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

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Manuscript received January 20, 1986
Revised copy accepted December 15, 1986

ABSTRACT

The $\delta a5$ mutation, a C→T change at position $-43$ of the $\lambda$ $p_{RE}$ promoter, results in a twofold increase in $p_{RE}$ activity in vivo. Smaller increases in $p_{RE}$ activity are found for the $\delta a2$ mutation, a T→C change at position $-1$ of $p_{RE}$, and the $\delta a3$ 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 $\lambda$ $p_{RE}$ promoter is activated by binding of the $\lambda$ $cII$ 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'-TTGCNTTTGC-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 TTTG sequences (Ho, Wulff and Rosenberg 1983). Mutations in the $-35$ region that affect $p_{RE}$ function extend over most of the 5'-TTGCNTTTGC-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 TTTG 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(litn6 cts87 cro27 Pam3) and UC6183(litn6 cts87 cro27 cll306 Pam3) (Fien et al. 1984).

Phage: The $p_{RE}$ strain $crl1 cy3008$ is described in Place et al. (1984). The $\delta a2$ and $\delta a3$ 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 $crl1 cy3008$ was passed through an E. coli mutD mutant strain (Fowler, Degnen and Cox 1974) by the method of Enquist and Weisberg (1977).

DNA sequence determinations: DNA sequences of Sam7...
addition to retaining the original mutations, had sequence analysis showed that the mutant strain, in forms plaques with lightly turbid centers. DNA sequence through an E. strain 3'-sequence that is the principle determinant in the which has a mutation in each of the 5'-derivatives of that is described in FIEN et al. (1984). Derivatives of pKM2 with data on mutations: cl13104 alterations are from unpublished (1984), and the clz alterations are from WULFF and ROSENBERG (1983). The cl1 gene is transcribed from the pRE promoter, which lies several hundred nucleotides to its left. The line labeled "S.D." indicates the Shine and Dalgarno (1974) homology for the cl1 gene. Hyphens have been omitted from the sequence for clarity.

derivatives of ct1 cy3008 dya5 and λdya5 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 pRE promoter governs expression of the E. coli galK gene, is described in FIEN et al. (1984). Derivatives of pKM2 with various mutations in the pRE DNA fragment were constructed in an identical fashion, using the appropriate mutant phage as sources of pRE DNA.

Galactokinase measurements: Galactokinase was assayed at 30 min after shifting a lysogenic UC6183[pKM2] derivative from 32°, at which cl1 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 ct1 cy3008, which has a mutation in each of the 5'-TTGCN₆TTGC-3' repeats, was mutagenized by passage through an E. coli mutD mutator strain, and the strain ct1 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'-TTGCN₆TTGC-3' sequence that is the principle determinant in the -35 region for binding by cl1 protein and RNA polymerase (HO, WULFF and ROSENBERG 1983). The p_{RE} promoter and the cl1 structural gene overlap (SCHMEISSNER et al. 1980), and the dya5 mutation also results in a GAG→GAA change in codon 10 of the cl1 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 cl1 gene.

Suitable genetic crosses were performed to separate the dya5 mutation from its original genetic background, and a recombinant strain of genotype λdya5 was easily isolated because it forms plaques with more deeply turbid centers than λ+. The genotype of λdya5 was confirmed by DNA sequence analysis.

The strains λdya2 ct1 cy3008 and λdya3 ct1 cy3008 were also isolated following mutD mutagenesis, and differ from λdya5 ct1 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 cl1 gene. Both mutations decrease the potential secondary structure which may be formed by cl1 mRNA, and dya2 also changes the sequence of cl1 mRNA which is complementary to the 3'-end of 16 S mRNA from 5'-UAGGGA-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 cl1' mutations that are characterized by inefficient translation of cl1 mRNA (DUL, MAHONEY and WULFF 1987). Strains of genotype λdya2 and λdya3 have been constructed, and, unlike λdya5, 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, cl1 protein from a derepressed defective prophage activates galactokinase expression on the multicopy plasmid pKM2, in which the λ pRE promoter governs expression of the E. coli galK gene. We constructed pKM2 derivatives of λct1 cy3008 dya5 and λdya5, as well as pKM2 derivatives of the corresponding dya2 and dya3 strains, and the parental λct1 cy3008 strain. These plasmids were then introduced into the appropriate lysogenic host strain, and galactokinase activities were determined following activation of cl1 gene expression. A p_{RE} plasmid of genotype ct1 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
Effects of dya mutations on promoter activity

