Constitutive Function of a Positively Regulated Promoter Reveals New Sequences Essential for Activity

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A consensus "-10" recognition sequence for RNA polymerase was created at the positively regulated λ Pre promoter by introducing three single base pair mutations. This altered promoter, Pre*, functions constitutively in vitro and in vivo at high efficiency despite very poor consensus "-35" region sequence homology. We examined the influence of the -35 region sequence information on promoter function by shifting the wild type -35 region ± 2 base pairs relative to the -10 region consensus sequence and by completely replacing it with alternative DNA sequences. In every case, the altered Pre* promoters retained transcriptional activity although differences in their transcriptional efficiencies were observed. Apparently the Pre* promoter does not require specific -35 region sequences for constitutive promoter activity, although the -35 region sequences can modulate overall promoter strength. In addition, by point mutation analysis we have identified bases immediately upstream of the -10 hexamer which are essential for constitutive function of the Pre* promoter. We propose that these mutants define an extended -10 region at Pre* that compensates for its poor -35 region sequence information by providing critical contacts that stabilize productive RNA polymerase binding.

The positively regulated λ Pre promoter is not recognized directly by RNA polymerase. Promoter function is completely dependent on the phage transcriptional activator protein, cII (1, 2). Activation of Pre by cII plays a vital role in achieving the delicate balance between lytic and lysogenic development observed for phage λ (3–5).

Analysis of the DNA sequences of promoters recognized by Escherichia coli RNA polymerase have identified two conserved 6-base pair sequences located approximately 10 and 35 bases upstream from the transcription start site. They are referred to as the "-10" and "-35" promoter regions, respectively (for reviews see Refs. 6–8). The importance of these hexanucleotide regions has been demonstrated by extensive mutational studies. In fact, almost all of the promoter point mutations that have been characterized map to the -10 or -35 hexanucleotide regions (8, 9). Another conserved promoter feature is the spacing distance between the -10 and -35 sequences. This spacer region has a conserved length of 17 base pairs and demonstrates little, if any, DNA sequence specificity (10–17).

At the level of DNA sequence, the λ Pre promoter has poor homology with consensus promoter sequences (18, 19). In the -10 region, Pre has only three of the six conserved bases, and in the -35 region, 17 base pairs upstream, there is essentially no homology. Thus, it is not surprising that the λ Pre promoter is not recognized directly by RNA polymerase and that transcription from Pre is dependent upon the λ activator protein, cII. The cII protein specifically recognizes a tetranucleotide repeat sequence, TTGC(N6)TTGC, separated by 6 base pairs, (N6), which corresponds to the -35 region of the λ Pre promoter (2, 20). The interaction of cII protein with this sequence is both necessary and sufficient for productive RNA polymerase binding and efficient transcription from the Pre promoter.

More than 35 point mutations in the λ Pre region have been characterized (21). However, despite considerable effort no cII-independent promoter mutations have been obtained. Presumably this is because a single point mutation would not be sufficient to allow independent RNA polymerase recognition. This contention is supported by the lack of consensus information at the Pre promoter site and the fact that a single base change toward consensus in either the -10 or -35 promoter region would not constitute a sufficient increase toward consensus. A truly constitutive λ Pre promoter mutant would probably require multiple point mutations within the confined regions of the -10 and/or -35 hexamer sequences.

In an effort to construct an efficient constitutive derivative of the λ Pre promoter we increased its homology with consensus sequences in the -10 region. Three point mutations were introduced into this region to create a perfect consensus -10 region sequence (TATAAT). We demonstrate that this altered promoter, Pre*, functions independently of the activator protein at high efficiency both in vivo and in vitro. This efficient constitutive promoter activity of Pre* occurs despite the fact that this promoter has essentially no homology with consensus sequence information in the -35 region. To determine the influence of -35 region sequences on the constitutive function of the Pre* promoter we replaced the original λ -35 region sequence with several alternative sequences. We show that no specific bases are required in any of the six positions of the -35 region to promote constitutive transcription from Pre*. Moreover, we identify bases just upstream of the -10 region which have become crucial for constitutive function of the Pre* promoter.

