by isolating a 150-base pair EcoRI and BamHI fragment from pRP16 (18) by agarose gel electrophoresis. This fragment, which bears wild-type 434 O$_R$, P$_R$, and P$_{RM}$, was inserted into pEMBL 8 (19) that had been previously cut with EcoRI and BamHI. The 450-bp transcription template was prepared by isolating the 450-base pair PvuII fragment from this plasmid. Point mutants in O$_R$, O$_3$ and/or promoter sequence (see Fig. 1 for sequences) were prepared by polymerase chain reaction mutagenesis as described in Ref. 20, using pJX as the polymerase chain reaction template. A control template fragment containing the RNA-I promoter was isolated from pJX and mixed in equimolar ratios with experimental template.

**Transcription in Vitro**—Transcription reaction were performed essentially as described (14). Briefly, each DNA template (5 nM) was incubated without or with varying amounts of 434 repressor for 10 min at 23 °C. After transcription buffer containing 100 mM KCl, 40 mM Tris, pH 7.9, 100 mM MgCl$_2$, and 10 mM dithiothreitol. Except for experiments to determine the time course of run-off transcription formation, RNA polymerase was added to a final concentration of 50 nM, and incubation was continued for another 15 min at 37 °C to allow the formation of open complexes. The transcription reaction was started by the addition of [m-$^32$P]UTP, GTP, and CTP, 0.04 mM UTP, and 10 pCi of [a-$^32$P]dATP and 0.1 mg/ml heparin. After 10 min of further incubation, the reactions were stopped by addition of formamid dye mix (90% formamide) and fractionated by two ethanol precipitations. The amounts of RNA transcripts resulting from initiation at P$_R$ and P$_{RM}$ were quantified relative to the amount of RNA-I transcript by PhosphorImager analysis of these gels.

**KmO$_4$ Footprinting**—KmO$_4$ footprinting was performed essentially as described (11). Briefly, a 400-base pair PvuII-HindIII DNA fragment derived from the desired pJX derivative was 3’ end-labeled using the Klenow fragment and [a-$^32$P]dATP. This fragment was incubated without or with varying concentrations of 434 repressor at 23 °C for 10 min, followed by the addition of RNA polymerase. After varying times of additional incubation at 37 °C, the DNA was exposed to 10 mM KMnO$_4$ for 1 min. The oxidation reaction was stopped by adding 1 M 2-mercaptoethanol, and the DNA was purified by two ethanol precipitations. The precipitated DNA fragments were dissolved in 100 µl of 1 M piperidine and incubated for 15 min at 90 °C to induce cleavage at the modified bases. The DNA was then diluted in the same volume of double distilled H$_2$O, lyophilized twice, dissolved in formamide dye mix, and fractionated on a 6% denaturing polyacrylamide gel. The products were visualized by PhosphorImager analysis.

**DNase I Footprinting**—DNase I footprinting assays were performed essentially as described in Ref. 21. The 400-base pair PvuII-HindIII DNA fragment was 3’ end-labeled as described above. The DNA was mixed with increasing amounts of 434 repressor in transcription buffer. After 10 min of incubation at 23 °C sufficient DNase I was added to give, on average, one cleavage/DNA molecule in 5 min of further incubation. The digested samples were precipitated with ethanol and butanol-2, dissolved in a formamide dye, and resolved on 6% denaturing gels.

