Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3233–3237, May 1986
Biochemistry

Identification of a positive retroregulator that stabilizes mRNAs in bacteria

(*cry* gene/mRNA metabolism/gene expression/transcription terminator)

HING C. WONG AND SHING CHANG

Department of Microbial Genetics, Cetus Corporation, Emeryville, CA 94608

Communicated by Hamilton O. Smith, January 13, 1986

ABSTRACT **A positive retroregulator that enhances the expression of an upstream gene(s) has been identified. It resides within a 381-base pair (bp) restriction fragment containing the transcriptional terminator of the crystal protein (*cry*) gene from *Bacillus thuringiensis* vs. *Kurstaki* HD-1. This fragment was fused to the distal ends of either the penicillinase (*penP*) gene of *Bacillus licheniformis* or the interleukin 2 cDNA from the human Jurkat cell line. In both cases, the half-lives of the mRNAs derived from the fusion genes were increased from ≈2 to 6 min in both *Escherichia coli* and *Bacillus subtilis*. Synthesis of the corresponding polypeptides in the bacteria carrying the fusion genes was also increased correspondingly. The enhancement of expression of the upstream genes was independent of the insertional orientation of the distal *cry* terminator fragment. Deletion analysis showed that the locus conferring the enhancing activity coincided with the terminator sequence and was located within a 89-bp fragment that includes an inverted repeat, the 19-bp upstream-, and the 27-bp downstream-flanking sequences. We propose that transcription of the retroregulator sequence leads to the incorporation of the corresponding stem-and-loop structure at the 3′ end of the mRNA; the presence of this structure protects the mRNAs from exonucleolytic degradation from the 3′ end and, thereby, increases the mRNA half-life and enhances protein synthesis of the target genes.**

An unusual mechanism of gene regulation has been identified through studies on the regulation of the coliphage λ integrase (*int*) gene. It has been shown that the *cis*-acting element *sib*, located distal to the *int* gene, negatively regulates the expression of the *int* gene (1, 2). This type of regulation of a target gene by a *cis*-acting element distal to it, i.e., *sib*-controlled *int* expression, has been termed retroregulation (3).

We report here the discovery of a positive retroregulatory mechanism for the control of the expression of the gene encoding the insecticidal parasporal crystal protein (*cry*) of *Bacillus thuringiensis*. Earlier work on the structural analysis of the cloned *cry* gene (4) suggested that a sequence in the vicinity of the transcriptional terminator of the *cry* gene may positively regulate the *cry* gene expression. It was observed that the insertion of a Tn*5* transposon at the 3′ end of the cloned *cry* gene resulted in a significant reduction of cry production in *Escherichia coli*. We have employed molecular cloning techniques to investigate the role of the 3′-end noncoding region of the *cry* gene in regulation of gene expression. The data presented here indicate that the sequence containing the transcriptional terminator of the *cry* gene functions as a positive retroregulator in controlling gene expression in both *Bacillus subtilis* and *E. coli*.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Bacterial and Bacteriophage Strains and Plasmids. The construction of bacteriophage M13 derivatives and *E. coli* strain JM103 (5) and *B. subtilis* strain PSL1 (6) have been described. CS412 (*hsdR, pro, leu, strA*) is derived from C600. *E. coli* strains N99 (*galK, sup°, str*^{r}) and MA166 (*galK, sup°, str*^{r}, *glyA*::Tn*5*, *rnc*105) were kindly provided by H. Echols. Plasmids and double-stranded DNA of M13 phages were prepared according to Wong *et al.* (4). Plasmids pES1 (7), pLP1201 (8), and pLW1 (9) have been described. Plasmid pSYC667 was constructed from plasmid pSYC660 (10) by eliminating the second *Bam*HI site located downstream from the *penP* gene. Plasmid pSYC795 is a derivative of plasmid pSYC423 (11) except that it contains the S_{27} (serine substitution at position 27) allele of the *penP* gene.

RNA Isolation and Filter Hybridization. *E. coli* and *B. subtilis* strains carrying various plasmids were grown in L broth at 37°C with shaking. When the cultures reached the mid-logarithmic phase of growth (A_{600} = 0.7), rifampicin was added to 1 mg/ml to block further initiation of transcription. Samples (7 ml) were then withdrawn from the cultures at 1-min intervals for RNA extraction. Total cellular RNA from *B. subtilis* and *E. coli* cells was extracted and purified as described (4, 12).

