Mutations that Improve the p_{RE} Promoter of Coliphage Lambda

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ABSTRACT

The *dya5* mutation, a C→T change at position −43 of the λ p_{RE} promoter, results in a twofold increase in p_{RE} activity *in vivo*. Smaller increases in p_{RE} activity are found for the *dya2* mutation, a T→C change at position −1 of p_{RE}, and the *dya3* mutation, an A→G change at +5 of p_{RE}. The mutant p_{RE} promoters retain complete dependence on *cII* protein for activity. These observations argue, at least for p_{RE}-like promoters, that promoter activities are influenced by nucleotide sequences at least eight nucleotides to the 5′-side of the conventional −35 region consensus sequence, and by nucleotide sequences near the start-site of transcription. Although HAWLEY and MCCLURE (1983) found A·T pairs more frequently than G·TC pairs in the region of −40 to −45 of prokaryotic promoters, other mutations that change a G·TC pair to an A·T pair at positions −41, −44 and −45 of p_{RE} do not result in increased promoter activity. We also found that a T→C change at position −42 results in a mild decrease in promoter activity. These observations argue that Ts at positions −42 and −43 of p_{RE} are required for maximum promoter activity, but do not support the hypothesis that As and Ts in the −40 to −45 region generally lead to higher promoter activities.

PROKARYOTIC promoters generally have two regions of sequence similarity, located at approximately ten bases (the −10 region) and 35 bases (the −35 region) before the initial base of the mRNA (ROSENBERG and COURT 1979; SIEBENLIST, SIMPSON and GILBERT 1980; HAWLEY and MCCLURE 1983). Most mutations that affect promoter activity lie in one of these two regions.

The λ p_{RE} promoter is activated by binding of the λ *cII* protein to the promoter region (SHIMATAKE and ROSENBERG 1981). The p_{RE} promoter sequence agrees with the prokaryotic consensus sequence in only three of six positions in the −10 region, and shows no homology with the consensus sequence in the −35 region (Figure 1) (SCHMEISSNER *et al.* 1980). This is perhaps not surprising since p_{RE} is not recognized as a promoter in the absence of *cII* protein. DNA binding and chemical protection studies indicate that the *cII* protein recognizes and binds to a 5′-TTGCN$_6$TTGC-3′ repeat sequence in the −35 region of p_{RE} (Figure 1) (HO, WULFF and ROSENBERG 1983). Binding of *cII* protein facilitates binding of RNA polymerase to the opposite face of the DNA double helix, where it makes contacts with the region of six intervening nucleotides between the two TTGC sequences (HO, WULFF and ROSENBERG 1983). Mutations in the −35 region that affect p_{RE} function extend over most of the 5′-TTGCN$_6$TTGC-3′ repeat sequence, a considerably longer region than that spanned by promoter mutations in the −35 regions of other promoters (WULFF *et al.* 1984). Mutations in the TTGC sequences greatly reduce binding by *cII* protein, while mutations in the intervening six nucleotides affect RNA polymerase contacts (HO, WULFF and ROSENBERG 1983).

No promoter mutations in the −40 to −45 region have been isolated in p_{RE} or in any prokaryotic promoter, although HAWLEY and MCCLURE (1983), in their compilation of prokaryotic promoter sequences, found that A·T base pairs are somewhat preferred over G·TC base pairs in this region. Similarly, no promoter mutations in the −5 to +5 region have been isolated in any prokaryotic promoter, although HAWLEY and MCCLURE (1983) found a weak consensus sequence of CAT at the adjacent positions −1, +1 and +2. In this paper we report the isolation and properties of mutations with up to twofold increases in promoter activity which are located at positions −43, −1 and +5 of p_{RE}.

MATERIALS AND METHODS

Bacteria: All strains are derivatives of *Escherichia coli*. Strain C600 (CAMPBELL 1961) was used for standard phage work, and for determination of plaque morphologies. For the galactokinase assay systems, plasmids were transformed into UC6183(λ*int6 cIts857 cro27 Pam3*) and UC6183(λ*int6 cIts857 cro27 cII3067 Pam3* (FIEN *et al.* 1984).

