Transcriptional Terminator Is a Positive Regulatory Element in the Expression of the Escherichia coli crp Gene

Transcriptional Terminator Is a Positive Regulatory Element in the Expression of the Escherichia coli crp Gene

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Plasmids were constructed that contain deletions in the stem region of the presumed rho-independent terminator of the cloned crp gene of Escherichia coli. The level of cyclic AMP binding activity and the amount of CRP in cells harboring the deletion plasmids were found to be significantly lower than those in cells harboring the wild-type crp plasmid. Quantitative S1 assays indicated that the steady-state levels of crp mRNA were markedly reduced in cells harboring the deletion plasmids. Evidence was also presented to show that the crp mRNAs produced from deletion plasmids are less stable than that from the intact crp gene. In vitro transcription assays revealed that the putative crp terminator is indeed a rho-independent terminator. Using the galK expression system and Northern blot analysis we showed that the crp terminator is functional in vivo. Moreover it was shown that the deletion mutations in the stem region of the crp terminator cause a significant readthrough. We conclude that the 3′-flanking sequence of the crp gene acts to stabilize its own mRNA as well as to terminate transcription.

The cyclic AMP (cAMP) receptor protein (CRP) of Escherichia coli regulates the transcription of a number of genes by binding to specific DNA sites within or near promoters when complexed with cAMP (1, 2). Although the native CRP requires cAMP to exert its regulatory function, mutations in the crp gene are known to eliminate the requirement of cAMP for the expression of catabolite-sensitive operons. Such mutations have been referred to as crp* (3, 4) crp (5), crp* (6), sup (7), and csm (8, 9). The nucleotide sequence analysis of several crp* mutants revealed the sites of mutations responsible for the cAMP independence (CRP* phenotype) were within the crp structural genes, as expected (10–12). Thus the crp* mutants produce an altered protein which have amino acid substitution(s) in CRP polypeptide.

George and Melton (13) described a peculiar crp* mutation (csm) which was shown to have 1-base insertion in the stem region of the presumed rho-independent transcription terminator of this gene. In addition, they reported that plasmids

Plasmids-Plasmid pHA7 is a derivative of pBR322 carrying the Escherichia coli crp gene and has been described (21). Plasmid pYA7 previously called pHAT*3 (10), is the same as pHAT except it carries crp* encoding an altered CRP partially independent of cAMP. The crp* has a T to G transversion at 148th codon of CRP (10).

EXPERIMENTAL PROCEDURES

Bacterial Strains and Media—E. coli strains used were TP7860 (F- xyl argH1 his λ Δcrp), TP2399 (F- xyl leu argH1 Δcrp XT4 Δcrp), and R594 (gal I gal-2 lac rpsI, supF3 (17)). HY 100 was constructed from TP7860 by random insertion of Tn5 (18) and its Crp- phenotype was checked on MacConkey lactose and MacConkey maltose plates containing cAMP (1 mM) and kanamycin. Bacteria were routinely grown at 37 °C in LB medium (19). For the assay of gelactokinase M56 medium (20) was used. MacConkey agar containing 1% of lactose or maltose were used to test fermentation of sugars. When used, antibiotics were added to the medium at the following concentrations: ampicillin, 50 μg/ml; kanamycin, 25 μg/ml.

Enzymes and Chemicals—Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo and Boehringer Mannheim. Kits for dideoxy sequencing, nick translation, and end labeling were obtained from Boehringer Mannheim. [α-32P]UTP (>5000 Ci/mmol), [α-32P]dCTP, and [3H]cAMP (37 Ci/mmol) were purchased from Amersham. Other chemicals were obtained from standard commercial sources.

Plasmids-Plasmid pHAT is a derivative of pBR322 carrying the E. coli crp gene and has been described (21). Plasmid pYA7, previously called pHAT*3 (10), is the same as pHAT except it carries crp* encoding an altered CRP partially independent of cAMP. The crp* has a T to G transversion at 148th codon of CRP (10).

Plasmids pHATD1 and pHATD2, derivatives of pHAT, contain a 4- and 49-bp deletions in the 3′-flanking region of the crp gene, respectively. To construct pHATD1, plasmid pHAT was cleaved with BshHII located in the stem region of the presumed terminator of crp (Figs. 1 and 2), treated with S1 nuclease and recircularized by ligation. The ligation mixture was used to transform TP7860 and the transformants were selected on MacConkey lactose plates containing 4-bp deletion in the same stem region or a larger deletion located 164 bp downstream from the translation stop codon of the crp gene mimics the csm mutation (13, 14). Thus they concluded that mutations in the 3′-flanking region of the crp gene rendered CRP capable of promoting the transcription of catabolite-sensitive genes in the absence of exogenous cAMP. The question of how mutations located outside the structural gene could allow CRP to function independently of cAMP prompted us to investigate further the nature of these mutations.

