Cyclic AMP-dependent Initiation and $\beta$-dependent Termination of Colicin E1 Gene Transcription*

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We have analyzed the initiation and termination sites of transcription in vivo of the colicin E1 gene in Escherichia coli cells by S1-mapping assay and RNA blot hybridization. According to the S1-mapping assay, the transcription was initiated at about 75 base pairs upstream from the NH$_2$-terminal codon of the gene. The initiation site corresponded with one of the two promoters which were previously determined by in vitro transcription experiments (Ebina, Y., Kishi, F., Miki, T., Kagamiyama, H., Nakazawa, T., and Nakazawa, A. (1981) Gene 15, 119-126). Transcription in vivo of the colicin E1 gene was stimulated by cyclic AMP in the adenylate cyclase-defective mutant cells. Two transcripts of the colicin E1 gene, approximately 1700 and 2200 nucleotides, were detected by the blot hybridization. Since initiation of the transcription started at one site in vivo, these results indicated two termination sites. The location of the termination sites was approximately 60 and 560 base pairs downstream from the COOH-terminal codon of the gene as judged by S1-mapping assay. In vitro transcription experiments with $\beta$-factor strongly suggested that the termination in the proximal terminator was $\beta$-dependent. In the terminator structure, there is the sequence CAAACAAA which is homologous to a common sequence CAATCAA found in other $\beta$-dependent terminators.

Colicin E1, an antibiotic protein, is encoded by ColE1. The synthesis of the protein is repressed under ordinary conditions in Escherichia coli cells. On treatment with DNA-damaging agents or inhibitors of DNA replication, such as ultraviolet light or mitomycin C, synthesis of the protein is induced. Induction of ColE1 is considered as one of the "SOS responses" which also include $\lambda$ prophage induction, induced DNA repair, mutagenesis, and filamentous growth of the cells (1, 2). Among the responses, the mechanism of $\lambda$ prophage induction has been most extensively studied at a molecular level (3). Induction of $\lambda$ prophage results from proteolytic cleavage of $\lambda$ repressor by recA protein that has been activated by products occurring after treatment by the agents that damage DNA (4). The activated recA protein also cleaves its own repressor, lexA protein (5). Consequently, recA protein is synthesized in a large amount, leading to acceleration of inactivation of the $\lambda$ repressor.

Although inducers and time courses of $\lambda$ prophage and colicin E1 inductions appear similar, colicin E1 induction differs from $\lambda$ prophage induction at least in regard to the following features. (i) Colicin E1 induction is stimulated by cAMP, whereas $\lambda$ prophage induction is not (6). (ii) Colicin E1 induction is delayed in the $\omega$B mutant, but $\lambda$ prophage induction is normal in the mutant (7). (iii) $\lambda$ prophage induction is inhibited in the recB recC recF sbeB mutant, but colicin E1 is induced in this recombination-deficient mutant (8). Recently, we have obtained evidence indicating that the colicin E1 gene is directly repressed by lexA protein (9, 10), which is in contrast to the repression mechanism of $\lambda$ prophage genes. The inactivation of lexA protein by the activated recA protein results in the derepression of the colicin E1 gene. Similar mechanism of onset of the gene expression has been reported to be involved in most of the SOS operons (11-14).

To elucidate further the mechanism of regulation of the colicin E1 gene expression, we determined the initiation and termination sites of transcription in vivo of the gene. This paper presents evidence indicating that transcription in vivo of the colicin E1 gene is initiated at one of the two promoters which were previously detected by in vitro transcription experiments (9), and that the transcription is stimulated by cAMP. Furthermore, we identified two termination sites of transcription of the colicin E1 gene. The termination proximal to the COOH-terminal codon of the colicin E1 gene was $\beta$-dependent as judged by in vitro transcription experiments. The terminator contained homologous sequence with that of other $\beta$-dependent terminators (15, 16).