Phage: The p_{RE}^- strain λ*ctr1 cy3008* is described in PLACE *et al.* (1984). The *dya2* and *dya3* mutations are described by DUL, MAHONEY and WULFF (1987). The *cII*$^-$ strains used in this work are from a collection of mutant strains for which DNA sequence changes have been determined.

General: Media, general phage techniques and phage crosses are described in WULFF (1976).

Mutagenesis: Lambda *ctr1 cy3008* was passed through an *E. coli mutD* mutator strain (FOWLER, DEGNEN and COX 1974) by the method of ENQUIST and WEISBERG (1977).

DNA sequence determinations: DNA sequences of *Sam7*

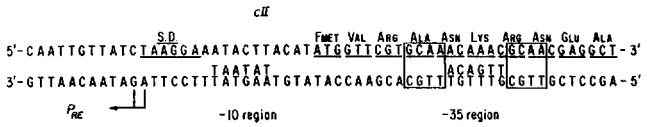

FIGURE 1.—DNA sequence of the p_{RE} promoter and NH_2-terminal region of the *cII* gene (SCHWARZ *et al.* 1978; ROSENBERG *et al.* 1978; SCHMEISSNER *et al.* 1980). The 6-base consensus sequences for the −10 and −35 regions of prokaryotic promoters are indicated between strands (ROSENBERG and COURT 1979; SIEBENLIST, SIMPSON and GILBERT 1980; HAWLEY and MCCLURE 1983). The TTGC sequences recognized by *cII* protein are outlined. Transcription from p_{RE} initiates at either of two sites, as indicated by the arrow (SCHMEISSNER *et al.* 1980). The *cII* gene is transcribed from the p_R promoter, which lies several hundred nucleotides to its left. The line labeled "S.D." indicates the SHINE and DALGARNO (1974) homology for the *cII* gene. Hyphens have been omitted from the sequence for clarity.

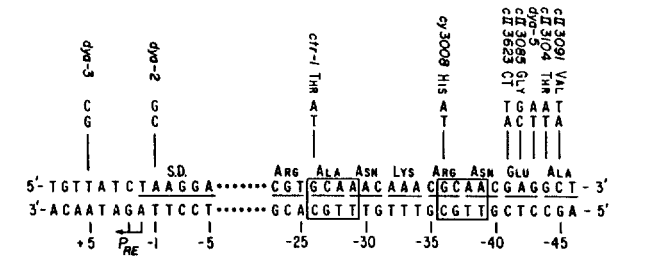

FIGURE 2.—DNA sequence changes for *dya-2*, *dya-3* and *dya-5*, and other mutations used in this study. The *cy3008* alteration is from WULFF *et al.* (1980), the *ctr1* alteration from PLACE *et al.* (1984), and the *cII3623* and *cII3085* alterations from WULFF *et al.* (1984). The *cII3104* and *cII3091* alterations are from unpublished data on *cII*⁻ mutations. The numbers below the line indicate the interval from the p_{RE} start site as designated in HO, WULFF and ROSENBERG (1983). Hyphens have been omitted for clarity.

derivatives of λ*ctr1 cy3008 dya5* and λ*dya5* were determined according to the method of MAXAM and GILBERT (1980) as described previously (WULFF *et al.* 1980, 1984).

Plasmids: The plasmid pKM2, in which the λ p_{RE} promoter governs expression of the *E. coli galK* gene, is described in FIEN *et al.* (1984). Derivatives of pKM2 with various mutations in the p_{RE} DNA fragment were constructed in an identical fashion, using the appropriate mutant phage as sources of p_{RE} DNA.

Galactokinase measurements: Galactokinase was assayed at 30 min after shifting a lysogenic UC6183[pKM2] derivative from 32°, at which *cII* gene expression is repressed, to 42°, as described by FIEN *et al.* (1984).