**RESULTS**

Since changes in the DNA sequence bound by the repressor affect the conformation of the 434 repressor-operator complex (1, 15), we hypothesized that these sequence changes may alter the ability of the repressor to interact with the RNA polymerase. This idea was tested by examining the ability of the repressor to activate P$_{RM}$ transcription when bound at wild-type and mutant O$_R$ regions. At wild-type O$_R$ in the absence of the repressor, only transcripts resulting from RNA polymerase initiation at the P$_R$ promoter are detectable (Fig. 2A). As shown previously (13, 14), adding increasing amounts of the 434 repressor to the reaction inhibits transcription from P$_R$ and stimulates transcription from P$_{RM}$. Maximum stimulation of P$_{RM}$ occurs at 800 nM repressor, the concentration corresponding to that needed to completely occupy O$_{91}$ and O$_{92}$ under these conditions (Figs. 2A and 3A). These findings illustrate the positive transcriptional regulatory role of the repressor at P$_{RM}$ and the negative effect of the repressor on P$_R$ transcription (14, 22). As was also shown previously, adding higher concentrations of the repressor (≥3.2 µM) results in repression of P$_{RM}$ transcription. Binding results show that repression of P$_{RM}$ results from O$_R$ becoming occupied by the repressor (Fig. 3A), thereby preventing the binding of RNA polymerase to the promoter.

With wild-type 434 O$_R$, the differences between the concentrations of the repressor required to stimulate P$_{RM}$ and those that repress it are not large (Fig. 2A). To increase this difference, we introduced a mutation in O$_{93}$ that decreases its affinity for the repressor without disturbing P$_{RM}$ (see Fig. 1 for sequences). Fig. 2B shows that the repressor activates P$_{RM}$ from this O$_{93}^+$ template as well as it does from the wild-type promoter. About 3-fold more repressor is needed to begin to see repression of P$_{RM}$ transcription on this template than on the wild-type template. As expected, the decreased ability of the repressor to repress P$_{RM}$ transcription is due to inhibition of the repressor binding to O$_{93}$ (Fig. 3B). To facilitate examination of the effects of O$_R$ mutations on transcription from P$_{RM}$ in the studies described below, we used the O$_{93}^-$ template as starting material.

Comparing Fig. 2, B and C, shows that transcription activation of P$_{RM}$ by the repressor bound at an O$_{92}$ bearing an A → G mutation at position 4 (see Fig. 1 for sequences) is reduced as compared with that seen when the repressor is bound to wild-type O$_{92}$. Fig. 3C shows that the ∼3-fold reduction in transcription stimulation is seen even though the repressor has completely occupied the mutant O$_{92}$. Fig. 3C also shows that the decreased activation is not due to the inhibitory effect of partial occupancy of O$_{93}$ by the repressor on these templates. On this template, the repressor appears to be unable to specifically bind O$_{93}$ at concentrations ≥ 6.4 µM. Therefore, the decreased activation by the repressor from the mutant O$_{92}$ is a result of a DNA sequence-induced disruption of the activating signal from the DNA-bound repressor to RNA polymerase and not by affecting binding site occupancy.

To begin to examine the mechanism of how position 4 mutation affects transcription activation by the repressor bound at O$_{92}$, we determined the salt concentration dependence of transcription activation by the repressor. Previous studies (1, 15) showed that both the conformation and stability of wild-type operator-repressor complexes are insensitive to salt concentration. However, we also showed that position 4 A → G mutation

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1 E. Ziedins and G. Koudelka, unpublished results.
increases the salt dependence of operator affinity for the repressor, with corresponding changes in the conformation of the repressor-operator complex (15). The data in Fig. 4 show that the level of repressor-activated PRM transcription from wild-type OR2 is unaffected by changing the salt concentration between 50 and 100 mM NaCl. As we anticipated from the binding studies (1), the concentration of repressor needed to maximally stimulate PRM is also unaffected by changes in salt concentration (not shown).

In contrast to the results obtained with the OR2 template, increasing salt concentration decreases the maximal level of repressor-stimulated transcription of PRM on the template bearing an A → G change at position 4 in OR2. Despite the increased sensitivity of the repressor’s binding to the mutant OR2, binding experiments show that at either 50 or 100 mM salt maximal PRM stimulation occurs when the repressor completely occupies the mutant OR2 (not shown). Hence, the effect of salt on transcription activation from the position 4 mutant operator mirrors its effect on binding to the repressor. We established previously that position 4 mutation affects affinity by changing the conformation of the repressor-operator complex (1). Thus, these position 4-induced conformational changes may also affect the repressor transcription activation.