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—An adenylate cyclase-defective strain of E. coli K12, KH720 (Δacy, pshS, trp, lac, rpsL, rpoB), was reported previously (17). ColE1 was introduced into the strain by the transformation procedure (18). KH720 (ColE1) was grown to log phase in the modified M9 medium in which 20 amino acids (75 μg/ml each) were substituted for vitamin-free casamino acids of the M9 medium previously used (6). Colicin E1 synthesis was induced by MTC (2 μg/ml) for 1 or 2 h with or without 1 mM cAMP as previously described (8).

**Preparation of RNA**—After adding 50 ml of the cold M9-salts solution (6) containing 0.02 M Na$_2$CO$_3$ to 50 ml of the culture, the cells were collected by centrifugation and resuspended in a 6-ml solution of 0.02 M sodium acetate (pH 5.0), 0.5% SDS, and 1 mM EDTA (acetic/SDS buffer). After addition of 12 ml of redistilled phenol (equilibrated with acetic/SDS buffer), the mixture was incubated at 60 °C for 10 min with vigorous shaking. After centrifugation, the aqueous phase was re-extracted by phenol at room temperature. RNA was precipitated by adding 3 volumes of ethanol to the aqueous phase, and the mixture was chilled at -70 °C for 30 min. The RNA precipitate was collected by centrifugation and redissolved in 3 ml of the acetic/SDS buffer. The ethanol precipitation was repeated. The final precipitate was dried in vacuo and dissolved in 0.5 ml of the 2×

7072
SSC solution (0.3 M NaCl and 30 mM sodium citrate). The RNA concentration was determined by measuring the absorbance at 260 nm. The quantity of DNA which contaminated the crude RNA preparation was less than 1% of the total RNA.

Preparation of DNA Fragments—The DNA fragment containing the colicin E1 operator-promoter region was isolated with 4% polyacrylamide gel electrophoresis after digesting ColEl with SmaI and HaeII endonucleases. The 5' ends of the fragments were labeled with [γ-32P]ATP by T4 polynucleotide kinase as previously described (9).

The labeled fragments were separated on 4% polyacrylamide gel after denaturation by dimethyl sulfoxide. The sense strand of the promoter-operator fragment was determined by DNA sequencing and by referring to the nucleotide sequence of this fragment previously reported (9).

The fragment containing the COOH-terminal region of the colicin E1 gene was obtained as follows. ColEl was cleaved with PstI, and the 3' ends of the fragments were labeled with [α-32P]ATP by terminal deoxynucleotidyltransferase as described previously (18). The labeled fragments were digested with HindIII and loaded on a 4% polyacrylamide gel. The labeled 979-bp fragment containing the COOH-terminal region of the colicin E1 gene (19, 20) was isolated.

For the isolation of the fragment for RNA blot hybridization, ColEl was cleaved with EcoRI and HaeIII, and the 670-bp fragment was isolated with 4% polyacrylamide gel electrophoresis. This fragment was corresponded to a part of the coding sequence for colicin E1 (20), was labeled by nick translation in the presence of [α-32P]dCTP, DNase I, and DNA polymerase I according to the published method (21). The sense strand of the fragment migrating as the upper strand on the 4% polyacrylamide gel was assigned by the result of sequencing of the same 5'-labeled strand.

S1-mapping Assay—The S1-mapping experiments were done as previously reported (22) with some modifications as follows. 32P-labeled DNA fragment and RNA were precipitated with cold ethanol and dried in vacuo. These materials were resuspended in 30 μl of hybridization buffer (80% formamide, 20 mM PIPES-N,N'-bis(2-ethanesulfonic acid), pH 6.5, and 0.4 mM NaCl). The mixture was incubated at 75 °C for 10 min and cooled down gradually in about 4 h to 37 °C. The incubation was further continued for 2 h at 37 °C.