The ≈1.0-kilobase *Eco*RI–*Cla* I fragment of PSYC795 carrying the 5′ portion of the *penP* gene was cloned into the *Sma* I–*Eco*RI sites of the replicative form DNA from phage M13mp11 after the *Cla* I terminus was blunt-ended by the polymerase repair reaction. The interleukin 2 (IL-2) cDNA was isolated as a *Hin*dIII–*Stu* I fragment from pLW1 and inserted into the *Hin*dIII–*Sma* I sites of M13mp10. In both cases, the genomes of the recombinant phages carry the sense strand (i.e., the strand that is complementary to the mRNAs) of the *penP* or the *IL-2* genes. To prepare labeled single-stranded DNA probes, the single-stranded DNAs from these recombinant phages were isolated (5) and digested with DNase I (0.05 μg/μg of DNA) for 20 min at 37°C. After phenol extractions, the single-stranded linear DNA fragments were dephosphorylated with bacterial alkaline phosphatase and labeled with [γ-^{32}P]ATP by polynucleotide kinase. The specific radioactivity was ≈3 × 10^6 dpm/μg of DNA.

RNAs were immobilized on 82-mm nitrocellulose filters (grade BA 85, Schleicher & Schuell). Prehybridization of the filters was carried out by the procedure of Woo (13). Processed filters were then hybridized with the specific probes (20 μg per filter) as described (14). To ensure that an excess of DNA probes was present in the hybridization solution, the experiment employed several filters with different amounts of adsorbed, immobilized RNA (25, 50, and 100 μg per sample).

Abbreviations: IL-2, interleukin 2; bp, base pair(s).

S1 Nuclease Mapping and DNA Sequencing. The derivative of M13mp9NP3 carrying a *Bgl* II restriction site located 87 base pairs (bp) upstream from the inverted repeat sequence in the *cry* terminator fragment was used for preparing the DNA probe. The 161-bp *Bgl* II–*Eco*RI fragment isolated from this recombinant M13 phage replicative form DNA was labeled at the 3′ end with [α-^{32}P]dATP using DNA polymerase I large fragment. S1 nuclease mapping of *in vivo* RNAs was performed essentially as described (4). Determination of nucleotide sequence by the chemical degradation method was performed as described (15).

Other Techniques. Methods for protein determination (16), for transformation and preparation of competent cells of *E. coli* (17) and *B. subtilis* (18), and for $NaDodSO_4$/polyacrylamide gel electrophoresis of protein samples (19) were carried out essentially as described. Penicillinase activity was determined as described (20) using logarithmic-phase culture. Specific activity of penicillinase was expressed as micromoles of pyridine-2-azo-*p*-dimethylaniline hydrolyzed per minute per gram of protein at 25°C. IL-2 expression was induced and assayed as described (9), except *B. subtilis* cells were grown in broth, and the constitutive level of IL-2 was determined from the cell extracts. Other methods used for DNA manipulations were as described by Maniatis *et al.* (17).

RESULTS

Structure of the Transcriptional-Terminator Region of the *cry* Gene. The restriction map of the recombinant plasmid pES1 containing the cloned *cry* gene from *B. thuringiensis* is shown in Fig. 1*A*. Transcription of the *cry* gene originates at the site about 350 bp to the left of the fourth *Eco*RI site and terminates 358 bp to the right of the third *Pvu* II site (refs. 4 and 21; Fig. 1*A*). The nucleotide sequence of the *Pvu* II–*Nde* I restriction fragment carrying the transcriptional terminator of *cry* (Fig. 1*B*) contains an inverted repeat sequence on the 5′ proximal side of the *Nde* I site. The transcript made from this region can potentially form a stem-and-loop structure. This putative stem-and-loop structure (Fig. 1*C*) is relatively rich in G+C content with a predicted ΔG value of −30.4 kcal, as calculated by the rules of Tinoco *et al.* (22).

To facilitate the construction of transcriptional fusions between the *cry* terminator-containing fragment and heterologous genes, the 381-bp *Pvu* II–*Nde* I fragment was subcloned into the *Sma* I site of the bacteriophage M13mp9 in the orientation such that the *Pvu* II site was adjacent to the *Bam*HI site in the polylinker sequence of the phage genome. The resulting recombinant phage was designated M13mp9-NP3; it contained unique *Eco*RI and *Bam*HI sites flanking the insert.