Results of previously reported binding experiments showed that the conformation of the repressor-operator complexes bearing position 4 substitution can be returned to a configura-
tion that resembles the wild-type complex by changing the identity of the base at position 6 (15). To verify that position 4 base sequence affects the repressor’s ability to activate transcription by altering the conformation of the repressor-operator complex, we changed the identity of the base at operator position 6 in different position 4 base sequence contexts and measured the repressor’s ability to activate PRM transcription on these templates. Fig. 5 shows that changing the position 6 base pair from GzC to either TzA or TzT partially overcomes the deleterious effect of the position 4 A3G change on transcriptional activation by the repressor. Consistent with the partial effect of position 6 mutation on correcting the repressor binding deficiency of a position 4 mutant binding site (15), the effect of position 6 mutation on restoring transcriptional activation by the repressor is not complete. This observation suggests a strong correlation between the conformation of the repressor-operator complex and the efficiency with which the repressor can activate transcription.

In contrast with the favorable effects of position 6 mutation on the repressor transcription activation on the position 4 mutant OR2 site, changing position 6 in the context of the wild-type position 4 sequence decreases the ability of the repressor to activate transcription. In the context of a position 4 A-T base sequence, changing the base at position 6 from G-C → T-A partially overcomes the deleterious effect of the position 4 A → T change on transcriptional activation by the repressor. Consistent with the partial effect of position 6 mutation on correcting the repressor binding deficiency of a position 4 mutant binding site (15), the effect of position 6 mutation on restoring transcriptional activation by the repressor is not complete. This observation suggests a strong correlation between the conformation of the repressor-operator complex and the efficiency with which the repressor can activate transcription.

To examine whether DNA sequence-induced structural changes uniquely affect transcription activation by the repressor, we examined the effect of changing Gln-33, one of the residues that contact operator position 4, to alanine. This protein sequence change relaxes the position 4 base specificity of the repressor (23). X-ray crystallographic analyses show that changing this residue causes the DNA phosphate backbone at operator position 4 to move closer to the protein and also changes the relative rotation of the two DNA-bound monomers.

**FIG. 3.** Binding of wild-type 434 repressor to on wild-type OR1, OR2, OR3 (A), OR1, OR2−4G, OR3− (B), and OR4−, OR2−4G, OR3− (C) templates. DNase I footprinting conditions are given under “Experimental Procedures.” Repressor concentrations were increased in 2-fold steps starting at 50 nM protein.

**FIG. 4.** Effect of salt concentration on transcriptional activation by 434 repressor at wild type and mutant OR2. Transcription reactions were performed at 50 or 100 mM KCl as indicated. All other reaction conditions were as described in the legend to Fig. 2. Plotted are the relative maximal amounts of PRM transcripts, normalized to RNA-I, and set equal to 100%, obtained in a repressor titration experiment (see Fig. 2). Error bars represent the S.D. values of the average of three separate determinations.

**FIG. 5.** Effect of position 6 substitution on transcriptional activation by 434 repressor. The amount of run-off transcripts from the various DNA templates in the presence of an optimally stimulating amount of the 434 repressor was determined from PhosphorImager analysis. The amounts of these transcripts were normalized relative to the level of an included RNA-I control template. The normalized data were then normalized relative to each other, with the amount of transcripts derived from the wild type template set equal to 100%. The asterisk denotes the OR1, OR2−4G, OR3− template. Error bars represent the S.D. values of the average of three separate determinations.
(see Ref. 26). Relative to the wild-type repressor, the Gln-33 → Ala change decreases the ability of the repressor to activate transcription from wild-type OR2 by 2.5-fold (data not shown). Interestingly, the effect of changing position 4 from A to G (A/TEG) has a smaller effect on transcriptional activation by the Ala-33 repressor than it does on the wild-type repressor. The smaller effect of the DNA mutation on activation by the Ala-33 repressor correlates with the decreased effect of the operator sequence change on binding of this protein. These observations are consistent with the idea that the position 4 A → G mutation influences the repressor’s ability to activate transcription by altering the conformation of the repressor-operator complex.