RESULTS

Isolation and properties of P_{RE} promoter-up mutations: The clear plaque p_{RE}^- strain λ*ctr1 cy3008*, which has a mutation in each of the 5′-TTGCN$_6$TTGC-3′ repeats, was mutagenized by passage through an *E. coli mutD* mutator strain, and the strain λ*ctr1 cy3008 dya5* was isolated as a variant which forms plaques with lightly turbid centers. DNA sequence analysis showed that the mutant strain, in addition to retaining the original mutations, had acquired a C→T change in position −43 of p_{RE} (Figure 2), four bases to the 5′-end of the 5′-TTGCN$_6$TTGC-3′ sequence that is the principle determinant in the −35 region for binding by *cII* protein and RNA polymerase (HO, WULFF and ROSENBERG 1983). The p_{RE} promoter and the *cII* structural gene overlap (SCHMEISSNER *et al.* 1980), and the *dya5* mutation also results in a GAG→GAA change in codon 10 of the *cII* gene. Since both GAG and GAA are glutamic acid codons which are recognized by the same iso-accepting species of tRNA (IKEMURA 1981a,b), it is unlikely that the *dya5* phenotype results from any change in the *cII* gene.

Suitable genetic crosses were performed to separate the *dya5* mutation from its original genetic background, and a recombinant strain of genotype λ*dya5* was easily isolated because it forms plaques with more deeply turbid centers than λ⁺. The genotype of λ*dya5* was confirmed by DNA sequence analysis.

The strains λ*dya2 ctr1 cy3008* and λ*dya3 ctr1 cy3008* were also isolated following *mutD* mutagenesis, and differ from λ*dya5 ctr1 cy3008* in that they form plaques with more lightly turbid centers (DUL, MAHONEY and WULFF 1987). The *dya2* and *dya3* mutations are T→C and A→G changes at positions −1 and +5, respectively, of p_{RE} (Figure 2). The *dya2* and *dya3* mutations lie in the region of overlap between p_{RE} and the ribosome recognition region of the *cII* gene. Both mutations decrease the potential secondary structure which may be formed by *cII* mRNA, and *dya2* also changes the sequence of *cII* mRNA which is complementary to the 3′-end of 16 S mRNA from 5′-UAAGGA-3′ to 5′-UGAGGA-3′ (DUL, MAHONEY and WULFF 1987). The *dya2* mutation, but not the *dya3* mutation, partially reverses the translation defects of certain *cII*⁻ mutations that are characterized by inefficient translation of *cII* mRNA (DUL, MAHONEY and WULFF 1987). Strains of genotype λ*dya2* and λ*dya3* have been constructed, and, unlike λ*dya5*, form plaques which are indistinguishable from those of λ⁺ (DUL, WULFF and MAHONEY, 1987).

We used the pKM2 system of FIEN *et al.* (1984) in order to establish that the *dya2*, *dya3* and *dya5* mutations confer increased promoter activity *in vivo*. In this system, *cII* protein from a derepressed defective prophage activates galactokinase expression on the multicopy plasmid pKM2, in which the λ p_{RE} promoter governs expression of the *E. coli galK* gene. We constructed pKM2 derivatives of λ*ctr1 cy3008 dya5* and λ*dya5*, as well as pKM2 derivatives of the corresponding *dya2* and *dya3* strains, and the parental λ*ctr1 cy3008* strain. These plasmids were then introduced into the appropriate lysogenic host strain, and galactokinase activities were determined following activation of *cII* gene expression. A p_{RE}^- plasmid of genotype *ctr1 cy3008* yields about 4% of the wild-type p_{RE}^+ activity (Table 1), which is increased two- to threefold by a *dya2*, *dya3* or *dya5* mutation. A plasmid with a *dya5* p_{RE} genotype shows a twofold greater activity

TABLE 1

Effects of *dya* mutations on promoter activity

Mutations on p_{RE} fragment	Relative galactokinase activity	
	cII$^+$ prophage	*cII*$^-$ prophage
None (p_{RE}^+)	100	<0.5
ctr1 cy3008	4	<0.5
ctr1 cy3008 dya2	9	<0.5
ctr1 cy3008 dya3	11	<0.5
ctr1 cy3008 dya5	12	<0.5
dya2	127	<0.5
dya3	153	<0.5
dya5	202	<0.5