We constructed plasmids with the crp gene containing deletions within the inverted repeat sequences in its presumable rho-independent terminator. By introducing mutant plasmids into E. coli cells we investigated the effects of the deletions on crp expression. Our results indicated that deletions in the stem of the 3′-flanking region of crp do not impact cAMP independent phenotype. Instead we found that the deletion mutations markedly reduce the expression of crp gene. This reduction in crp expression was shown to be due to the decreased stability of crp mRNA. We also found that the presumable rho-independent terminator is functional to terminate transcription both in vitro and in vivo and the deletions in the stem region significantly reduce the termination function.
ampicillin. Plasmids were isolated and tested for deletions by restriction analyses. To construct pHA7/D2, the BstHI-cleaved pHA7 was partially digested with EcoRV. The digests were fractionated by electrophoresis on a 0.8% agarose gel. The large DNA fragment which lacked the portion between BssHII and EcoRV sites (Fig. 1) was extracted from the gel. The fragment was treated with S1 nuclease and renatured by ligatation. Transformation of TP7800 were obtained and the plasmids were analyzed as mentioned above. Similarly, plasmids pYAD1 and pYAD2, which carry the crp* gene containing deletions in its 3‘-flanking region, were constructed from pYAT7. The precise structure of deletion plasmids was verified by DNA sequencing.

**Plasmid pLT1 (Fig. 6)** was constructed by inserting the 140-bp Sau3A-TaqI fragment containing the putative crp terminator region (Fig. 1) between the BamHI and AccI sites of pUC18 (23). The Sau3A-TaqI fragment was derived from pHA7. Similarly, plasmids pLTD1 and pLTD2 were constructed by using the sau3A-TaqI fragments derived from pHA7D1 and pHA7D2, respectively.

Plasmid pKG100 is a terminator-cloning vector (24). The 333-bp HaeIII fragment containing the putative crp terminator was isolated from pHA7 and ligated into the Smal site of pKG100 (Fig. 8). One clone with the “native” orientation was referred to as pTK76. Similarly, the HaeIII fragments from pHA7D1 and pHA7D2 were inserted into the Smal site of pKG100 to construct plasmids pTD11 and pTD24, respectively.

Plasmid DNA and DNA fragments were prepared, analyzed, and manipulated by standard procedures (19).

**Preparation of Cell Extracts**—Cell extracts were prepared essentially as described by Lupaki et al. (25). Briefly, cells were grown in LB medium (30 ml) at 37 °C to an A660 of 0.6. After centrifugation, the cells were washed with 1 ml of 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 2.5 μg of bovine serum albumin, 200 ng of RNA polymerase, and 20 ng of DNA fragment in a total volume of 30 μl. Where indicated, 0.1 mM cAMP and 20 ng of CRP were added to the reaction mixture. Following incubation for 5 min at 37 °C, 3 μl of a ribonuclease mixture containing 0.1 mM [α-32P]UTP (5 μCi) and 1 mM each of ATP, CTP, and GTP were added. After 15 min of incubation at 37 °C, transcription was terminated by adding 50 μl of phenol, 30 μl of 0.6 mM sodium acetate (pH 5.5), 20 mM EDTA, and 100 μg/ml of tRNA. The products were precipitated with ethanol and analyzed on an 8% polyacrylamide gel in 8 M urea gels. CRP was purified from E. coli strain PP47 harboring pHA7 (21) by the procedure of Ellen et al. (30). RNA polymerase was purified from E. coli strain W3350 essentially according to the method of Fukuda et al. (31).

**RESULTS**

**Construction of Plasmids Containing Deletions in the Stem Region**—The 3‘-flanking region of the crp gene has the characteristics of a rho-independent terminator, with inverted repeat sequences of the 3‘-flanking region of the crp gene are partly deleted in these plasmids.

**Expression of lac in Strains Harboring the Deletion Plasmids**—George and Melton (13) observed that a plasmid carrying a 450-bp deletion in the crp region is polarized by induction of the lac operon. As shown in Fig. 1, the inserted repeat sequences of the 3‘-flanking region of the crp gene are partly deleted in these plasmids.

