Effects of Ribosomal RNA Promoters on Plasmid Replication

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Abstract

The effects of promoters of ribosomal RNA operons in the replication origin region of the *Bacillus subtilis* chromosome have been studied on the replication of plasmids in *Escherichia coli* as a host cell. It has been already found that plasmids carrying these promoters can not be replicated in *B. subtilis*, therefore the mechanism of inhibition of replication can not be examined in this bacterium. In this study we found that even in *E. coli* there are inhibitory effects on plasmid replication depending on site and direction of insertion of the promoters. In pBR vectors (*E. coli* plasmids), the promoters can be inserted either in one direction or in both directions depending on gene constitutions around the site of insertion. Stability of the plasmid is also affected by the site and direction of the insertion. In a composite vector between pBR and pUB (*Staphylococcus aureus* plasmid), the insertion of the promoters alone does not take place but occurs with an accompanying DNA fragment, always located downstream from the promoters. This fragment is derived from the *E. coli* chromosome and can be replaced by DNA fragments containing known terminators of transcription. Subsequently we have newly constructed a vector that may be useful as a terminator searching plasmid. The mechanism of inhibition caused by these promoters on plasmid replication was discussed.

Key words cloning, rRNA promoters, plasmid replication

Introduction

During the study of the structure and function of the origin region of replication of *Bacillus subtilis* in our laboratory, a DNA fragment (B7), which exhibited some inhibitory functions on DNA replication was found within this region (1). By sequencing a part of this DNA fragment a set of two tandem promoters was discovered (2). These promoters are the main loci responsible for the suppression of autonomous replication of plasmids carrying this fragment in *B. subtilis*. They exercised strong inhibition on plasmid replication in *Escherichia coli* and caused rapid segregation of cells which had already lost the plasmid (3).

It was found that B7 contains a part of a ribosomal RNA operon (*rrnO*) and that the two tandem promoters in this fragment were promoters of rRNA operon (*rrn* promoters) (4, 5). We wanted to know if the inhibitory effects caused by *rrn*O promoters would be common for other *rrn* promoters and to understand the mechanism of inhibition due to these promoters.

In general, *rrn* promoters are considered strong promoters since they are frequently

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transcribed by the RNA polymerase. Until now more than 100 different promoter sequences have been identified. Promoters from several bacterial and viral genomes have been easily cloned in *E. coli* and their strength has been studied (6, 7). However cloning of DNA fragments containing strong promoters alone, such as bacteriophage T5 promoters (8) or *rrn* promoters of *E. coli* (9), have failed.

The mechanism of this unclonability of the strong promoter has not been elucidated yet. In this paper we describe the insertion of *rrn* promoters in various plasmids and its effects on plasmid replication in *E. coli*. Some vectors constructed in this study may have the potential to be used as plasmids searching for transcriptional terminators. The mechanism of inhibition caused by strong promoters is also discussed.

Materials and Methods

Bacterial strains. *E. coli* C600 (*thy, leu, thi, tonA, lac, supF*) and *E. coli* 1037 (*recA*$^-$, *dnaA*-ts), were used as hosts for the cloning of plasmids containing *rrn* promoters of *B. subtilis*. Whole chromcsomal DNA of *B. subtilis* 168 LTT (*leu, trpC2, thy*) was used for Southern hybridization experiments.

Plasmids. Plasmids used in this study are summarized in Table 1. The construction of pLAR111 and pLAR331 is illustrated in Fig. 5.

Phages. Lambda ch-BSOl containing *rrn*O operon and lambda ch-BS11 containing *rrn*A operon (Fig. 1) have been previously described (13).