After adding 240 μl of H2O and 30 μl of the 10× S1 buffer (0.3 M sodium acetate, pH 4.6, 0.5 M NaCl, 10 mM ZnSO4, and 50% glycerol), the reaction mixture was treated with 100 to 900 units of S1 nuclease for 20 or 45 min. The reaction was terminated by adding 10 μg of tRNA and 300 μl of phenol which had been saturated with 1× SSC containing 0.1% SDS. After phenol extraction, the reaction products were precipitated with ethanol and dried in vacuo. The precipitate was dissolved in 30 μl of 0.8% formamide, 10 mM NaOH, 1 mM EDTA, 0.05% N-laurylsarcosine, 0.025% xylene cyanol, and 0.025% bromphenol blue, and the mixture was heated at 70 °C for 2 min, and was electrophoresed on a 5% polyacrylamide gel in 8 M urea/Tris/borate/EDTA buffer (9).

Analysis of RNA by Blot Hybridization—The blot hybridization was performed by the method previously reported (23). RNA denatured by glyoxal was electrophoresed on a 1.2% agarose gel in 10 mM phosphate buffer, pH 7.0. The RNA was blotted to nitrocellulose sheets, and hybridized to nick-translated 32P-labeled DNA.

In Vitro Transcription—The standard reaction mixture for transcription (15 μl) contained 50 mM Tris-HCl (pH 7.9), 0.1 mM dithiothreitol, 10 mM MgCl2, 50 mM NaCl, 1 mM each ATP, CTP, and UTP, 50 μM GTP, 35 μCi of [γ-32P]GTP, 3 μg of RNA polymerase (3 pmol), 1 pmol of DNA template, and 0.6 μg of ρ-factor. After incubation for 30 min at 37 °C, the reaction mixture was treated as described previously (9), and products were separated on a 3% polyacrylamide, 8 μl gel.

Materials—Restriction endonucleases and T4 polynucleotide kinase were purchased from Takara Shuzo (Kyoto), Nuclease S1 and DNase I were obtained from Boehringer. DNA polymerase I and calf thymus terminal deoxynucleotidyltransferase were products of Bethesda Research Laboratories and P-L Biochemicals, respectively. [γ-32P]ATP and [γ-32P]GTP were prepared as described (9). [α-32P]ATP (635 Ci/mmol) and [α-32P]dCTP (3000 Ci/mmol) were purchased from The Radiochemical Centre (Amerham). RNA polymerase was purified by a modification of the method of Chamberlin and Berg (24). ρ-Factor was kindly provided by K. Shigesada (Kyoto University).

RESULTS AND DISCUSSION

Initiation Site of Transcription in Vivo under Induced Conditions—From in vitro transcription experiments using the DNA fragment containing the promoter-operator region of the colicin E1 gene, we previously demonstrated two initiation sites for transcription, and determined their exact locations by sequencing each transcript (9). The sites were located 75 bp upstream (I1) and 10 bp downstream (I2) from the NH2-terminal codon of the gene. Although I2 was within the coding region for colicin E1, the transcription from I2 was four times as efficient as that from I1 in vitro (9). To understand the seemingly incomprehensible results derived from the in vitro experiments, we tried to determine the in vivo transcription initiation site of the colicin E1 gene by S1-mapping assay. The rationale for the assay is illustrated in Fig. 1. The single-stranded DNA probe which is complementary to the mRNA (sense strand) was uniquely labeled at the SmaI end. I1 and I2 are 289 and 205 nucleotides away from the SmaI...
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end, respectively (9). The labeled probe was hybridized to colicin E1 mRNA. The hybrid was treated with S1 nuclease to remove the unhybridized RNA and DNA tails. After denaturation of the hybrid, the size of the protected DNA probe was determined by gel electrophoresis and autoradiography. An RNA species originating from I₁ should protect around 289 nucleotides of the probe, whereas an RNA species from I₂ should protect about 205 nucleotides.

Cells of strain KH720, a deletion mutant in the adenylate cyclase gene, harboring ColE1, were incubated for 1 h in the presence of cAMP and MTC. Under these conditions, colicin E1 was actively synthesized. The total cellular RNA was extracted from the cells by the hot phenol procedure as described in “Experimental Procedures,” and the DNA probe was hybridized to various quantities of the RNA. After S1 nuclease digestion and electrophoresis, the intense band of around 289 nucleotides which corresponded to the transcript initiated from I₁ could be seen, but the band of 205 nucleotides which would come from transcript from I₂ could not be detected under these conditions (Fig. 2a). These results indicated that the synthesis of colicin E1 mRNA started only at I₁, in vivo under induced conditions.