Derivatives of the plasmid pKM2 carrying the designated mutations were transformed into UC6183(λ*int6 cIts857 cro27 Pam3*) and UC6183(λ*int6 cIts857 cro27 cII3067 Pam3*) (FIEN *et al.* 1984). Galactokinase was assayed 30 min after shifting a log phase culture from 32° to 42°, as described by FIEN *et al.* (1984). A relative activity of 100 equals 440 units of galactokinase.

than a wild-type p_{RE}^+ plasmid, and plasmids with *dya2* p_{RE} and *dya3* p_{RE} genotypes show somewhat smaller increases over a wild-type p_{RE}^+ plasmid. All of these results are consonant with the plaque morphologies described above. These results establish that the *dya2*, *dya3* and *dya5* mutations confer increased promoter activity, both in the original *ctr1 cy3008* genetic background and in a λ$^+$ background.

In order to ascertain if the increased promoter activities conferred by the *dya* mutations are dependent upon *cII* function, we repeated the above galactokinase measurements with the plasmids in a *cII*$^-$ host strain of identical construction to the *cII*$^+$ host, except for the *cII3067* mutation, a AUG→ACG alteration in the initiation codon of the *cII* gene (WULFF *et al.* 1984). The results show that the promoter activities associated with the *dya* mutations are fully dependent upon *cII* function (Table 1).

Effects of other mutations in the −40 region of P_{RE} on P_{RE} function: Four additional mutations have been obtained in the −40 region of p_{RE} as *cII* mutations in the region of overlap between the *cII* gene and p_{RE}, and we wished to see if these had effects on p_{RE} function similar to that of the *dya5* mutation. These mutations were tested in the galactokinase assay system as above. The results (Table 2) show little effect for a C→A change at position −41 of p_{RE} (*cII3623*, see Figure 2), a C→T change at position −44 (*cII3104*) and a G→A change at position −45 (*cII3091*). In contrast a T→C change at position −42 (*cII3085*) resulted in about a 40% decrease in promoter activity.

TABLE 2

Effects of various mutations on promoter activity

Mutations on p_{RE} fragment	Relative galactokinase activity
None (p_{RE}^+)	100
cII3623 (C→A at −41)	96
cII3085 (T→C at −42)	61
dya5 (C→T at −43)	202
cII3104 (C→T at −44)	116
cII3091 (G→A at −45)	104

Experiments were performed as described in Table 1. The mutations are depicted in Figure 2.

DISCUSSION

The conclusion that the *dya2*, *dya3* and *dya5* mutations confer increased activity to the p_{RE} promoter, rather than create a new promoter activity with a new transcriptional start site, is based upon three considerations. (1) The mutations do not result in sequences with any semblance of a promoter sequence, or of a binding site for *cII* protein. (2) The increased activities associated with the mutations are fully dependent upon *cII* protein (Table 1). (3) The *cII* protein binds to the 5′-TTGCN$_6$TTGC-3′ sequence which flanks the −35 region of the p_{RE} promoter. If a *dya* mutation were creating a new promoter with a different transcriptional start site, then the positioning of *cII* protein with respect to this new start site and to RNA polymerase would have to be different from that in every other *cII*-dependent promoter, an exceedingly unlikely possibility. That the increased transcription associated with the *dya* mutations is indeed caused by binding of *cII* protein to the identical binding site as in p_{RE} is argued by (a) no other *cII* binding site is present in this region of DNA, and (b) the *ctr1* and *cy3008* mutations, which decrease the binding affinity of *cII* protein for the TTGCN$_6$TTGC site (HO, WULFF and ROSENBERG 1983; PLACE *et al.* 1984), cause a large decrease in the absolute magnitude of the *dya* effect on galactokinase activity (Table 1).