Transcriptional Fusion Between the *penP* Gene and the *cry* Terminator Fragment. The recombinant plasmid pSYC667 carrying the penicillinase gene from *B. licheniformis* was employed for this study (Fig. 2*A*). This *penP* gene contains a point mutation (S_{27}) that blocks the maturation pathway leading to the formation of penicillinase in the lipoprotein form but still allows the synthesis and secretion of the soluble forms of mature penicillinase (10). S1 mapping data (not shown) indicated that penP mRNA, isolated from *B. subtilis* cells containing plasmid pSYC667, terminates in the region located downstream from the *Bcl* I site as is expected from studying the sequence data (23).

To construct a transcriptional fusion of the *penP* coding sequence and the *cry* terminator-containing fragment, we replaced the *Bcl* I–*Nru* I fragment on pSYC667 containing the *penP* terminator with the *Bam*HI–*Eco*RI fragment from the phage M13mp9NP3 replicative form DNA that carried the *cry* terminator (Fig. 2*A*). This generated the bifunctional plasmid pHCW-A3. Similar to the parental pSYC667 plasmid, it replicates in *E. coli* and *B. subtilis* and confers resistance to

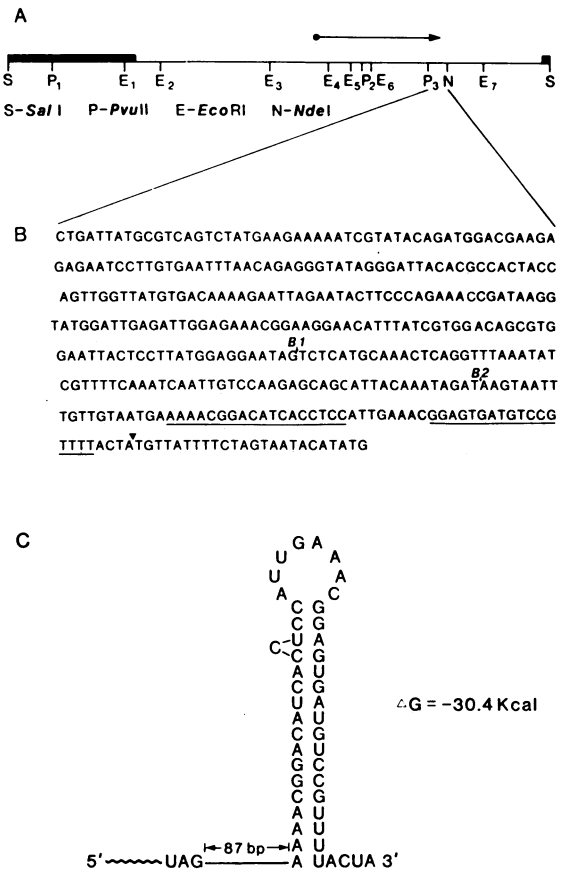

FIG. 1. Structure of the crystal protein (*cry*) gene and the potential secondary structure at the 3′ end of its mRNA. (*A*) Physical map of the *cry* gene in plasmid pES1. The horizontal arrow indicates the coding region and the transcriptional direction of the *cry* gene. The thick lines represent the pBR322-derived sequences. (*B*) The DNA sequence of the distal portion of the *cry* gene. The symbol ▼ represents the transcriptional stop site. The inverted repeat sequences are underlined. B1 and B2 indicate locations where new *Bgl* II sites were generated by insertion of an A or CT at the sites, respectively, using oligonucleotide-directed site-specific mutagenesis. (*C*) Potential secondary structure at the 3′ end of the cry mRNA.

chloramphenicol in these hosts. As shown in Table 1, the *E. coli* and *B. subtilis* strains containing the pHCW-A3 plasmid accumulated 2.6- to 5.3-fold greater amounts of penicillinase than did the two strains carrying the parental plasmid pSYC667. This was further confirmed by directly measuring the penicillinase protein either in the respective *E. coli* cell extracts or in the culture supernatants of the *B. subtilis* strains (data not shown). To rule out the possibility that the differences in *penP* expression level were caused by differences in plasmid copy number, we determined the enzyme activity for the chloramphenicol acetyltransferase encoded by these plasmids. Within the same *E. coli* or *B. subtilis* host strain, these two plasmids specified similar levels of chloramphenicol acetyltransferase activity. Thus the enhanced expression of the *penP* gene in strains carrying plasmid pHCW-A3 resulted directly from the replacement of the *penP* terminator with the *cry* terminator fragment. We conclude from these data that the *cry* terminator-containing fragment positively regulates the expression of the immediately proximal penicillinase gene.