After detection of the radioactive band by autoradiography, portions of the gel corresponding to the 289-nucleotide band were cut out and counted for radioactivity. The radioactivity of S1-protected DNA band was in proportion to the quantity of colicin E1 mRNA, when the amount of total cellular RNA added was less than 6 μg (Fig. 2b).

Effect of cAMP on Transcription in Vivo—We have previously reported that colicin E1 induction is dependent on cAMP in the adenylate cyclase-deficient mutant cells (6). Therefore, whether cAMP stimulates transcription in vivo of the colicin E1 gene was examined by S1-mapping assay. The labeled DNA probe was hybridized to 3 μg of the cellular RNA isolated from KH720 (ColE1) cells in which colicin E1 synthesis was induced by MTC for 1 (Fig. 3, lane 2) and 2 h (lane 3) in the presence (lane a) and absence (lane b) of cAMP. Judging from the radioactivities of the bands, the colicin E1 transcription was stimulated by cAMP about 10 times. Approximately 60 and 85 bp upstream from I₁, we found the sequence 5’TGTGA3’, which is homologous to the binding site for the complex of cAMP and cAMP receptor protein in the operator region of the lac and gal operons (25). However, in the in vitro experiments, the colicin E1 gene was transcribed even in the absence of cAMP and cAMP receptor protein, and the transcription was not stimulated by these factors (9). Other conditions for the in vitro experiments seem to be necessary for demonstrating the transcription stimulation by cAMP and cAMP receptor protein.

Since many steps existed in the induced process of colicin

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**Fig. 3 (left). The effect of cAMP on transcription in vivo of the colicin E1 gene.** The 32P-labeled fragment (150,000 cpm/30 ng) was hybridized to the mixture of 3 μg of RNA isolated from cells under various conditions plus 47 μg of RNA from the noninduced cells. The amount of S1 nuclease used was 500 units. RNAs were isolated from KH720(ColE1) 1 h after addition of MTC in the presence (lane a) and absence (lane b) of cAMP. Lane 1, is for marker DNA fragments. Colicin E1 activities of the cells in lanes 2a, 2b, 3a, and 3b were 11, 0.2, 516, and 5 units/absorbance at 660 nm of the culture, respectively.

**Fig. 4 (right). Colicin E1 mRNA detected by RNA blot hybridization.** Total cellular RNA or purified ColE1 DNA was fractionated on a 1.2% agarose gel after denaturation by glyoxal and transferred to nitrocellulose paper. The papers were hybridized with the nick-translated sense strand of a part of the colicin E1 gene (EcoRI-HaeIII fragment of 670 bp; 2.2 × 10⁶ cpm/75 ng). Lane 1, 10 μg of RNA from KH720(ColE1) 1 h after addition of MTC and cAMP. Lane 2, 10 μg of RNA from the same strain 1 h after addition of only MTC; lane 3, ColE1 DNA (0.6 μg). The 16 S and 23 S ribosomal RNAs of E. coli which were estimated to be approximately 1650 and 3300 nucleotides, respectively (33), were stained by acridine orange (34) and the positions are indicated on the left.
E1 synthesis, cAMP might stimulate a step other than transcription of the colicin E1 gene. For instance, if the recA gene, which plays the central role in the SOS responses, is activated by cAMP, the same results shown above could be obtained. However, this is not the case, because the expression of recA mRNA was not dependent on cAMP and cAMP receptor protein in the \textit{in vivo} and \textit{in vitro} experiments.\textsuperscript{2}

Aiba et al. (22) reported that the presence of cAMP and cAMP receptor protein selected the use of one of the two functional promoters in the \textit{gal} operon. To examine whether the similar selection of promoters by cAMP occurs in the colicin E1 gene expression, the labeled DNA probe was hybridized to a large amount (50 \(\mu\)g) of the cellular RNA isolated from the KH720 (ColEI) cells after incubation with or without mitomycin C in the absence of cAMP. From the S1-mapping assay, we could not detect the 205-nucleotide band but found one intense 289-nucleotide band under all conditions we tested (data not shown). Therefore, in the case of the colicin E1 gene, only one of the initiation sites that were detected \textit{in vitro} was utilized \textit{in vivo} either in the presence or absence of cAMP.