The *dya5* mutation, at position −43 of p_{RE}, is outside of the region implicated for *cII* protein contact by methyl protection studies, and on the periphery of the region implicated for contact by RNA polymerase in the presence of *cII* protein (HO, WULFF and ROSENBERG 1983). Thus, in the presence of *cII* protein alone, the Gs at positions −36 and −37 are fully protected from methylation, the G at position −40 is partially protected, and the Gs at positions −41, −43 and −44 are not protected at all. The *cII* protein makes contact with the major groove of the DNA helix, but the major groove at position −43, the site of the *dya5* mutation, is on the opposite face of DNA double helix from the site of *cII* binding. In the presence of RNA polymerase and *cII* protein, the Gs at positions −40 and −41 are protected from methylation, but not the Gs at positions −43 and −44. RNA polymerase also makes contact with the major groove of the DNA helix, on the opposite side from the *cII* protein and on the same side as the major groove at position −43. Therefore it is more likely that *dya5* affects primarily RNA polymerase binding, and not *cII* binding.

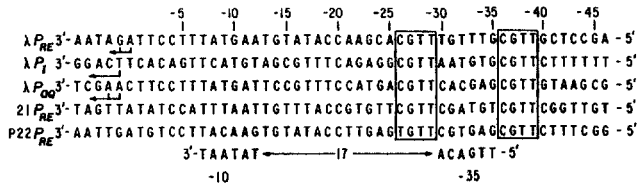

FIGURE 3.—Nucleotide sequences of λ p_{RE} (SCHWARZ *et al.* 1978; ROSENBERG *et al.* 1978; SCHMEISSNER *et al.* 1980), λ p_I (ABRAHAM *et al.* 1980; HOESS *et al.* 1980; DAVIES 1980; SCHMEISSNER *et al.* 1981), λ p_{aQ} (HOOPES and MCCLURE 1985; HO and ROSENBERG 1985), 21 p_{RE} (SCHWARZ 1980), and P22 p_{RE} (BACKHAUS and PETRI 1984; POTEETE, HEHIR and SAUER 1986). The consensus sequences at the −10 and −35 regions (HAWLEY and MCCLURE, 1983) are also included.

All of the p_{RE}^- mutations in the −35 region are found between positions −27 and −39, and promoter mutations in the −35 region of prokaryotic promoters in general are confined to the −35 consensus region itself (corresponding to positions −30 to −35 of p_{RE}) (HAWLEY and MCCLURE 1983). The *dya5* mutation demonstrates that the p_{RE} promoter is influenced by nucleotide sequence at least eight nucleotides to the 5′-side of the region in which promoter mutations are confined in other prokaryotic promoters.

HAWLEY and MCCLURE (1983), in their compilation of prokaryotic promoter sequences, found a mild preference for A·T over G·C pairs in the region of −40 to −45. Although one might suppose that GC→AT changes in this region would generally result in promoter-up mutations like *dya5*, we found that GC→AT changes at positions −45, −44, −41 were all without measureable effect on p_{RE} activity (Table 2). The G in the G·C pair that is altered in the −41 mutation is one of the bases that RNA polymerase protects from methylation, indicating that the protein makes close contact with this base pair even though the transcriptional activity is independent of whether it is a G·C or a T·A pair. A similar situation was previously found for the *ctrl* mutation, which affects a nucleotide pair that *cII* protein protects from methylation (PLACE *et al.* 1984). In contrast to the above results, a T→C change at position −42 resulted in about a 40% decrease in activity.

It is interesting to compare the sequence of the λ p_{RE} promoter with other promoters activated by *cII* protein or a *cII*-like protein. We note in Figure 3 that of five such promoters (λ p_{RE}, λ p_I, λ p_{aQ}, 21 p_{RE} and P22 p_{RE}), three have T at position −43, one has A and only λ p_{RE} has C. The *dya5* mutation therefore represents a mutation to the consensus nucleotide for p_{RE}-like promoters at position −43. In contrast, of the three mutations in the −40 region that were found to have no effect on p_{RE} activity, none is a mutation to a consensus nucleotide. The T→C change at position −42 that results in a 40% decrease in promoter activity eliminates the agreement of λ p_{RE} with the consensus T (found in three of the five promoters) at this position. The mutational studies and the comparisons with p_{RE}-like promoters therefore lead to the conclusion that Ts at both positions −42 and −43 are required for maximum promoter activity, but they do not support the idea that As and Ts in the −40 to −45 region generally lead to higher promoter activities.