The Transcriptional Terminator of the *cry* Gene Functions in Both *E. coli* and *B. subtilis*. To ascertain that the *cry*-derived sequence in pHCW-A3 is cotranscribed with the *penP* gene, we determined the transcriptional stop sites of the penP mRNAs produced from the plasmid pHCW-A3 in *E. coli* and *B. subtilis*. As shown in Fig. 3, *cry*-specific RNA is present

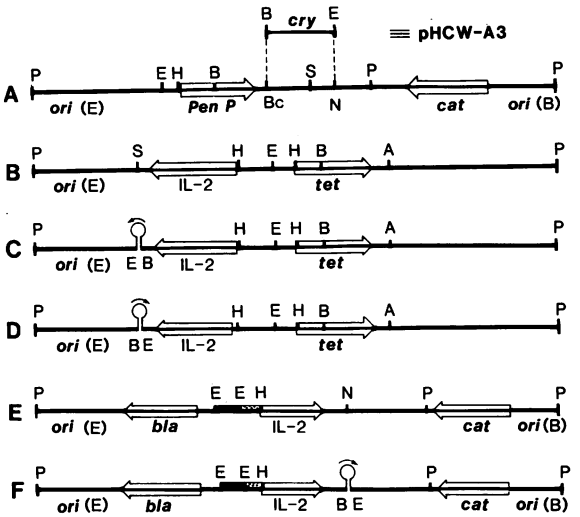

FIG. 2. The construction of a transcriptional fusion of the *penP* and *IL-2* genes to the *cry* gene transcriptional terminator. (*A*) Plasmids pSYC667 and pHCW-A3. (*B–D*) *E. coli* plasmids, pLW1 (*B*), pHCW701 (*C*), and pHCW702 (*D*), carrying the transcriptional fusions of the human IL-2 cDNA sequence and the *cry* transcriptional terminator. (*E* and *F*) Structures of the IL-2 expression plasmids pHCW300 (*E*) and pHCW301 (*F*). The locations of the coding regions, the transcriptional directions of the penicillinase (*penP*), the chloramphenicol acetyltransferase (*cat*), the tetracycline-resistance (*tet*) and the β-lactamase (*bla*) genes are marked by arrows. The direction of the arrows drawn above the terminator region indicate the "native" orientation of the terminator; the "native" orientation is the one similar to that found in the cry mRNA. The *trp* promoter-controlled *IL-2* sequence and the replication origins in *E. coli* [*ori*(E)] and in *B. subtilis* [*ori*(B)] are shown. The solid and dot-filled boxes represent the promoter P_{156} and the synthetic ribosome-binding site sequence, respectively. Symbols A, B, Bc, E, H, N, P, and S represent the sites recognized by the restriction enzymes *Ava* I, *Bam*HI, *Bcl* I, *Eco*RI, *Hin*dIII, *Nru* I, *Pvu* II, and *Stu* I, respectively. The maps are not drawn to scale.

in the samples prepared from the *E. coli* and *B. subtilis* strains carrying the *penP-cry* fusion on plasmid pHCW-A3, indicating that the *cry*-derived sequence is cotranscribed with the *penP* gene in both of these bacterial hosts. The termination site of the transcript is located in the *cry*-derived sequence 4 bp downstream from the inverted repeat region (Fig. 1*C*). This is identical to the stop site of the *cry* transcript produced in *B. thuringiensis* (21). Therefore, the *cry* terminator is correctly recognized by the transcriptional apparatus of both *E. coli* and *B. subtilis*.