![Fig. 1. Restriction map of the crp gene and DNA fragments used in this study](image)

The partial restriction map of the crp gene in pHA7 is shown. Plasmid pHA7 contains a 950-bp DNA region encoding CRP (21). The open bar represents the crp structural gene. The arrow indicates the start site and direction of transcription. DNA fragments used are shown below the restriction map.
Regulation of crp Expression by Terminator

The nucleotide sequence of the 3'-flanking region of the wild-type and mutant crp genes is shown in Table I. The inverted repeat sequences are shown by horizontal arrows. The dotted lines represent the deletions. The translation termination codon (TAA) is underlined. The arrowheads represent the endpoints of transcription determined by S1 nuclease assay.

Table I

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Fermentation of lactose*</th>
<th>β-Galactosidase activity*</th>
<th>cAMP binding activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pHA7</td>
<td>53</td>
<td>1514</td>
<td>3983</td>
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<td>pHA7D1</td>
<td>-</td>
<td>377</td>
<td>4130</td>
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<tr>
<td>pHA7D2</td>
<td>-</td>
<td>299</td>
<td>4193</td>
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<tr>
<td>pYA7</td>
<td>+</td>
<td>608</td>
<td>3926</td>
</tr>
<tr>
<td>pYA7D1</td>
<td>±</td>
<td>122</td>
<td>3886</td>
</tr>
<tr>
<td>pYA7D2</td>
<td>±</td>
<td>130</td>
<td>3965</td>
</tr>
</tbody>
</table>

*Fermentation responses of TP7860 (ΔcyA) or HY100 (ΔcyA crp-) harboring plasmids were examined on MacConkey agar plates containing 1% lactose and 50 μg of ampicillin, after 16 h incubation at 37°C; +, red; -, white with red center; --, white.

1 HY100 harboring plasmids were grown in LB medium containing 50 μg/ml of ampicillin, 1 mM isoprropyl-β-D-thiogalactopyranoside, and indicated amount of cAMP. The β-galactosidase activities were expressed in Miller units (20).

2 The cAMP binding activities were measured with cell extracts of TP2393 harboring plasmids. The values are relative to the β-lactamase activities of the same extracts. No significant variation in β-lactamase activities was observed for cells harboring these plasmids.

3 Cell growth was inhibited.

Deletions in Stem Region Reduce the Production of CRP—

To determine directly the levels of CRP protein in cells, we analyzed total proteins of TP2339 harboring plasmids by SDS-polyacrylamide gel electrophoresis. Since plasmid pHA7 overproduce CRP, the CRP polypeptide of 23,619 daltons (21) can be easily detected on a gel stained with Coomassie Brilliant Blue. The data clearly show a significant reduction in CRP levels when cells are carrying either pHA7D1 or pHA7D2 (Fig. 3). Similar results were obtained for the proteins from cells carrying pYA7 series plasmids (data not shown). We conclude that the introduction of deletion in the stem region of the presumed terminator of crp markedly reduce the production of CRP in cells.

Steady-state Levels of crp mRNA—The effect of the deletion mutations on the crp expression was further investigated by assaying for the steady-state levels of crp mRNA in cells. Total RNAs from TP2339 harboring pHA7, pHA7D1, and pHA7D2 were analyzed by a quantitative S1 nuclease assay, using the lower strand of the 488-bp HpaII fragment 3' labeled at its 5' end as a DNA probe. In pHA7 the crp gene is transcribed from one of the bla promoters located immediately upstream of the HindIII site (21). When RNAs from cells harboring pHA7 were analyzed by S1 assay, an S1-resistant DNA band of about 380 bases which corresponds to the difference in CRP levels in cells.

We also investigated the properties of cells carrying pYA7 series plasmids. TP7860 or HY100 harboring the pYA7 exhibited a Lac + phenotype, since these cells contain a cAMP-independent form of CRP (CRP*3) derived from pYA7. However the transformants harboring either pYA7D1 or pYA7D2 exhibited a weak Lac + phenotype. In fact the β-galactosidase levels in HY100 harboring deletion plasmids were reduced to about 30% of the level found in strain harboring pYA7. All of these results suggest that the introduction of deletions in the stem region of the 3'-flanking region of crp significantly affect the expression of CRP.