With respect to selection of the two potential \textit{in vitro} promoters, we have obtained evidence indicating that the structure of the DNA template directs the use of a particular promoter. As will be shown later, transcription \textit{in vitro} from I\textsubscript{1} occurred with the closed circular DNA template, whereas it started at I\textsubscript{2} with the linear DNA template (Fig. 9). Therefore, a similar mechanism for selection of promoters may be involved \textit{in vivo}. On the other hand, we could not detect RNA species whose transcription started at I\textsubscript{2} and stopped before the Smal site under the conditions of the S1-mapping assay described above. Such a transcript from I\textsubscript{2} might participate in the \textit{in vivo} regulation of the colicin E1 gene expression.

Two mRNA Transcripts Detected by RNA Blot Hybridization—We have previously reported that the structural gene for colicin E1 consists of 1566 bp (20). The colicin E1 mRNA should cover this region. Total cellular RNAs from KH720 (ColEI) cells under induced (Fig. 4, lane 1) or noninduced conditions (lane 2) were fractionated with agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with the nick-translated sense strand of a part of the colicin E1 gene (670 bp). We could detect two intense bands which correspond with the predicted termination sites (Fig. 5). The nucleotide sequence of the region between the PstI and Hinfl sites has been previously determined (19, 20). The locations of the COOH terminus of the colicin E1 gene (19, 20), the immunity gene (19), the RNA-1 gene (19), and the initiation site for transcription of primer RNA for DNA replication (26) have been previously reported. The PstI-Hinfl fragment (979 bp) was labeled at the 3’ end of the PstI site as described under “Experimental Procedures,” and used as the probe of S1 mapping. The two arrows represent the two presumed termination sites (T\textsubscript{1} and T\textsubscript{2}) which were calculated from sizes of two colicin E1 mRNAs determined by blot hybridization (see Fig. 4).

Transcription Termination Sites of Colicin E1 Gene—To determine transcription termination sites more precisely, we used the S1-mapping assay. From the DNA sequence analysis (20), the number of nucleotides from the transcription initiation site I\textsubscript{1} to the PstI site near the COOH terminus of the colicin E1 gene was 1497 nucleotides. Since each of two transcripts of the colicin E1 gene was initiated at I\textsubscript{1} and the sizes of the two transcripts detected by the blot hybridization (Fig. 4) were about 1700 and 2200 nucleotides, two termination sites of the mRNA could be calculated about 200 and 700 nucleotides away from the PstI site. Therefore, the PstI-Hinfl fragment (979 bp) which was labeled at the 3’ end of the PstI site was used for the probe of the S1-mapping assay to determine these two termination sites (Fig. 5). An RNA species terminated at T\textsubscript{1} should protect about 200 nucleotides of the probe after the RNA-DNA hybrid was treated with S1 nuclease, whereas an RNA species terminated at T\textsubscript{2} should protect about 700 nucleotides. As shown in Fig. 6, lane 2, two species of about 215- and 715-nucleotide bands were detected, both of which corresponded well with the presumed termination sites described above. Two discrete bands of about 979 nucleotides and longer than this seem to be due to undigested single- and double-stranded probe by S1 nuclease. Thus, two termination sites of the colicin E1 transcription appeared to be located about 215 (T\textsubscript{1}) and 715 (T\textsubscript{2}) nucleotides away from the PstI site.

\textsuperscript{2} Y. Ebina, F. Kishi, and A. Nakazawa, unpublished results.
(T₁) away from the PstI site. The T₁ site is located in the immunity gene (19), and the T₂ site is adjacent to the initiation site of the primer RNA for replication of ColE1 (26), as shown in Fig. 5. The shorter mRNA seems to be synthesized in larger amounts than the longer one as judged by the results of the S1-mapping assay, but we obtained opposite results by blot hybridization. Therefore, exact comparison of efficiencies in transcription terminations at these sites awaits further experiments.