A trivial explanation for the effect of position 4 mutation on transcriptional activation is that the A → G change affects the ability of RNA polymerase to bind the promoter. The change in OR2 is 46 base pairs upstream from the transcription start site of P_{RM} and is thereby in a position unlikely to affect interaction of the promoter DNA with the σ70 subunit. However, DNA base changes in this region of the P_{RM} promoter may affect protein contacts made by the carboxyl-terminal domain of the α-subunit of RNA polymerase (24, 25). To test this idea, we examined the effect of OR2 mutation on the ability of the repressor to activate transcription of an RNA polymerase bearing an α-subunit in which the carboxyl-terminal domain of this subunit has been deleted. Our results show that changing position 4 from A to G similarly decreases repressor-activated transcription by both wild-type RNA polymerase and RNA polymerase bearing the truncated α-subunit (data not shown). The identical effect of the position 4 sequence change on the amount of repressor-activated transcription by these two polymerases shows that the transcription defect is not due to effects on the protein DNA interactions of the α-subunit.

Insight into how the position 4 sequence changes affect the repressor’s ability to activate transcription requires an understanding of how the repressor normally stimulates transcription. The repressor could function by recruiting RNA polymerase to the promoter, or it may stimulate transcription by increasing the rate of an RNA polymerase-promoter complex isomerization step. To begin to distinguish between these alternatives, we altered the sequence of the −35 region of P_{RM} and characterized the ability of RNA polymerase to transcribe these templates in the absence and presence of repressor (see Fig. 1 for sequences).

Inspection of the sequence of P_{RM} reveals that this promoter bears nonconsensus sequences in both −35 and −10 regions. Changing the sequence of the −35 region of P_{RM} toward consensus greatly increases the ability of RNA polymerase to transcribe this DNA in the absence of the repressor (Fig. 6A). Since the −35 regions of P_R and P_{RM} overlap, changing the −35 sequence of P_{RM} changes the −35 sequence of P_R away from consensus. As expected from this sequence change, Fig. 6A also shows that the mutation decreases the ability of RNA polymerase to initiate transcription at P_R. The repressor is unable to stimulate transcription on the template bearing the improved P_{RM} −35 region (Fig. 6B). Both footprinting (data not shown) and the observed inhibition of P_R transcription (Fig. 6B) indicate that repressor binding is unperturbed by the −35 change in P_{RM}. These observations, together with the finding that changing the sequence of the −10 region of the P_{RM} promoter toward consensus has no effect on transcription initiation in the absence of repressor (data not shown), are consistent with the idea that the low level of transcription from wild-type P_{RM} may be due to imperfect interactions between RNA polymerase and the −35 region of the promoter. In the wild-type promoter, the repressor may function to replace or stabilize these nonideal protein-DNA contacts (see below).

The observation that the repressor is unable to activate transcription from the −35 mutant promoter suggests that the repressor activates transcription by recruiting RNA polymerase to P_{RM}. This idea was tested by following the time course of open complex formation in parallel with the time course for the formation of run-off transcripts in the presence of the repressor on templates bearing either wild-type or mutant OR2. Fig. 7B shows that the amount of open complex formed, as measured by KMnO_4 sensitivity, increases with time on both wild-type and mutant templates. These increases are matched by increases in the amount of run-off transcript formed (Fig. 7A). Analysis of these data reveals that the rates at which both open complexes and run-off transcripts are formed are similar and unaffected by the sequence of OR2. If the OR2 mutation affected the repressor’s ability to stimulate a rate-limiting RNA polymerase isomerization step that precedes open complex formation, we would expect that the rate of open complex and transcript formation would be decreased. This is clearly not seen. Moreover, the observation that the rate of run-off transcription matches the rate of open complex formation on both the wild-type and mutant templates shows that the OR2 mutation is not affecting any potential repressor-stimulated events in the tran-
position of RNA polymerase from the initiating to elongating states.