Deletions in Stem Region Reduce the Production of CRP—

In order to examine the effect of deletions on the expression of crp, we introduced plasmids into Δcrp ΔcyA cells (TP2339) and determined cAMP binding activities of the transformants. The relative cAMP binding activities, normalized to β-lactamase activities, are shown in Table I. Strain TP2339 harboring a control plasmid pBR322 exhibits essentially no cAMP binding activity, whereas the levels of cAMP binding activity in cells harboring either pHA7 or pYA7 are remarkable. It is noteworthy that the activity in cells harboring pHA7 is significantly higher than that in cells harboring pYA7. This is because pYA7 produces CRP*3 that has an increased affinity for cAMP. When we introduced the deletion plasmids in TP2339, the levels of cAMP binding activity of cells markedly decreased compared with that of cells harboring the respective parent plasmid. This suggests that the deletion in the presumed terminator result in the reduction of CRP expression.

To determine directly the levels of CRP protein in cells, we analyzed total proteins of TP2339 harboring plasmids by SDS-polyacrylamide gel electrophoresis. Since plasmid pHA7 overproduce CRP, the CRP polypeptide of 23,619 daltons (21) can be easily detected on a gel stained with Coomassie Brilliant Blue. The data clearly show a significant reduction in CRP levels when cells are carrying either pHA7D1 or pHA7D2 (Fig. 3). Similar results were obtained for the proteins from cells carrying pYA7 series plasmids (data not shown). We conclude that the introduction of deletion in the stem region of the presumed terminator of crp markedly reduce the production of CRP in cells.

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crp RNA was obtained (Fig. 4, lane 2). When the RNAs from cells harboring either pHATD1 or pHATD2 were used, the intensity of the S1-resistant DNA band was reduced to about one-third compared with the DNA protected by RNA from cells harboring pHAT (Fig. 4, lanes 3 and 4). The data indicate that the introduction of the deletions in the stem region cause a significant decrease in the level of the crp RNA.

The Presumed Terminator Stabilize crp mRNA—We postulated that the lower levels of crp expression from deletion plasmids may be due to the decreased stability of RNA. To test this hypothesis, we have determined the rate of decay of the crp RNAs derived from the wild-type and mutant genes. Cells harboring plasmids pHAT or pHATD1 were grown to exponential phase and rifampicin was added to prevent further initiation of transcription. Then cellular RNAs were isolated at various times after the addition of rifampicin. These RNAs were subjected to the quantitative S1 assay (Fig. 5). To estimate the half-lives of crp RNAs, the intensity of the S1-resistant DNA band in each lane was quantified by densitometric scanning. The results are also shown in Fig. 5. The half-lives of crp RNAs produced from pHAT, pHATD1, and pHATD2 were calculated to be 3.6, 1.4, and 1.4 min, respectively. It is apparent that the removal of the stem region renders the crp RNA less stable. Thus the decreased stability of RNA can account at least partly for the reduction of the crp expression from the deletion plasmids. In other words, the presumed rho-independent terminator is acting as a positive regulator to stabilize its own mRNA.

The Presumed Terminator Functions In Vitro—Is the presumed rho-independent terminator of the crp gene a real terminator for transcription? To examine this, we have conducted in vitro transcription assays. The 140-bp Sau3AI-Taq1 fragment containing the presumed crp terminator region derived from pHAT was cloned between the BamHI and AccI sites of pUC18 to construct pLT1 (Fig. 6). In pLT1 the putative crp terminator is located just downstream of the lac promoter. Similarly, pLTD1 and pLTD2 which contain deletions in the presumed crp terminator were constructed by using the Sau3AI-Taq1 fragments from pHATD1 and pHATD2, respectively. For in vitro transcription assays the PouII-HindIII and PouII-PouII fragments were prepared from these fusion plasmids. The lac promoter, that is dependent on CRP-CAMP, is the only functional promoter existed on these fragments.