By the S1-mapping assay, we could not define the precise nucleotide at which transcription stopped. However, the T₁ site could be defined fairly precisely, since an about 215-nucleotide band, which corresponded to the transcription terminated at T₁, was just above the 210-nucleotide single-stranded DNA which was derived from the PstI-AvaII fragment. As shown in Fig. 7, the T₁ site is located close to and on the 3’ side from the AvaII site. We could not find other terminator structures in the neighborhood of each termination site. The possible terminator structures for the colicin E1 mRNA are shown in Fig. 7. Although the T₂ site is located in the promoter region of the primer RNA for the replication of ColE1, the effect of the termination at T₂ on the transcription initiation of the primer RNA is unclear.

*p*-dependent Termination of Transcription in Vitro—In the neighboring region of T₁ (Fig. 7), there are sequences CAATCCAAAT and CAAACAAA, which are homologous to a common sequence CAATCCAA found in other *p*-dependent terminators such as λₐ₉₆ and tRNAₜ₉ (15, 16). Therefore, we examined by in vitro transcription experiments whether the transcription termination at T₁ was dependent on *p*-factor.

From two initiation sites (I₁ and I₂) and two termination sites (T₁ and T₂), four transcripts were expected to be synthesized in vitro using ColE1 as a template. The sizes of the transcripts were calculated as follows; about 1624 and 2124 nucleotides started from I₁ and terminated at T₁ and T₂, respectively, and about 1540 and 2040 nucleotides started from I₂ and terminated at T₁ and T₂. However, it was difficult to measure precisely these large transcripts by gel electrophoresis. Therefore, the deletion plasmid of ColE1, which lost the region between the promoters and the terminators of the colicin E₁ gene, was constructed. The 1.3-kb region between the promoters and the terminators of the colicin E₁ structural gene was deleted in the plasmid. The deletion plasmid (ColE1-ΔPstI DNA) was selected by colicin E₁ immunity. The predicted transcripts which are produced from the two promoters and the numbers of base lengths are indicated.

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\begin{align*}
\text{Transcription in Vitro} & \text{—} \text{In the absence of } p, \text{ the } 850-\text{nucleotide transcript was synthesized in the absence of } p \text{ and the } 930-\text{nucleotide transcript was synthesized in the presence of } p. \\
\end{align*}
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**Fig. 8.** The deletion plasmid used for in vitro *p*-dependent termination studies of the colicin E₁ gene. The two initiation sites of the colicin E₁ gene (I₁ and I₂) were detected by in vitro experiments. Terminators (T₁ and T₂) were derived from *in vivo* studies. To obtain a deletion plasmid of ColE1, ColE1 was digested by PstI, and the largest fragment (5.5 kb) was ligated. The 1.3 kb of the colicin E₁ structural gene was deleted in the plasmid. The deletion plasmid (ColE1-ΔPstI DNA) was selected by colicin E₁ immunity. The predicted transcripts which are produced from the two promoters and the numbers of base lengths are indicated.

**Fig. 9.** The effects of *p*-factor on transcription in vitro of ColE₁-ΔPstI DNA. a, in vitro transcription experiments were performed using closed circular ColE₁-ΔPstI DNA as a template in the absence (lane 1) and presence (lane 2) of *p* as described under “Experimental Procedures.” b, linear ColE₁-ΔPstI DNA, which was cleaved by AccI at a site adjacent to the replication origin of ColE₁-ΔPstI DNA (19), was transcribed in the absence (lane 1) and presence (lane 2) of *p*. Sizes of marker RNAs, which were obtained by transcription in vitro using HaeIII-A (1185 bp) and HaeIII-SmaI (871 bp) fragments of ColE1 as templates (20), are indicated in the middle. The approximate sizes of the major transcripts are indicated on the right and left sides.
disappeared, and an about 350-nucleotide transcript was detected (Fig. 9b, lane 2). These transcripts probably started from I2 and terminated at T1 and T2. It appears that I1 of the colicin E1 gene is mainly utilized when the covalently closed circular DNA is transcribed, and I2 is preferentially utilized when the template is the linear form. Furthermore, the termination seems to be ρ-dependent at T1, but not at T2, at least in vitro, irrespective of the structure of the template.