The *cry* Gene Retroregulator Stabilizes mRNA. The rate of decay of the penP mRNAs transcribed from plasmid pHCW-A3 and from its parental plasmid pSYC667 in *E. coli* and *B. subtilis* was determined. Cellular RNAs were isolated from exponentially growing cultures of *E. coli* and *B. subtilis* carrying either plasmid pHCW-A3 or pSYC667, and they were fixed on nitrocellulose filters. ^{32}P-labeled single-stranded M13 DNA containing the sense strand of *penP* gene was then used as the hybridization probe to quantitatively measure the penP-specific RNA among the samples. The results of these hybridization experiments are summarized in Fig. 4. The half-lives of the penP mRNAs produced from plasmid pSYC667 in *E. coli* and *B. subtilis* were estimated to be 2.8 and 2.0 min, respectively; that from pHCW-A3 in these bacteria was 6 min. Our analysis also revealed that the cells carrying plasmid pHCW-A3 had a higher steady-state level of penP mRNA than did the cells harboring plasmid pSYC667 (Fig. 4). This result shows that the *cry*-derived sequence stabilizes the mRNAs of the cotranscribed genes. Since the increase in penP mRNA stability matches closely the magnitude of increase in penicillinase enzyme in both of these bacterial hosts, the *cry* gene fragment enhances gene expression primarily through its influence on mRNA stability.

Table 1. Effect of the *cry* terminator on the expression of upstream genes in *E. coli* and *B. subtilis*

Host strain	Plasmid	*cry*-derived fragment length, bp	Penicillinase or IL-2* specific activity
Penicillinase gene			
E. coli CS412	—	—	<150
	pSYC667	—	1,055
	pHCW-A3	381	2,762
	pHCW-A4	157	2,661
	pHCW-A5	89	2,831
B. subtilis PSL1	—	—	<150
	pSYC667	—	3,280
	pHCW-A3	381	17,510
	pHCW-A4	157	18,600
	pHCW-A5	89	17,822
E. coli N99	—	—	<150
	pSYC667	—	1,126
	pHCW-A3	381	2,983
E. coli MA166	—	—	<150
	pSYC667	—	1,590
	pHCW-A3	381	4,131
IL-2 cDNA sequence			
E. coli CS412	pLW1	—	2.0×10^5
	pHCW701	381	1.4×10^6
	pHCW702	381	9.2×10^5
B. subtilis PSL1	pHCW300	—	1.0×10^4
	pHCW301	381	3.0×10^4

*Specific activity for IL-2 is defined as units per 150 mg of cellular proteins.

Enhancement of Gene Expression by the *cry* Terminator

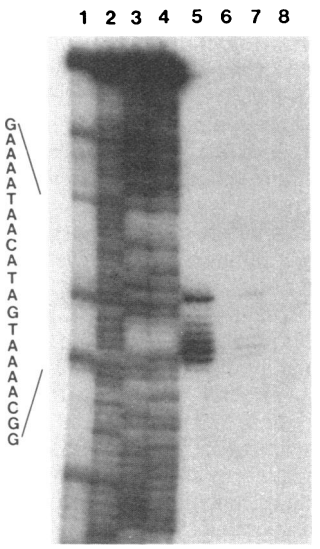

FIG. 3. S1 nuclease mapping analysis of the penP transcripts derived from plasmid pSYC667 and pHCW-A3. The 161-bp *Bgl* II–*Eco*RI fragment carrying the distal portion of the *cry* gene was 3′ end-labeled at the *Bgl* II terminus and hybridized to the RNA samples as indicated. After treatment with S1 nuclease, the samples were analyzed on a DNA sequencing gel. Base-specific chemical cleavages of this same labeled fragment are shown. Lane 1, G reaction. Lane 2, G + A reaction. Lane 3, C + T reaction. Lane 4, C reaction. Lanes 5 and 6, RNA samples were extracted from *B. subtilis* containing plasmids pHCW-A3 and pSYC667, respectively. Lanes 7 and 8, *E. coli* harboring plasmids pHCW-A3 and pSYC667, respectively.