MATERIALS AND METHODS

Bacterial Strains—The E. coli strains used in this work are all K12 derivatives designated as follows: uc6183(F- galK- hflI-1 recA- strA- supE), uc6183063 red-3 cI-857 cro-27 Pam-3), uc6183(int-6 red-3 cI-857 cro-27 Pam-3) (22). They were kindly provided by D. W. Wulf of the State University of New York at Albany.

Plasmid Constructions—The galK expression vector used in this study, pKO-SK, is a modified version of the plasmid pKO-11 in which the Nde-1 and EcoRI restriction sites at positions 1936 and 4000,
respectively, were destroyed by fill-in reactions with the Klenow fragment of DNA polymerase (25). The resulting vector, pKO-SK, is suitable for both in vivo and in vitro transcriptional studies. The λ Pre promoter region extending from position +2 to −93 was cloned on a 95-base pair Nde-I (18342)/Dde-I (18345) fragment which had been filled in with the Klenow fragment of DNA polymerase and ligated into the Sma-1 site of the galK expression vector, pKO-SK, to construct the plasmid pKO-Pre (Fig. 1, A and B). The plasmid pKO-Pre was cleaved at the unique EcoRI and Nde-1 sites to remove the wild type promoter sequences between positions +2 and −16. Then to construct the plasmid pKO-Pre*, we replaced the wild type promoter sequence from positions +2 to −16 with a synthetic oligonucleotide that contained the identical promoter sequences except for three single base changes in the −10 region. The introduction of these point mutations at positions −12, −10, and −9 created a perfect consensus −10 promoter region at Pre. Another synthetic oligomer with the wild type λ Pre sequence from positions +2 to −16 was also reconstructed to serve as an exact control for the Pre* promoter construct. This plasmid is called pKO-Pre+.

To create the 2-base pair insertion and deletion mutants at the Pre and Pre* promoters, the position of the −35 region was shifted relative to the −10 region by linearizing each plasmid at the unique Nde-1 site in the intermittent region. The insertion mutants, pKO-Pre+2 and pKO-Pre+2+, were constructed by filling in the 2-base pair overhang with the Klenow fragment of DNA polymerase. The deletion mutants, pKO-Pre−2 and pKO-Pre−2, were constructed by removing the 2-base pair overhang with Mung bean nuclease. These constructions were all confirmed by Maxam and Gilbert DNA sequence analysis (24) (data not shown).

Several derivatives of the Pre* promoter with the alternative −35 region sequences were constructed. The first derivative was made by filling in the Nde-1 and EcoRI ends of the plasmid pKO-Pre+ with the Klenow fragment of DNA polymerase. Then this 18-base pair fragment which contained the promoter sequences from positions +2 to −16 and the consensus −10 sequence was ligated directly into the Sma-1 site of the vector pKO-SK to generate the plasmid pKO-Pre+. Two other plasmids with alternative −35 regions, pKO-Pre+2 and pKO-Pre−3, were constructed by linearizing the Pre* plasmid with Nde-1 and inserting a 555-base pair fragment which had a 190-base pair DNA fragment that contained the transcription terminator, toop, inserted at the unique Nru-1 site (see "Experimental Procedures" for details). The constructions were verified by fine restriction mapping and loss of the unique Nde-1 restriction site.

The transcription terminator, toop, into the unique Nru-1 site 180 base pairs downstream of the transcription start sites of the promoters. The resulting plasmids, pKO-Pre and pKO-Pre+2, were used as supercoiled DNA templates for in vitro run-off transcription experiments, described below.

### Transcription in Vitro

The in vitro run-off transcription reactions were performed as described previously (1, 2). All transcription reactions contained 1.0 μg of plasmid DNA linearized at the unique Nru-1 site (180 base pairs downstream of the promoter regions) unless otherwise noted. The transcription mixtures contained 0.5 mM ATP, UTP, and GTP and 0.2 mM UTP, 20 μCi of α-labeled [35S]UTP (specific activity, 410 Ci/mmol), 25 mM Tris·HCl, pH 7.5, 80 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, and 1.0 mM dithiothreitol. Purified λ cII protein was added to the reactions as indicated. Reactions were initiated by addition of limiting (0.5 unit) or excess (2.0 units) amounts of E. coli RNA polymerase purchased from Pharmacia P-L Biochemicals (specific activity, 342 units/mg) and incubated at 37 °C for 15 min. Reactions were stopped by addition of 50 μL (8 M urea, 0.5 × TBE, 0.05% sodium dodecyl sulfate, 0.25% xylene cyanol/ bromphenol blue), denatured at 65 °C for 2 min, and then 5–10 μL was loaded onto a 5% polyacrylamide urea gel and subjected to electrophoresis.