The results in Fig. 7, A and B, show that both the amount of run-off transcripts formed and extent of open complexes detected on the mutant OR2 templates are lower than those observed with the wild-type OR2 template. This finding is consistent with a lowered affinity of RNA polymerase for the mutant template-repressor complex, as compared with the wild-type case. These results suggest that the OR2A3G mutation alters the ability of the repressor to stabilize RNA polymerase contacts with the promoter and not RNA polymerase isomerization steps. Moreover, the observation that the overall yield of transcripts is affected to a greater extent by OR2 mutation than is the amount of open complexes formed is also consistent with this suggestion. Decreasing the amount of initial RNA polymerase promoter complexes decreases the overall flux through the pathway toward transcript formation. Since open complex formation precedes transcript initiation, the amount of open complexes should be less affected by decreased RNA polymerase binding affinity than is transcript formation.

DISCUSSION

The data presented here indicate that the ability of a regulatory protein to activate transcription depends upon the sequence of its DNA binding site. Changing the sequences of contacted or noncontacted bases in the binding site of the 434 repressor alters its ability to activate transcription from P_{RM}. These DNA sequence changes appear to affect transcription by altering the conformation of the protein-DNA complex. Having shown that the conformation of a protein-DNA complex can regulate its ability to activate transcription, we would like to ascertain how the change in conformation disrupts transmission of the activating signal from the 434 repressor to RNA polymerase. However, before we can do this, we must first know the nature of the activation signal transmitted by the repressor. Two models for the signal transmission can be imagined. In the first model, the repressor may stimulate transcription from P_{RM} by inducing the formation of a particular DNA structure. Changing the DNA sequence, and thereby its structure, may disrupt the signal. Alternatively, the repressor may stimulate transcription by directly interacting with RNA polymerase. Changing the conformation of the repressor-operator complex may compromise the quality of this interaction by decreasing the accessibility of the surface of the repressor that is responsible for interacting with RNA polymerase.

Two lines of evidence suggest that the repressor stimulates transcription via a direct protein contact with RNA polymerase. First, Bushman et al. (27) identified mutations in the 434 repressor that do not affect DNA binding, but are defective in transcriptional activation. Therefore, the DNA binding and transcriptional activation functions of the repressor are separable, consistent with the idea that the repressor activates transcription by making a protein-protein contact with RNA polymerase. Together with structural studies (28, 29), the results of biochemical and genetic experiments indicate that these repressor mutations lie in a region that should be in direct apposition with the σ70 subunit of RNA polymerase (14, 30). Second, Fig. 5 shows that identical position 6 sequence changes made in two different position 4 backgrounds have opposite effects on transcriptional activation by DNA-bound 434 repressor. It is difficult to imagine that similar sequence changes could affect DNA structure in precisely opposite ways depending on the sequence at position 4. Hence, the 434 repressor appears to activate transcription by making a direct protein contact with RNA polymerase. Thus, changes in the
conformation of the repressor-operator complex appears to interfere with transcriptional activation by the repressor by altering the geometry of the interaction between the repressor and RNA polymerase.

Knowing that the repressor-operator complex structure modulates the interaction between the repressor and RNA polymerase permits us to ask how DNA sequence changes give rise to the transcriptional effects. Our previous work shows that changing the sequence of bases at operator positions 4 and/or 6 can result in an alteration of the relative position of the protein with respect to the DNA phosphate backbone (15). How might these structural alterations affect the 434 repressor-RNA polymerase interaction? One possibility is that the alteration in the relative position of the repressor with respect to the DNA phosphate backbone misaligns RNA polymerase with the activating surface of the repressor. We tested this idea by measuring the distance between a residue in the activating surface of the repressor and the DNA phosphate that is known to be contacted by both RNA polymerase and the repressor in the ternary transcription complex. We analyzed the three-dimensional structures of the 434 repressor and its mutants bound to several different DNA binding sites (28, 29, 31) with attention to the distance between Glu-19, the residue in part responsible for transcription activation by the 434 repressor, and the “shared” phosphate that is 5′ to the position 9 base. These measurements show that this distance is 4 Å longer in the wild-type repressor complex (31) than it is in complexes between repressor and operators bearing a G-C base pair at position 4 (29). The smaller distance between these two groups is also found in complexes between the wild-type operator and the repressor bearing the Gln-33→Ala substitution. These observations suggest that the structural change imparted by the protein and DNA sequence alterations decreases the repressor’s ability to activate transcription by preventing RNA polymerase from easily accessing the activating surface of the repressor.