Table 1. Properties of the plasmids used in this study

Plasmid	Size (kb)	Composition	Genetic markers	Source
pBR322	4.36		Amp^r, Tc^r	(10)
pBR328	4.90		Amp^r, Tc^r, Cm^r	(11)
pUB110	5.00		Km^r	(12)
pMS102′	7.70	pBR322+pUB110	Am^r, Km^r	(3)
pNO1010	5.66	pBR322+E4′[1]	Amp^r, Tc^r	provided by Dr. Ogasawara
pMS102′-B6	13.65	pMS102′+B6[2]	Amp^r, Km^r	provided by Dr. Ogasawara
pLOp-1	7.17	pBR322+$\lambda t_{R1}/t_0$[3]	Amp^r	provided by Dr. Masamune
pLAR9	6.20	pBR322+E19 (b)[4]	Amp^r, Tc^r	this communication
pLAR12	7.21	pBR322+E14 (b)	Amp^r, Tc^r	this communication
pNO1012	6.73	pBR328+E19 (a)	Amp^r, Tc^r	(13)
pNO1013	6.73	pBR328+E19 (b)	Amp^r, Tc^r	(13)
pNO2003	7.75	pBR328+E14 (a)	Amp^r, Tc^r	(13)
pNO2004	7.75	pBR328+E14 (b)	Amp^r, Tc^r	(13)
pBR322-B7	9.90	pBR322+B7	Amp^r	provided by Dr. Ogasawara
pLAR10	11.36	pMS102′+E19+X (a)	Amp^r, Km^r	this communication
pLAR11	13.40	pMS102′+E14+Y (a)	Amp^r, Km^r	this communication
pLAR33	13.40	pMS102′+E14+Y′ (b)	Amp^r, Km^r	this communication
pLAR13	16.25	pMS102′+2 [E14]+Y (c)	Amp^r, Km^r	this communication
pLAR111	13.40	pLAR11[5]	Amp^r, Km^r	this communication
pLAR331	13.40	pLAR33[5]	Amp^r, Km^r	this communication

[1] : pNO1010 has a 1.3 kb fragment (E4′) containing *rrn*O terminator introduced into the *Hind*III site of pBR322. [2] : pMS102′-B6 has a 5.95 kb fragment (B6) (13) introduced into the *Bam*HI site of pMS102′. B6 contains E3′ in which the *rrn*A terminator is located. [3] : pLOp-l contains terminators t_{R1} and t_0 also genes O and P of lambda phage. [4] : The inserted fragments are at the *Eco*RI site of the vector. (a) . Direction of promoters is towards Amp^r gene. (b) : Direction of promoters is towards Tc^r gene in pBR328 and pBR322 or towards pUB110 origin in pMS102′. (c) : Two E14 fragments separated by a DNA fragment (Y) with the direction of promoters facing each other. X, Y and Y′ are DNA fragments derived from the *E. coli* chromosome (see text). [5] : The *Eco*RI site upstream from the promoters is modified. (kb) : Molecular size of DNA in kilobase pairs.

Chemicals and enzymes. [α-^{32}P] dCTP (PB10165, 400 Ci/mmole) was purchased from Amersham International Ltd. (Amersham, UK). T4 DNA polymerase, T4 DNA ligase, bacterial alkaline phosphatase and restriction enzymes were from Takara Shuzo Co., Ltd. (Kyoto, Japan). DNA polymerase I and DNase I, used for nick translation, were from Amersham International Ltd. (Amersham, UK). *Eco*RI linker was from Genex Corporation (Rockville, Md., USA). Low melting point agarose was from Takara Shuzo Co., Ltd. (Kyoto, Japan).

Construction of plasmids carrying *rrn* promoters of *B. subtilis*. Lambda ch-BS01 and lambda ch-BS11 were digested with *Eco*RI and fractionated by electrophoresis in 0.8% low melting point agarose. Fragments E19 from *rrn*O and E14 from *rrn*A (Fig. 1) were extracted from the agarose following instructions specified by the manufacturer. All the plasmids used for the cloning were dephosphorylated with bacterial alkaline phosphatase, as described (14). E19 or E14 fragment was inserted into the *Eco*RI site of pBR322, pBR328 or pMS102'. The conditions for the ligation were as described (15). Transformation of *E. coli* competent cells was performed as described (16). Transformants were selected on LB agar plates containing 30 μg/ml of ampicillin (Amp) or 15 μg/ml of tetracycline (Tc).

Segregation assay. *E. coli* cells were grown in LB medium with Amp until 20 Klett units. One portion was diluted 10^6 times and plated on LB plates to obtain 100-500 colonies/plate and then either replicated on LB+Amp plate or streaked on LB+Amp plate to determine percentage of Amp sensitive colonies ("time 0"). The other portion was diluted 10^3 times in LB medium, grown until 20 Klett units (for 10 generations) and then the same procedure as the "time 0" sample was repeated. The percentage of colonies that could not grow in the presence of Amp was taken as the percentage of segregation.