### Results

#### Pre* Functions Constitutively in Vitro

In an effort to isolate a constitutive derivative of the λ Pre promoter we introduced three base changes in the −10 region creating a consensus −10 sequence (TATAAT) (Fig. 2). This altered promoter, Pre*, was tested to determine if the increase in −10 region homology was sufficient to alleviate the cII dependence of the promoter, i.e. create a constitutive promoter. A wild type λ Pre sequence was also reconstructed to serve as a control for these experiments (see "Experimental Procedures" for details).

![Diagram of the Pre* promoter region](image)

**Fig. 1.** PKO-SK and the Pre* promoter region. **A,** this diagram of the plasmid pKO-SK shows the Pre promoter region insert (not to scale) with the direction of transcription indicated by an arrow, the E. coli galactokinase gene, gal K downstream of the promoter, several unique restriction sites, the ampicillin resistance gene, ampR, and the heat shock protein holoK. The size of the PKO-SK vector is 4002 base pairs. **B,** the base sequence of the noncoding strand of the Pre promoter region insert extends from position −93 to +42 relative to the transcription start site which is denoted with an arrow. The −10 and −35 region hexamers are indicated as is the unique Nde-1 restriction site in the spacer region.

**Fig. 2.** Promoter DNA sequences. The antisense strands of the 10 promoter constructions used in this study are shown with the −10 and −35 region sequences in darkened type; point mutations are denoted with an asterisk (the first time they appear, and the underline bases represent the 2-base pair insertion and deletion mutations in the spacer region.)
These constructions were tested for transcriptional activity using linearized templates in standard in vitro run-off transcription assays. We found that the wild type -10 region construct, pKO-Pre+, retained complete cII dependence and functioned identically to the original λ Pre construct, pKO-Pre. Therefore, it was an appropriate control for wild type Pre function. In contrast, the Pre+ promoter functioned independently of cII protein, and we found that its activity was approximately equivalent to the activity of the λ Pre promoter maximally activated by cII protein (Fig. 3). In transcription reactions using excess RNA polymerase, cII protein had no additional effect on transcription from the Pre+ promoter (data not shown). However, in transcription reactions containing limiting amounts of RNA polymerase, the addition of cII protein reproducibly increased the level of transcription approximately 2-fold (Fig. 3). Apparently cII protein still recognizes the Pre+ promoter and helps it compete for enzyme when RNA polymerase is limiting.

**Pre+ Functions Constitutively in Vivo**—The same plasmid DNAs that were used as templates in the in vitro run-off transcription experiments were also used to examine and compare Pre and Pre+ function in vivo. These vectors are derivatives of the galK fusion vector system, and thus their relative promoter efficiencies can be compared directly in vivo by assaying galactokinase activity (25). Each plasmid was transformed into the appropriate galK- host to allow measurement of galK enzyme activity produced in the presence and absence of cII protein (see "Experimental Procedures" for details). The results obtained in vivo were completely consistent with those found in vitro. Whereas expression from the wild type Pre promoter was completely cII dependent, the Pre+ promoter functioned independently of cII protein (Table I). Moreover, the Pre+ promoter expressed galactokinase at essentially the same levels as did the cII-activated λ Pre promoter. Clearly the three base changes in the -10 region make the Pre+ promoter function constitutively and efficiently.

It is of interest to note that in vivo the activity of the Pre+ promoter was increased almost 2-fold in the presence of cII protein (Table I). This increase is comparable with that observed in vitro when the concentration of RNA polymerase in the transcription reactions was limiting. Apparently cII protein helps the Pre+ promoter compete for limiting RNA polymerase in vivo as well as in vitro.