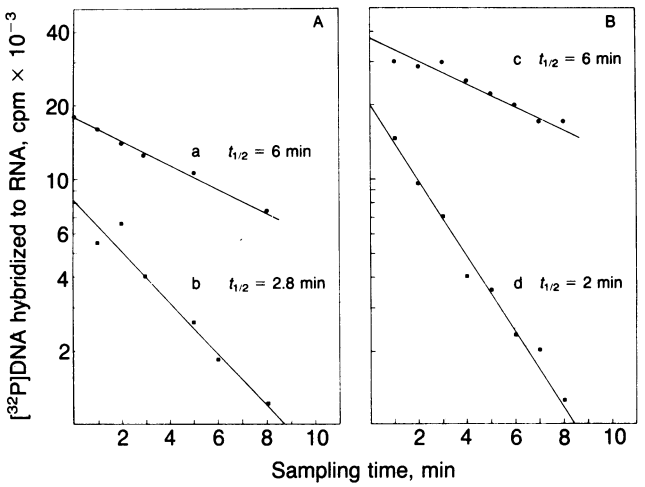

FIG. 4. Decay of the penP mRNAs produced by *E. coli* and *B. subtilis* harboring the plasmids pSYC667 and pHCW-A3. Samples containing total cellular RNA isolated 1–8 min after rifampicin treatment of *E. coli* (*A*) or *B. subtilis* (*B*) carrying the plasmid pHCW-A3 (curves a and c) and pSYC667 (curves b and d) were fixed on nitrocellulose filters. The penP-specific RNAs among the samples were then quantitatively measured by hybridization using the ^{32}P-labeled single-stranded M13 DNA containing the sense strand of the *penP* gene as the probe.

Fragment Is a General Phenomenon. To further examine the specificity of the enhancement effect of the *cry* terminator fragment, we constructed fusions between the cDNA encoding the human IL-2 protein and the *cry* terminator fragment. A restriction map of the recombinant plasmid pLW1 bearing the cDNA of human *IL-2* gene is shown in Fig. 2*B*. The expression of the *IL-2* sequence is under the control of the *E. coli trp* promoter and translational initiation signal carried on this plasmid (9). The only *Stu* I site located just beyond the coding region of *IL-2* on pLW1 was employed for the insertion of the terminator-carrying *Eco*RI–*Bam*HI fragment from M13mp9NP3. Ligation of the blunt-ended fragment carrying the *cry* terminator to the *Stu* I-digested pLW1 DNA regenerated both the *Eco*RI and the *Bam*HI recognition sequences. One orientation of insertion resulted in positioning the *Bam*HI site proximal to the *IL-2* coding sequence, and the plasmid was designated pHCW701. This orientation is similar to that found in the cry mRNA, and we refer to it here as the "native" orientation. The other orientation of insertion resulted in the recombinant plasmid designated pHCW702 (see Fig. 2 *C* and *D*).

E. coli strains containing either pHCW701 or pHCW702 produced higher levels (4.6- to 7-fold) of IL-2 activity than did the strain carrying pLW1 (Table 1). This was confirmed independently by directly measuring the amount of IL-2 protein present in these cell extracts (data not shown). Since the *IL-2* gene is derived from eukaryotic source, and its expression is regulated by the *trp* promoter in *E. coli*, these results suggest that the enhancement activity associated with the terminator fragment has a broad range with regard to the target genes and the associated promoters that initiate the transcription of these genes. Furthermore, the enhancement effect of the terminator fragment is independent of its orientation to the direction of transcription of the upstream target gene.

Plasmid pHCW300 was constructed from the bifunctional plasmid pLP1201 to test the enhancement of *IL-2* gene expression in *B. subtilis*. The *IL-2* gene was under the control of the *B. subtilis* promoter P156, an early promoter isolated from the phage SP82, and a synthetic ribosome-binding-site sequence (detailed construction of this will be published elsewhere). Plasmid pHCW301 was generated by inserting the *cry*-derived terminator fragment into the *Stu* I site in its native orientation. The structure of these plasmids is shown in Fig. 2 *E* and *F*. Biological activity of IL-2 in cell extracts prepared from the strains harboring these plasmids was analyzed, and the results are summarized in Table 1. A higher level of *IL-2* gene expression was observed from the strain PSL1(pHCW301) than was from the strain harboring the parental plasmid pHCW300. Thus the *cry* terminator-containing fragment enhances the expression of target genes in both the Gram-negative *E. coli* and the Gram-positive *B. subtilis*.

The half-lives of the IL-2 mRNAs produced by the plasmids pLW1 and pHCW701 in *E. coli* were also determined (data not shown) by the method described. The IL-2 mRNA from the strain carrying plasmid pHCW701, which contained the retroregulator, has a longer half-life in *E. coli* than the IL-2 mRNA synthesized from the strain harboring the parental plasmid pLW1 (6 min vs. 2 min).