The *dya2* and *dya3* mutations, at positions −1 and +5 of p_{RE}, are far from the site of *cII* binding and must affect interaction with RNA polymerase. Promoter mutations near the start sites of transcription have not previously been reported in prokaryotic promoters, but we suspect that the isolation of promoter mutants in these positions of p_{RE} is due to our ability to detect variants with small differences in promoter activity, rather than to a difference between p_{RE} and other kinds of promoters. HAWLEY and MCCLURE (1983) found a weak consensus sequence of CAT at start sites of prokaryotic promoters, and we note that the *dya2* mutation represents a TAG→CAG change in the corresponding p_{RE} sequence, which makes it conform more closely to the consensus sequence. However, HAWLEY and MCCLURE find no preferred sequences in the +5 region of prokaryotic promoters, and would not have predicted that the *dya3* mutation, an A→G change at +5 of p_{RE}, would have an effect on promoter activity. Comparison with other promoters activated by *cII* protein or a *cII*-like protein does not lead to prediction of the increased promoter activities associated with the *dya2* and *dya3* mutations. Both mutations decrease the agreement of the λ p_{RE} promoter with the consensus sequence for p_{RE}-like promoters, and one might have predicted decreased, rather than increased promoter activities.

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LITERATURE CITED

ABRAHAM J., D. MASCARENHAS, R. FISCHER, M. BENEDIK, A. CAMPBELL and H. ECHOLS, 1980 DNA sequence of the regulatory region for the integration gene of bacteriophage λ. Proc. Natl. Acad. Sci. USA **77:** 2477–2481.

BACKHAUS H. and J. B. PETRI, 1984 Sequence analysis of a region from the early right operon in phage P22 including the replication genes *18* and *12*. Gene **32:** 289–303.

CAMPBELL, A., 1961 Sensitive mutants of bacteriophage λ. Virology **14:** 22–32.

DAVIES, R. W., 1980 DNA sequence of the *Int-xis-PI* region of bacteriophage lambda: overlap of the *int* and *xis* genes. Nucleic Acids Res. **8:** 1765–1782.

DUL, E., M. E. MAHONEY and D. L. WULFF, 1987 Mutations that affect the efficiency of translation of mRNA for the *cII* gene of coliphage lambda. Genetics **115:** 585–590.

ENQUIST, L. W. and R. A. WEISBERG, 1977 A genetic analysis of the *att-int-xis* region of coliphage lambda. J. Mol. Biol. **111:** 97–120.

FIEN, K., A. TURCK, I. KANG, S. KIELTY, D. L. WULFF, K. MC-

KENNEY and M. ROSENBERG, 1984 CII-dependent activation of the p_{RE} promoter of coliphage lambda fused to the *Escherichia coli galK* gene. Gene **32:** 141–150.

FOWLER, R., G. DEGNEN and E. COX, 1977 Mutational specificity of a conditional *Escherichia coli* mutator, *mutD5*. Mol. Gen. Genet. **133:** 179–191.

HAWLEY, D. K. and W. R. MCCLURE, 1983 Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11:** 2237–2255.

HO, Y. S. and M. ROSENBERG, 1985 Characterization of a third, *c*II-dependent coordinately activated promoter on phage λ involved in lysogenic development. J. Biol. Chem. **260:** 11838–11844.

HO, Y. S., D. L. WULFF and M. ROSENBERG, 1983 Bacteriophage λ protein *c*II binds promoters on the opposite face of the DNA helix from RNA polymerase. Nature **304:** 703–708.