**Effects of Spacer Length on Promoter Function**—It was somewhat surprising that the constitutive Pre+ promoter functioned as efficiently as the fully cII-activated λ Pre promoter. The cII-activated Pre promoter is known to be a rather efficient promoter, and the Pre+ promoter functions as efficiently although it has essentially no homology with the -35 region consensus sequence. In order to examine the contribution of sequences in the -35 region to Pre+ function we made alterations in the length of the spacer region separating the -10 and -35 promoter regions. It is well established that promoter activity is extremely sensitive to alterations in the spacer length (10–17). At Pre+ we altered this distance by insertion or deletion of 2 base pairs from the Nde-1 site within the spacer region to construct the pKO-Pre+2 and pKO-Pre+2 promoters, respectively (see "Materials and Methods" for details). These constructions were examined as above for their effect on Pre+ function both in vivo and in vitro.

Transcription experiments carried out in vitro and in vivo (Fig. 4, Table I) indicated that both the Pre+2 and Pre+2 promoters retained the ability to function constitutively and efficiently. In fact, the Pre+2 promoter was more active than the Pre+ promoter (Table I). This increased activity is probably due to the fact that the 2-base pair shift in the Pre+2 promoter places the sequence (TTGTTT) in the appropriate -35 region position (Fig. 2) and thereby increases the homology with the consensus sequences (TGACA). The Pre+2 promoter is almost as active as the Pre+ promoter (Table I), despite the fact that it has completely different nonconsensus sequence information upstream of position -16 due to the 2-

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**TABLE I**

**Summary of the relative promoter activities assayed in vivo**

The relative transcriptional efficiencies of the indicated promoters fused to the E. coli galactokinase gene on the pKO series multicopy plasmids were determined by assaying galactokinase expression in isogenic lysogens with either cII+ or cII- genotypes as described in Refs. 22 and 23. Briefly, lysogens were grown to log phase at 32 °C and then shifted to 42 °C to induce cII gene expression. Then 30 min after induction, cell extracts were prepared and galactokinase activities determined. The percent galactokinase expression is given relative to fully cII-activated λ Pre which is 100% and equal to 565 units of galactokinase/15 min at 30 °C. Each number represents the average of at least three independent experiments using different cell extract preparations in which the calculated galactokinase units varied by less than 10%.

| Promoter | cII- | cII+ |
|----------|------|------|
| 1. Pre   | 3    | 100  |
| 2. Pre+  | 4    | 100  |
| 3. Pre+  | 97   | 175  |
| 4. Pre+2 | 28   | 20   |
| 5. Pre-2 | 4    | 2    |
| 6. Pre*+2| 200  | 165  |
| 7. Pre*-2| 80   | 58   |
| 8. Pre+1 | ND*  | 60   |
| 9. Pre*2 | ND   | 23   |
| 10. Pre*3| ND   | 62   |
| 11. P+T  | 5    | 100  |
| 12. P+C  | 12   | 100  |
| 13. P+C  | 12   | 88   |

*ND*, not determined.
the supercoiled integrity of the DNA template, as Pre+2 promoter activity was only observed in vitro with uncut supercoiled template (Fig. 5). Addition of cII protein also had an inhibitory effect on transcription from the Pre+2 promoter, similar to that observed with the Pre+2 and Pre-2 promoters (Figs. 4A and 5, Table I). These results support our contention that spatial positioning is crucial for positive activation by cII protein and that changing the spacer length by +2 base pairs leads to nonproductive cII binding which sterically interferes with the interaction of RNA polymerase at these promoter sites.

Effects of Alternative DNA Sequences in the −35 Region of Pre∗—The fact that the wild type −35 region sequence of Pre∗ has little to no homology with the consensus −35 sequence and that neither insertion nor deletion mutations severely affected promoter function suggested that a specific sequence in this region was not critically important for promoter function. To further test the importance of −35 region sequences on Pre∗ function we constructed three promoters, Pre+1, Pre+2, and Pre-3, in which the normal −35 region of Pre∗ was completely replaced with three different DNA sequences (Fig. 2).

The Pre+1 construct contained Pre∗ sequences from position −17 to +2. All other upstream and downstream sequence information was contributed by the pKO-SK vector sequences. Remarkably, this sequence functioned constitutively both in vitro and in vivo despite its poor −35 region sequence information (Fig. 6, Table I). The efficiency of the Pre+1 promoter was 60–65% that of the Pre+∗ promoter. This result indicates that although the sequences upstream of position −17 quantitatively affect promoter strength they were cer-

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**Fig. 4.** In vitro analysis of Pre and Pre+∗ promoters with insertion and deletion mutations in the spacer region. A, autoradiogram of a polyacrylamide gel analysis of the RNA transcripts synthesized in vitro with limiting RNA polymerase in the absence and presence (0.2 μg/reaction, 400 nM) of purified cII protein. The DNA sequences of the promoters on the plasmids 1, pKO-Pre+∗; 2, pKO-Pre+; and 3, pKO-Pre−2 are shown in Fig. 2 and described in the text. Templates were linearized at the unique Nru-1 restriction site, position 293, respectively. The estimated size of the 180-base run-off transcript is 290 bases.