Deletion Mapping of the Retroregulator. Deletion studies were carried out to define the sequence within the *cry* terminator region responsible for the enhancement activity. Oligonucleotide-directed site-specific mutagenesis (24) was employed to introduce, separately, two *Bgl* II restriction sites at the locations 19 bp and 87 bp upstream from the inverted repeat sequence in the *cry* terminator fragment (Fig. 1*B*). Shortened terminator-containing fragments were excised from these modified M13mp9NP3 phage genomes by *Bgl* II–*Eco*RI digestion and were cloned into pSYC667 at the *Bcl* I–*Nru* I site generating plasmids pHCW-A4 and pHCW-A5. *B. subtilis* and *E. coli* strains harboring these plasmids were analyzed for their ability to express the cloned *penP* gene. The data are presented in Table 1. It is clear that the shortened fragments still contain the regulatory function previously observed. Since the two newly created *Bgl* II sites are outside the *cry* coding sequence, these data demonstrate that the locus that confers the enhancement activity is located in the 3′ noncoding region of the *cry* gene and probably coincides with the transcriptional terminator of the *cry* gene.

DISCUSSION

We employed the *penP* gene to study the enhancement effect of the *cry* terminator in both *E. coli* and *B. subtilis*. The *penP* gene transcript terminates shortly beyond the coding region with a typical ρ-independent terminator sequence. The measured half-life for the penP mRNA is 2.0–2.8 min, which is similar to most bacterial mRNAs. When the *penP* terminator is replaced by the *cry* terminator, the chimeric mRNA exhibits a high degree of stability comparable to that of the cry mRNA in *B. thuringiensis*. Since the magnitude in the increase of penicillinase activity roughly matches that of the penP mRNA stability, stabilization of the mRNAs of the target genes can account for the observed enhancement conferred by the *cry* retroregulator. Our data on the enhancement of the *IL-2* gene expression in these bacteria further demonstrate that this effect is not restricted to a small class of target genes or to a small group of promoters. It appears that cotranscription of the *cry* retroregulator with the upstream target gene enhances the expression of the upstream gene. This is an example demonstrating that mRNA half-lives in bacteria can be elevated by a positive retroregulator to the level significantly beyond the 2-min average value for most mRNA species. We have tested the terminators from two additional bacterial genes that encode stable mRNAs, the *lpp* gene of *E. coli* and the *ery* gene from the *Staphylococcus aureus* plasmid pE194. The mRNA half-lives for these genes were reported to be 11.5 and >22 min, respectively (25, 26). However, no enhancement on the expression of the *penP* gene was obtained (Sheng-Yung Chang and S.C., unpublished results); the *penP-lpp* and *penP-ery* fusions expressed *penP* at the same level as did the native *penP* gene in both *E.*

coli and *B. subtilis*. Therefore, the *cry* terminator is unique in its ability to modulate gene expression as a retroregulator. This further indicates that there exist several mechanisms in bacteria that enhance mRNA stability.

Based on data presented here, we propose that the stem-and-loop structure in the mRNA, which corresponds to the inverted repeat sequence in the *cry* retroregulator fragment, is the sequence that enhances the mRNA stability. This hypothesis is supported by the observation that the enhancement activity of the retroregulator fragment is independent of its orientation of insertion with respect to the target gene. When present in either orientation, the only identical sequence present between the two respective transcripts is the inverted repeat involved in base pairing to form the stem in the mRNA. Secondary structure in mRNA has been implicated in its metabolism, for example, and an inverted repeat sequence has been identified in the *rxcA* transcript from *Rhodopseudomonas capsulata*, which may be responsible for the differential expression of the photosynthesis gene (27). On the other hand, the *cry* terminator is different from the T_J terminator of bacteriophage ϕX174 that have also been found to affect the mRNA stability of fusion gene (28). In the case of the T_J terminator, deletion and insertion studies indicated that the hairpin structure is not the primary requirement for the enhancing activity.