When the 357-bp PouII-HindIII fragment prepared from pLTI was transcribed in the presence of CAMP-CRP, two prominent transcripts (RNA1 and RNA2) were observed (Fig. 7, lane 3). These RNAs were not made in the absence of CRP-CAMP (Fig. 7, lane 2), indicating that two RNAs originate at the lac promoter. The size of RNA1 corresponds to the distance (159 bases) between the transcription start site of the lac promoter and the first T just after the G-C-rich dyad symmetry of the crp gene, whereas the size of RNA2 is in good agreement with the distance (236 bases) between the lac start site and the HindIII end of the fragment. This suggests that the shorter RNA1 is the product terminated at the crp terminator while the longer one (RNA2) is the readthrough product. If this were correct, one might expect that the 450-bp PouII-PouII fragment from pLTI1 also produces RNA1 along with a readthrough RNA of about 330 bases in the presence of CRP-CAMP. The result shown in Fig. 7, lane 4, is perfectly consistent with this prediction, indicating that the cloned 3'-flanking segment of the crp gene is functional to terminate transcription in vitro. Based on the relative intensities and sizes of RNA bands, we estimated that termination at the crp terminator is approximately 80% effective in our in vitro system. We also analyzed RNA transcripts produced from DNA fragments containing the mutant terminators. The major transcript of the PouII-PouII fragment from pLTD1 was a readthrough RNA of 325 bases, although the terminator was still capable to terminate transcription to some extent (Fig. 7, lane 5). However the PouII-PouII fragment derived from pLTD2 only produced a readthrough RNA of 278 bases (Fig. 7, lane 6). Taken together, we conclude that the putative crp terminator acts efficiently as a rho-independent terminator in vitro and the mutations in the stem region reduce markedly the termination function.

Action of the crp Terminator in Vivo—In order to test the function of the crp terminator and to study the effect of the mutations in the stem region on transcription termination, we have cloned DNA fragments containing the crp terminator derived from pHAT, pHATD1, and pHATD2 between the gal promoter and galK gene of pKG1000 to create pT6, pT1D1, and pT2D4, respectively (Fig. 8). These plasmids were introduced into cells of R594 and galactokinase activities of the cells were determined. In these plasmids, the expression of galactokinase gene is dependent on the termination signal inserted. As shown in Table II, the galactokinase level in cells
carrying pT6 is 20-fold lower than that in cells carrying the control plasmid pKG100. This means that the crp terminator functions in vivo. Moreover, less efficient terminations were observed for pTD11 and pTD24, indicating the deletions in the stem region of the crp impair the function of the terminator to cause a significant readthrough.

To investigate further the function of the rho-independent terminator in vivo, we examined the crp mRNAs produced from a series of plasmids by Northern blot analysis. In this experiment the nick-translated 736-bp HindIII-EcoRV fragment was used as a DNA probe. Total cellular RNAs from TP2339 harboring pHA7 gave a strong hybridization signal which corresponds to the crp RNA (Fig. 9, lane 1). The size of crp transcript was estimated to be about 0.7 kilobase. This implies that the rho-independent terminator of crp gene on the chromosome DNA is also functional to terminate the crp transcription. To determine more precisely the termination points, we conducted an S1 mapping experiment using the 450-bp HpaII-EcoRI fragment labeled at its HpaII 3' end with 32P as a DNA probe. The major termination sites (shown in Fig. 2) were mapped in the T-rich region immediately downstream of the inverted repeat sequences (data not shown). When RNAs from cells harboring either pHA7D1 or pHA7D2 were analyzed by Northern blot analysis, longer and heterogeneous crp RNAs were detected (Fig. 9, lanes 2 and 3). This result is consistent with that of the galactokinase assay shown in Table II. In addition the data show that the levels of crp RNA produced from pHA7D1 or pHA7D2 are significantly lower than those from pHA7, indicating again that the expression of the crp is markedly reduced by the introduction of deletions in the terminator region.

**DISCUSSION**

The issue we addressed in this paper is the effects of mutations in the crp terminator on the expression of the crp gene. We generated deletions in the terminator region of the cloned crp gene on a plasmid. Then we investigated the properties of these mutant plasmids in cells. The major conclusions of the present study are as follows. 1) The introduction of deletion mutations in the stem region of the presumed terminator of crp gene does not allow CRP to activate the luc expression in the absence of CAMP. 2) The steady-state levels of CRP as well as crp mRNA in cells harboring the deletion plasmids are significantly lower than those in cells harboring the wild-type crp plasmid. 3) The lower levels of crp expression in the deletion mutants are primarily due to the decreased stability of crp RNA. 4) The presumed rho-independent terminator of the crp gene is indeed functional to terminate
Regulation of crp Expression by Terminator

**Fig. 6. Diagram of plasmids carrying the crp terminator fused to the lac promoter.** Plasmids pLT1, pLTD1, and pLTD2 contain the crp terminator region derived from pHA7, pHA7D1, and pHA7D2, respectively. Construction of plasmids are described under "Experimental Procedures." In these plasmids the crp terminator is located near downstream of the lac promoter. The relevant restriction sites are indicated and numbered relative to the lac transcription initiation site that is shown by an open triangle. The bold bars represent the cloned fragments containing the crp terminator region. The open boxes represent the extents of deletions. Wavy lines represent RNA transcripts produced the 357-bp PvuII-HindIII and the 450-bp PvuII-PvuII fragments of pLT1 (Fig. 1). These transcripts originate at the lac promoter that is activated by CRP-cAMP. RNA1 is the transcript terminated at the crp terminator. RNA2 and RNA3 are the readthrough transcripts.