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**Fig. 5.** Activity of the Pre+2 promoter is dependent on DNA supercoiling. Autoradiogram of a polyacrylamide gel analysis of the RNA transcripts synthesized in vitro from the plasmid templates A, pKO-Pre; and B, pKO-Pre+2t described in the text. Transcription reactions were performed with limiting RNA polymerase in the absence and presence (0.2 μg/reaction, 400 nM) of purified cII protein with uncut supercoiled template (sc) or template DNA linearized (L) at the Bgl-I restriction site, position 3121, or the Hind-3 restriction site, position 293, respectively. The estimated size of the run-off transcript is 290 bases.
plasmids purified of a polyacrylamide gel analysis of the RNA transcripts synthesized in vitro immediately upstream of the −10 region hexamer revealed sequence homology with bases that show weak conservation in other promoters (i.e., guanine at position −14 and thymine at position −15) (8). To determine the influence of these bases on Pre* function we constructed several derivatives of Pre* which contained point mutations at positions −14, P14T*, P14C*, and −15, P15C* (Fig. 2) and tested these Pre* mutants in our in vitro and in vivo transcription assays.

In vitro all three of these single point mutations completely eliminated constitutive promoter function (Fig. 7). In vivo these mutants also severely affected constitutive promoter activity (Table I). These results indicate that the thymine and guanine nucleotides present at positions −15 and −14 of Pre*, respectively, are essential for constitutive promoter function. Apparently, in the absence of consensus −35 region sequence information these bases become critical components of the RNA polymerase recognition and initiation signal. We further examined the ability of these mutant Pre* promoters to respond to CII activation. In the presence of CII protein, these promoters functioned at essentially the same efficiency as λ Pre (Fig. 7, Table I). Thus, CII can compensate for the deficiency introduced into the Pre* promoter by these position −14 and −15 point mutations just as CII compensates for poor consensus sequence information at λ Pre.

**FIG. 6. In vitro activity of the Pre* promoters with substituted −35 region sequences.** This figure shows an autoradiogram of a polyacrylamide gel analysis of the RNA transcripts synthesized in vitro with limiting RNA polymerase from the promoters on the plasmids 1, pKO-Pre; 2, pKO-Pre*; 3, pKO-Pre*; and 4, pKO-Pre* in the absence and presence (0.2 µg/reaction, 400 nM) of purified CII protein as noted. The base sequences of the promoter regions are shown in Fig. 2 and described in the text. Templates were linearized at the unique Nru-1 restriction site, 180 base pairs downstream of the transcription start site of the indicated promoters to produce 180-base run-off transcripts.

**FIG. 7. In vitro activity of the point mutations in the spacer region of the Pre* promoter.** This figure shows an autoradiogram of a polyacrylamide gel analysis of the RNA transcripts synthesized in vitro with limiting RNA polymerase from the promoters on the plasmids 1, pKO-Pre*; 2, pKO-P14C; and 3, pKO-P15C in the absence and presence (0.2 µg/reaction, 400 nM) of purified CII protein. The base sequences of the promoter regions are shown in Fig. 2 and described in the text. Templates were linearized at the unique Nru-1 restriction site, 180 base pairs downstream of the transcription start site of the indicated promoters to produce 180-base run-off transcripts.
This constitutive promoter, Pre+, functions as efficiently as the fully cII-activated λ Pre promoter both in vivo and in vitro. The efficient activity of the Pre+ promoter was rather unexpected since its −35 region sequence (GTGTTGT) has little to no homology with the consensus −35 region sequence (TGGACA) that is required by most other constitutive prokaryotic promoters for activity. In vivo and in vitro transcriptional analyses of five Pre+ derivatives with alternative −35 region sequences showed that every derivative retained constitutive promoter function. The activity of these promoters was affected by the particular sequences which were placed in the −35 region indicating that they participate in modulating promoter activity. Furthermore, sequences which generally improved the homology of the −35 region with that of consensus (e.g. Pre+2, TTGTTT) increased promoter efficiency, and those which had poor homology showed somewhat less activity. However, even the Pre+2 promoter which contains a most unusual nonconsensus −35 region sequence (GGGGCG) had only a 4–5-fold down effect on Pre+ activity and thus retained quite reasonable levels of promoter activity. These findings clearly demonstrate that the Pre+ promoter does not require specific −35 region sequence information for constitutive promoter function, although sequences there may certainly exert a positive or negative effect on overall promoter efficiency.