The structural explanation for the transcription data suggests an inherent inflexibility in the activation mechanism of the 434 repressor that is distinct from other prokaryotic activators such as CAP (32). As mentioned above, the mutations in the 434 repressor that decrease its ability to activate transcription are located in the first helix of its helix-turn-helix unit. This structural unit is also responsible for governing DNA binding by the repressor. At least one of the targets for the activation signal is likely to be located in the segment of the αC subunit of RNA polymerase that contacts the −35 region of the promoter (33–36). The constraints imposed by proximity of the DNA binding regions with protein-protein interfaces in both repressor and RNA polymerase, and the necessity for DNA binding by each, imply that changes in the geometry of the repressor’s interaction with operator and/or the orientation of the two repressor monomers on DNA would profoundly affect the geometry of the protein-protein contact.

The DNA-induced alterations in repressor-operator conformation appear to affect transcription activation by preventing the repressor from stabilizing the RNA polymerase-promoter interaction. Since the repressor is unable to stimulate transcription by RNA polymerase strongly bound at the 1RM promoter bearing a consensus −35 region (Fig. 7), this suggests that the wild-type repressor-operator complex may stimulate transcription from wild-type 1RM by increasing the affinity of RNA polymerase for the promoter. Kinetic experiments show that the highly homologous λ repressor stimulates transcription from a λPRM by increasing the rate of an isomerization step that is RNA polymerase concentration-independent (37, 38). Positive control mutations in the two proteins lie at homologous positions (27, 39), and the structure of the two repressor-operator complexes are highly similar (26, 28, 40, 41); it is therefore difficult to reconcile their apparent differences in activation mechanisms.

Two non-exclusive hypotheses may resolve this discrepancy. The first hypothesis involves the realization that the formation of an open promoter complex is a multistep process. The rate of each forward and reverse step in the pathway to form an open complex is characterized by a rate constant that varies with promoter sequence. For different promoters, the RNA polymerase-promoter species that are in rapid equilibrium with the free protein and DNA may differ with sequence. Hence, the rate of transformation between two identical types of promoter complexes may be dependent or independent of RNA polymerase concentration. By this argument, λ and 434 repressor could catalyze the same chemical step, but whether this step is observed as an effect on the affinity of RNA polymerase for the promoter or isomerization of a tightly bound promoter complex may vary with promoter sequence. The second hypothesis is based on the finding that the distance between the phosphate that is shared by both RNA polymerase and repressor binding sites and the surface of the repressor that contacts RNA polymerase differs between λ and 434 repressor-operator complexes (29, 41). This observation suggests that the target of the activating regions of the repressors on the RNA polymerase molecule may be different. Such a proposal is not unprecedented, several other non-lambda repressor transcriptional activators have distinct targets on the αC subunit (42). The possible differences between the activation surface on the RNA polymerase targeted by the two proteins may contribute to the observed differences in their activation mechanisms.

The results we present here expand our ideas about how sequence differences between binding sites for a particular activator protein contribute to its activation function. It is well established that sequence differences allow an activator protein to discriminate between various binding sites along a strand of DNA and that this discrimination is crucial for proper biological functioning of the activator. The data presented here show that in addition to regulating binding affinity, binding site sequence can alter the activation efficiency of the protein.

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