To confirm further that the termination at T1 of the colicin E1 gene is ρ-dependent in vitro, the 1216-bp HpaII-HpaII fragment carrying the colicin E1 promoter and terminator regions was isolated from ColE1-ΔPstI DNA and used as a template for transcription (Fig. 10a). The predicted transcripts from the template are illustrated in Fig. 10a. In vitro transcription experiments were done at the NaCl concentrations of 50 and 100 mM in the reaction mixture. A broad band around 850 nucleotides seems to represent transcripts which started from I1 and terminated at both T1 and T2 of the template fragment (Fig. 10b, lanes 1 and 3). Under these conditions, the termination at T2 appeared to be inefficient. An intense band around 660 nucleotides was considered to be the transcript which started from a promoter near the EcoRI site and terminated at the end of the template. It was previously reported that an internal promoter was detected near the EcoRI site of the colicin E1 gene by in vitro transcription experiments, and that the direction of the transcription was the same as that of the colicin E1 gene (27). In the presence of ρ, these two transcripts disappeared and an about 350-nucleotide band appeared as shown in lanes 2 and 4 of Fig. 10b. This transcript probably started from I2 and terminated at T1. Since the size of the transcript which started from the internal promoter near the EcoRI site and terminated at T1 was calculated to be about 100 nucleotides from the nucleotide sequence (20), this transcript could not be detected on the gel in the present experiments. The transcript which started from the internal promoter near the EcoRI site and terminated at T2 could not be detected in the experiments described in Fig. 9. The internal promoter might be efficiently used when the template is a short linear DNA such as the HpaII-HpaII fragment.

From these results, we concluded that the transcription termination at T1 is ρ-dependent and that at T2 is ρ-independent in vitro.

Sequences for ρ-dependent Termination—Three ρ-dependent termination sites have been previously characterized: λR1 (15), the tRNA<sup>Tyr</sup> terminator (16), and a site within the λ<sub>rod</sub> gene called λ<sub>ro</sub> (28). These sites share the following features: (i) dyad symmetry preceding the termination point; (ii) A- and U-rich sequence at the terminus of the RNA; and (iii) the common sequences CAATCAA in λ<sub>R1</sub> and the tRNA<sup>Tyr</sup> and the ATCAACAA in λ<sub>ro</sub> just preceding the stop site. Three features described above are satisfied in the case of T1 of the colicin E1 gene. The homologous sequence CAAACAAA between 211 and 218 nucleotides from the PstI site seems to be attributable to the ρ-dependent terminator at T1.

The homologous sequences which are so far found in ρ-dependent terminators can be listed as follows: λ<sub>R1</sub>, 5′TTCAATCAAT3′; tRNA<sup>Tyr</sup>, 5′CGCAATCAAT3′; ColE1 T1, 5′ACCAACAAAG3′; and λ<sub>ro</sub>, 5′ATCAACAGGC3′. The consensus sequence obtained from the list is 5′CAAA<sub>3</sub>AA<sub>3</sub>′. The underlined region of the sequence seems to be most important in these termination sites.

However, not all the ρ-dependent terminators contain the sequence. A less homologous sequence, 5′CGGATCAT3′, was found in the typical ρ-dependent terminator of λ<sub>LI</sub> (29). Furthermore, the transcription termination at trp t of the tryptophan operon which was proved to be ρ-dependent in vitro does not have this consensus sequence. The terminator
has the dyad symmetry preceding the termination point and an A- and T-rich sequence at the terminus (30). Therefore, the consensus sequence appears not always necessary for ρ-dependent termination. The contribution of nusA protein to the terminations at trp t and λt has been reported (31, 32). Further research should be necessary to elucidate the relationship between such consensus sequences and ρ-dependent termination.

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