**Fig. 7. In vitro transcriptions of DNA fragments containing the crp terminator preceded by the lac promoter.** The PvuII-HindIII fragment from pLT1 was transcribed in the presence (lane 3) and absence (lane 2) of CRP-cAMP. The PvuII-PvuII fragments derived from pLT1 (lane 5), pLTD1 (lane 5), and pLTD2 (lane 6) were transcribed in the presence of CRP-cAMP. RNAs1–5 are the lac promoted transcripts: RNA1, transcript terminated at the crp terminator; RNAs2–5, readthrough transcripts. Lane 1 shows the mixture of in vitro transcriptions of DNA fragments containing the crp promoter (Ref. 29 and H. Alba, A. Hanamura, and H. Yamano, unpublished data) as RNA size markers. The numbers on the left represent base lengths. Transcription reactions were carried out as described under "Experimental Procedures."

transcription both in vitro and in vivo. 5) The deletions in the stem region markedly reduce the termination efficiency.

Our results are inconsistent with those of George and Melton (13) who showed in their studies that the insertion or deletion of base-pairs in the stem region of the crp terminator allow the expression of catabolite-sensitive operons in the absence of cAMP. It is particularly noteworthy that one of our deletion plasmids, pHA7D1, appears to be the same as pGM459 which was reported to confer the cAMP-independent phenotype. The reasons for this apparent discrepancy are unclear. One explanation is that the csm mutation and pGM459 may have a second mutation in the crp structural gene which is responsible for the CRP* phenotype. Alternatively, the cya crp strain (CA8445) used by George and Melton (13) may allow the plasmids containing deletions in the crp terminator to exhibit CRP* phenotype by unknown mechanisms.

The rate of RNA degradation is now recognized to play an important role in the control of gene expression (33, 34). A number of studies have shown that the stability of mRNA in bacteria is controlled by structures of RNA transcripts. Among these, dyad symmetry sequences in several operons are known to play some roles in stabilization of mRNA, presumably by encoding segments that form exonuclease-resistant stem structure. For example, the repetitive extragenic palindromic sequences, which exist in a number of E. coli operons, have been shown to stabilize upstream mRNA segments (35). The intercistronic inverted repeat sequences
TABLE II

Efficiency of the wild-type and mutant terminators of the crp

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Galactokinase</th>
<th>Termination %</th>
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<tbody>
<tr>
<td>pBR322</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>pKG100</td>
<td>315</td>
<td>0</td>
</tr>
<tr>
<td>pT6</td>
<td>14</td>
<td>96</td>
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<tr>
<td>pTD11</td>
<td>45</td>
<td>86</td>
</tr>
<tr>
<td>pTD24</td>
<td>178</td>
<td>43</td>
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</table>

Regulation of crp Expression by Terminator

Assays were done in R594 cells. The galactokinase units are expressed as nanomoles of galactose phosphorylated per min/A^260. The percent termination is calculated from the ratio of the galactokinase units of each plasmid to the galactokinase units of pKG100.

in the E. coli rpsU-dnaG-rpoD operon (36) and the rxcA operon of Rhodopseudomonas capsulata (37) also appears to confer stability to their upstream RNAs. The rho-independent transcription termination signal, which consist of GC-rich inverted repeat sequences followed by a run of Ts, are also known to participate in gene regulation through mRNA stability. A well known example for this is the repressor in lambda phage (38), in which the rho-independent terminator region stabilizes the expression of the int gene from mRNA degradation. The terminators of Bacillus thuringiensis crystal protein gene (39) and a bacteriophage 6X174 (40) were shown directly to confer stability to the transcripts. In addition the rho-independent terminators of trp (41) and gba (42) of E. coli seem to play a role in stabilizing mRNA, although no experiments with mRNA stability have been carried out in these cases.

In this paper we presented evidence that the rho-independ-