HOESS, R. H., C. FOELLER, K. BIDWELL and A. LANDY, 1980 Site-specific recombination functions of bacteriophage λ: DNA sequence of the regulatory regions and overlapping structural genes for Int and Xis. Proc. Natl. Acad. Sci. USA **77:** 2482–2486.

HOOPES, B. C. and W. R. MCCLURE, 1985 A *c*II-dependent promoter is located within the *Q* gene of bacteriophage λ. Proc. Natl. Acad. Sci. USA **82:** 3134–3138.

IKEMURA, T., 1981a Correlation between the abundance of *Escherichia coli* transfer RNAs and the occurrence of the respective codons in its protein genes. J. Mol. Biol. **146:** 1–21.

IKEMURA, T., 1981b Correlation between the abundance of *Escherichia coli* transfer RNAs and the occurrence of the respective codons in its protein genes: a proposal for a synonymous codon choice that is optimal for the *E. coli* translation system. J. Mol. Biol. **151:** 389–409.

MAXAM, A and W. GILBERT, 1980 Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. **65:** 499–559.

PLACE, N., K. FIEN, M. E. MAHONEY, D. L. WULFF, Y. S. HO, C. DEBOUCK, M. ROSENBERG, M.-C. SHIH and G. N. GUSSIN, 1984 Mutations that alter the DNA binding site for the bacteriophage λ *c*II protein and affect the translation efficiency of the *c*II gene. J. Mol. Biol. **180:** 865–880.

POTEETE, A. R., K. HEHIR and R. T. SAUER, 1986 Bacteriophage P22 Cro protein: sequence, purification and properties. Biochemistry **25:** 251–256.

ROSENBERG, M. and D. COURT, 1979 Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:** 319–353.

ROSENBERG, M., D. COURT, H. SHIMATAKE, C. BRADY and D. L. WULFF, 1978 The relationship between function and DNA sequence in an intercistronic regulatory region in phage lambda. Nature **272:** 414–423.

SCHMEISSNER, U., D. COURT, H. SHIMATAKE and M. ROSENBERG, 1980 Promoter for the establishment of repressor synthesis in bacteriophage λ. Proc. Natl. Acad. Sci. USA **77:** 3191–3195.

SCHMEISSNER, U., D. COURT, K. MCKENNEY and M. ROSENBERG, 1981 Positively activated transcription of λ integrase gene initiates with UTP *in vivo*. Nature **292:** 173–175.

SCHWARTZ, E., 1980 Sequenzanalyse der DNA lambdoider Bakteriophagen: Gene and Signalstrukturen der Transkriptionskontrolle and der DNA-Replikation. Ph.D. thesis, University of Freiburg.

SCHWARZ, E., G. SCHERER, G. HOBOM and H. KOSSEL, 1978 Nucleotide sequence of *cro*, *c*II and part of the *O* gene in phage λ DNA. Nature **272:** 410–414.

SHIMATAKE, H. and M. ROSENBERG, 1981 Purified λ regulatory protein *c*II positively activates promoters for lysogenic development. Nature **292:** 128–132.

SHINE, J. and L. DALGARNO, 1974 The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:** 1342–1346.

SIEBENLIST, U., R. B. SIMPSON and W. GILBERT, 1980 *E. coli* RNA polymerase interacts homologously with two different promoters. Cell **20:** 269–281.

WULFF, D. L., 1976 Lambda *cin*-1, a new mutation which enhances lysogenization by bacteriophage lambda, and the genetic structure of the lambda *cy* region. Genetics **82:** 401–416.

WULFF, D. L., M. BEHER, S. IZUMI, J. BECK, M. MAHONEY, H. SHIMATAKE, C. BRADY, D. COURT and M. ROSENBERG, 1980 Structure and function of the *cy* control region of bacteriophage lambda. J. Mol. Biol. **138:** 209–230.

WULFF, D. L., M. MAHONEY, A. SHATZMAN and M. ROSENBERG, 1984 Mutational analysis of a regulatory region in bacteriophage λ that has overlapping signals for the initiation of transcription and translation. Proc. Natl. Acad. Sci. USA **81:** 555–559.

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