<table>
<thead>
<tr>
<th>Mutations on pRE fragment</th>
<th>Relative galactokinase activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>cll+ prophage</td>
</tr>
<tr>
<td>None (pRE*)</td>
<td>100</td>
</tr>
<tr>
<td>ctr1 cy3008</td>
<td>4</td>
</tr>
<tr>
<td>ctr1 cy3008 dya2</td>
<td>9</td>
</tr>
<tr>
<td>ctr1 cy3008 dya3</td>
<td>11</td>
</tr>
<tr>
<td>ctr1 cy3008 dya5</td>
<td>12</td>
</tr>
<tr>
<td>dya2</td>
<td>127</td>
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<tr>
<td>dya3</td>
<td>153</td>
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<tr>
<td>dya5</td>
<td>202</td>
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</tbody>
</table>

Derivatives of the plasmid pKM2 carrying the designated mutations were transformed into UC6183(Xint6 clts857 cro27 Pam3) and UC6183(Xint6 clts857 cro27 clt3067 Pam3) (Fien et al. 1984). Galactokinase was assayed 30 min after shifting a log phase culture from 32°C to 42°C, as described by Fien et al. (1984). A relative activity of 100 equals 440 units of galactokinase.

than a wild-type pRE+ plasmid, and plasmids with dya2 pRE and dya3 pRE genotypes show somewhat smaller increases over a wild-type pRE+ 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 cll function, we repeated the above galactokinase measurements with the plasmids in a cll- host strain of identical construction to the cll+ host, except for the cll3067 mutation, a AUG→ACG alteration in the initiation codon of the cll gene (Wulff et al. 1984). The results show that the promoter activities associated with the dya mutations are fully dependent upon cll function (Table 1).

Effects of other mutations in the -40 region of PRE on PRE function: Four additional mutations have been obtained in the -40 region of pRE as cll mutations in the region of overlap between the cll gene and pRE, and we wished to see if these had effects on PRe 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 pRE (cll3623, see Figure 2), a C→T change at position -44 (cll3104) and a G→A change at position -45 (cll3091). In contrast a T→C change at position -42 (cll3085) resulted in about a 40% decrease in promoter activity.

DISCUSSION

The conclusion that the dya2, dya3 and dya5 mutations confer increased activity to the pRE 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 cll protein. (2) The increased activities associated with the mutations are fully dependent upon cll protein (Table 1). (3) The cll protein binds to the 5'-TTGCN₆TTGC-3' sequence which flanks the -35 region of the pRE promoter. If a dya mutation were creating a new promoter with a different transcriptional start site, then the positioning of cll protein with respect to this new start site and to RNA polymerase would have to be different from that in every other cll-dependent promoter, an exceedingly unlikely possibility. That the increased transcription associated with the dya mutations is indeed caused by binding of cll protein to the identical binding site as in pRE is argued by (a) no other cll binding site is present in this region of DNA, and (b) the ctr1 and cy3008 mutations, which decrease the binding affinity of cll protein for the TTGCN₆TTGC 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 pRE, is outside of the region implicated for cll protein contact by methyl protection studies, and on the periphery of the region implicated for contact by RNA polymerase in the presence of cll protein (Ho, Wulff and Rosenberg 1983). Thus, in the presence of cll protein alone, the Gs at positions -56 and -57 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 cll 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 cll binding. In the presence of RNA polymerase and cll 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 cll 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 cll binding.
$\mu$-like promoters at position -43. In contrast, of the three mutations in the -35 region that results in a 40% decrease in promoter activity eliminates the agreement of XPRE with the consensus nucleotide for A. T (found in three of the five promoters) at this position. The mutational studies and the comparisons with pRE-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 pRE, 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 pRE is due to our ability to detect variants with small differences in promoter activity, rather than to a difference between pRE 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 pRE 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 pRE, 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 pRE promoter with the consensus sequence for pRE-like promoters, and one might have predicted decreased, rather than increased promoter activities.

The two dya5 plasmids were constructed by L. Bogard. We thank G. Pinchbeck for technical assistance and K. Schuff for typing the manuscript. This research was supported by grant GM28370 from the National Institutes of Health.

LITERATURE CITED


FIEN, K., A. Turck, I. Kang, S. Kielty, D. L. Wulff, K. Mc-
Lambda Promoters


Communicating editor: I. HERSKOWITZ