The metabolism of bacterial mRNA has been the subject of considerable study (ref. 29 and references listed therein), but the factors that determine mRNA stability are not well understood yet. A number of ribonucleases have been identified in *E. coli* (30–32). However, their physiological roles in mRNA degradation and RNA processing are still unclear. Although there are implications that RNase III is involved in the retroregulation of the λ *int* gene, our analysis of the penP and penP-cry chimeric mRNAs indicates that it does not participate directly in the enhancement effect mediated by the *cry* gene retroregulator (see Table 1). If, as we propose, the *cry* terminator fragment stabilizes mRNA through the incorporation of the stem-and-loop structure into the 3′ end of the mRNA of the target genes, our data suggest that the degradation of the cry, penP, and IL-2 mRNAs in *E. coli* and *Bacillus* is primarily mediated by 3′-to-5′ exonucleases.

We thank Drs. H. Echols (University of California, Berkeley) and D. Gelfand for helpful discussions and for providing strains, S.-Y. Chang for providing unpublished results, Dr. C. Levenson and members of Cetus Organic Synthesis Group for providing oligonucleotides used in this work, Cetus Cell Biology Assay Group for performing IL-2 assays, and Edna McCallan and Judy Davis for preparation of the manuscript.

1. Guarneros, G. & Galindo, J. M. (1979) *Virology* **95,** 119–126.
2. Guarneros, G., Montanez, C., Hernandez, T. & Court, D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 238–242.
3. Schindler, D. & Echols, H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4475–4479.
4. Wong, H. C., Schnepf, H. E. & Whiteley, H. R. (1983) *J. Biol. Chem.* **258,** 1960–1967.
5. Messing, J. & Vieira, J. (1982) *Gene* **19,** 269–276.
6. Ostroff, G. R. & Pene, J. (1983) *J. Bacteriol.* **156,** 934–936.
7. Schnepf, H. E. & Whiteley, H. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2893–2897.
8. Ostroff, G. R. & Pene, J. (1984) *Mol. Gen. Genet.* **193,** 306–311.
9. Rosenberg, S. A., Grimm, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, E. & Mark, D. F. (1984) *Science* **223,** 1412–1415.
10. Hayashi, S., Chang, S.-Y., Chang, S. & Wu, H. C. (1984) *J. Biol. Chem.* **259,** 10448–10454.
11. McLaughlin, J. R., Chang, S.-Y. & Chang, S. (1982) *Nucleic Acids Res.* **10,** 3905–3919.
12. von Gabain, A., Belasco, J. G., Schottel, J. L., Chang, A. C. Y. & Cohen, S. N. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 653–657.
13. Woo, S. L. C. (1979) *Methods Enzymol.* **68,** 389–395.
14. Thomas, P. (1983) *Methods Enzymol.* **100,** 255–266.
15. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
16. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
18. Anagnostopoulous, C. & Spizizen, J. (1961) *J. Bacteriol.* **81,** 741–746.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Schindler, P. & Huber, G. (1980) in *Enzyme Inhibitors*, ed. Brobeck, U. (Verlag Chemie, Weinheim, F. R. G.), pp. 169–176.
21. Schnepf, H. R., Wong, H. C. & Whiteley, H. R. (1985) *J. Biol. Chem.* **260,** 6264–6272.
22. Tinoco, I., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Corthers, O. M. & Gralla, J. (1973) *Nature (London) New Biol.* **246,** 40–41.
23. Neugebauer, K., Sprengel, R. & Schaller, H. (1981) *Nucleic Acids Res.* **9,** 2577–2588.
24. Zoller, M. J. & Smith, M. (1983) *Methods Enzymol.* **100,** 468–500.
25. DiRienzo, J. M., Nakamura, K. & Inouye, M. (1978) *Annu. Rev. Biochem.* **47,** 481–532.
26. Schivakumar, A. G., Hahn, J., Grandi, G., Kozlov, Y. & Dubnau, D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3903–3907.
27. Belasco, J. G., Beatty, J. T., Adams, C. W., von Grabain, A. & Cohen, S. N. (1985) *Cell* **40,** 171–181.
28. Hayashi, M. N. & Hayashi, M. (1985) *Nucleic Acids Res.* **13,** 5937–5948.
29. Chanda, P. K., Ono, M., Kawano, M. & Kung, H.-F. (1985) *J. Bacteriol.* **161,** 446–449.
30. Ono, M., Kuwano, M. & Horiuchi, T. (1977) *Mol. Gen. Genet.* **153,** 1–4.
31. Reiner, A. M. (1969) *J. Bacteriol.* **97,** 1522–1523.
32. Studier, F. W. (1975) *J. Bacteriol.* **124,** 307–316.