Our results indicate that the Pre+ promoter segment spanning from position −16 to +2 is sufficient for constitutive promoter function. Clearly the consensus −10 region hexamer within this segment is a primary determinant of promoter activity. However, we have identified additional sequences at Pre+ just upstream of the −10 region hexamer (i.e. the extended −10 region) which are crucial for constitutive promoter function. Point mutations at either position −14 or −15 dramatically reduce constitutive Pre+ activity. Very few mutations have been obtained at analogous sites in other promoter signals. Moreover, the few mutations which have been characterized usually have only minor effects on promoter efficiency (3, 10, 26–29). We suspect that mutations in this extended −10 region of the Pre+ promoter eliminate certain contact sites for RNA polymerase and that in the absence of a sufficient −35 region recognition sequence, these contact sites have become critical for promoter recognition and function.

Several lines of evidence support our above contention. First, the extended −10 region does exhibit some weak sequence conservation when all promoter signals are compared (8). However, among those promoters which lack Pre+ do not require specific consensus −35 region sequence information for activity, such as gal P1, gal P2 (30), omp F (31), and uvr A (32), this region, and in particular the G:C and T:A base pairs at positions −14 and −15, respectively, exhibit strict sequence conservation. This data implies that the extended −10 region may actually compensate for the lack of consensus sequence information in the −35 region.

This contention is supported directly by results obtained in mutational studies with the P22 Pant promoter. This promoter has highly conserved consensus sequences in both the −10 and −35 region hexamers but does not contain an extended −10 sequence. Many promoter down mutations have been isolated in Pant and the vast majority mapped to the −10 and −35 region hexamers (9). However, when second site revertants were selected starting with a Pant promoter mutant that contained severe −35 region down mutations, promoter activity was recovered by an A:T to G:C transition at position −14 (33). Hence, the creation of an extended −10 region similar to that defined here at Pre+ was able to compensate for the −35 region defect at Pant.

The extended −10 region is also important for efficient promoter function of the λ Pre promoter. The Pre mutation cy3019 is a G:C to A:T transition at position −14 which reduces promoter activity (3). Similar point mutations in the extended −10 region of the Pre+ promoter respond normally to cII activation, and hence an RNA polymerase contact rather than a cII protein contact has been eliminated by the cy3019 mutation. We conclude that the extended −10 region usually plays a relatively minor role in most promoters which contain adequate −35 region sequence information. However, this region does become an important determinant of promoter function in the absence of conserved −35 region sequences.

We also characterized the responsiveness of some of the promoter constructs to activation by cII protein. Transcription from the Pre+ promoter was increased about 2-fold in vitro when RNA polymerase was limiting. In the presence of excess RNA polymerase, however, no effect of cII protein was observed. This suggests that cII protein enhances promoter recognition and that this effect can be overcome completely by simply increasing the RNA polymerase concentration. In vitro, again we observed a 2-fold activation of transcription from Pre+ by cII protein. This implies that in the bacterial cell the amount of available RNA polymerase is also limiting and that cII functions by helping the Pre+ promoter compete for RNA polymerase more effectively.

In those Pre+ promoters in which the −35 region had been shifted by either a 2-base pair insertion or deletion mutation we observed inhibition rather than enhancement of transcription by cII protein. In vivo we observed minor reductions in the constitutive activity of these promoters in the presence of cII protein; however, in vitro using saturating concentrations of cII protein a greater degree of inhibition (50%) was observed. Apparently, cII binding actually interferes or competes with RNA polymerase for binding if the cII binding site is displaced within the promoter site. Consistent with this idea was the fact that the identical insertion and deletion mutations at λ Pre resulted in complete elimination of cII activation. These results demonstrate that the precise positioning of the cII binding site within the promoter signal is critical for cII-activated transcription from λ Pre and the Pre+ promoter.

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REFERENCES
1. Shimakawa, H., and Rosenberg, M. (1981) Nature 292, 128
2. Ho, Y.-S., Wulff, D. L., and Rosenberg, M. (1983) Nature 304, 703
3. Rosenberg, M., Court, D., Shimakawa, H., Brady, C., and Wulff, D. L. (1978) Nature 272, 414
4. Herskovits, I., and Hagen, D. (1980) Annu. Rev. Genet. 14, 399
5. Wulff, D. L., and Rosenberg, M. (1983) in The Bacteriophage Lambda (Hendrix, J., Roberts, J., Stahl, F., and Weinberg, R., eds) Vol. 2, p. 53, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. Rosenberg, M., and Court, D. (1979) Annu. Rev. Genet. 13, 319
7. Siebenlist, U., Simpson, R., and Gilbert, W. (1980) Cell 20, 269
8. Hawley, D., and McClure, W. (1982) Nucleic Acids Res. 11, 2237
9. Youderian, P., Bouvier, S., and Suskind, M. (1982) Cell 30, 843
10. Berman, M. L., and Landy, A. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4303
11. Jaurin, B., Grundström, T., Edlund, T., and Normark, S. (1981) Nature 290, 221
12. Stefano, J. E., and Gralla, J. D. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 1069
13. Mandecki, W., and Reznikoff, W. S. (1982) Nucleic Acids Res. 10, 903
14. Russel, D. R., and Bennett, G. N. (1982) Gene 20, 231
15. Aoyama, T., Takanami, M., Ohtsuka, E., Taniyama, Y., Marumato, R., Suto, H., and Ikehara, M. (1983) Nucleic Acids Res. 11, 5855
16. Mulligan, M. E., Brosius, J., and McClure, W. R. (1985) J. Biol. Chem. 260, 3529–3538
17. Brosius, J., Erfle, M., and Storella, J. (1985) J. Biol. Chem. 260, 3539–3541
18. Schwarz, E., Scherer, G., Hobom, G., and Kössel, H. (1978) Nature 272, 410
19. Schmeissner, U., Court, D., Shimitake, H., and Rosenberg, M. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 391
20. Ho, Y. S., and Rosenberg, M. (1985) J. Biol. Chem. 260, 11838–11844
21. Wulff, D. L., Mahoney, M., Shatzman, A., and Rosenberg, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 555
22. Fien, K., Turck, A., Kang, I., Keilty, S., Wulff, D. L., McKenney, K., and Rosenberg, M. (1984) Gene 32, 141
23. Adams, C. W., and Hatfield, G. W. (1984) J. Biol. Chem. 259, 7399–7403
24. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499
25. Brady, C., and Rosenberg, M. (1982) in Gene Amplification and Analysis: Analysis of Nucleic Acids (Chirkjian, J. G., and Papas, T., eds) p. 385, Elsevier/North-Holland Biomedical Press, Amsterdam
26. Rosen, E. D., Hartley, J. L., Matz, K., Nichols, B. P., Young, K. M., Donnelson, J. E., and Gussin, G. N. (1980) Gene 11, 197
27. Busby, S. J., Aiba, H., and de Crombrugghe, B. (1982) J. Mol. Biol. 154, 211
28. Piette, J., Cunin, R., Boyen, A., Charlier, D., Crabeel, M. van Vliet, F., Squires, C., Glansdorff, N., and Squires, C. L. (1982) Nucleic Acids Res. 10, 8031
29. Munson, L. M., Mandecki, W., Caruthers, M. H., and Reznikoff, W. (1984) Nucleic Acids Res. 12, 4011
30. Ponnambalam, S., Webster, C., Bingham, A., and Busby, S. (1986) J. Biol. Chem. 261, 16043–16048
31. Inokuchi, K., Furukawa, H., Nakamura, K., and Mizushima, S. (1984) J. Mol. Biol. 178, 653
32. Backendorf, C., Brandsma, J. A., Kartasova, T., and van de Putte, P. (1983) Nucleic Acids Res. 11, 5795
33. Grana, D., Youderian, P., and Susskind, M. (1985) Genetics 110, 1