Southern hybridization. The procedure was based on the one described in (17). 1 μg of DNA of different sources was digested with appropriate restriction enzymes and fractionated by electrophoresis in 1% agarose in buffer TAE (40 mM Tris-HCl, 20 mM Na acetate, 2 mM EDTA, pH 8.0). The fragments in the gel were transferred to and fixed on nitrocellulose filter sheets (Schleicher & Schuell) by a trans-blott electrophoretic blotting apparatus

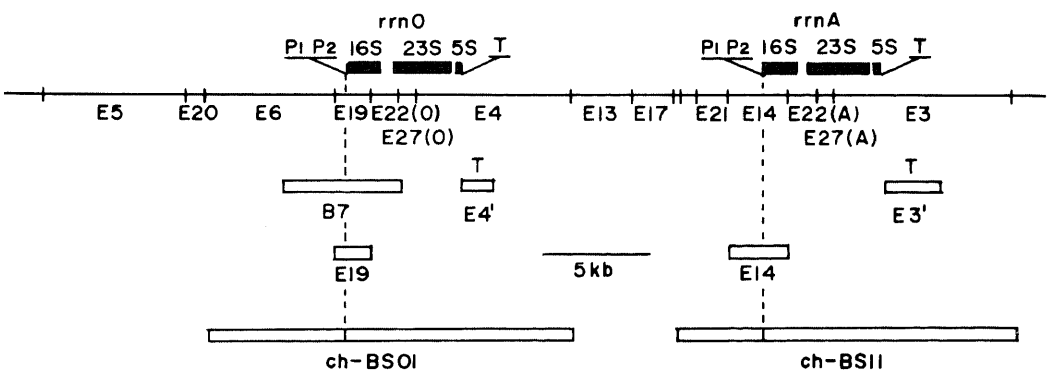

Fig. 1. Map of rRNA operons in the replication origin region of the *B. subtilis* chromosome (5). A map of *Eco*RI cleavage sites is shown. The fragments produced by digestion with *Eco*RI (E) are numbered as described (18). Each operon, *rrn*O and *rrn*A, has a set of two tandem promoters (P1, P2), 16S rRNA gene, 23S and 5S rRNA genes and terminator (T). B7, E19 and E14 are promoter containing fragments. E4' and E3' are terminator containing fragments. These two operons were cloned in lambda charon 28 phage giving rise to ch-*BS01* carrying *rrn*O and ch-BS11 carrying *rrn*A (13). The molecular size of the DNA is in kilobase pairs (kb).

(Bio-Rad Labs) following the instructions by the manufacturer. DNA fragments used as probes for hybridization were labeled with [α-^{32}P] dCTP by nick translation using DNAase I and DNA polymerase I. The probe was denatured at 65°C for 5 min. in 100μl of buffer TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4). The hybridization solution is 50% formamide, 1X Dendhardt's solution (0.02% ficoll, 0.02% polyvinylpyrrolidone, 0.02% nuclease free BSA), 5X SSPE (1X SSPE : 0.18 M Na Cl, 0.01 M Na phosphate, 1 mM EDTA, pH 7.7), containing the denatured probe and 50-100μg/ml of sonicated calf thymus DNA. The nitrocellulose filter was incubated in the hybridization solution for 24 to 48 hrs. at 42°C. The filter sheets were washed for 15 min. twice each in 2X SSPE with 0.1% SDS and 0.1X SSPE with 0.1% SDS, followed by exposure to kodak X-ray film (XAR-5).

Results

Insertion of *B. subtilis rrn* promoters in pBR322 and pBR328.

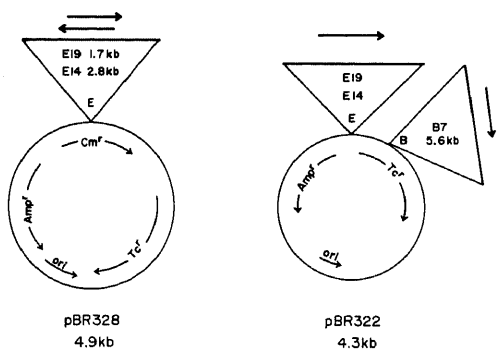

Fig. 2. Construction of plasmids carrying *B. subtilis rrn*O (E19, B7) and *rrn*A promoters (E14). Lambda ch-BS01 and lambda ch-BS11 were digested with *Eco*RI. Fragments E19 and E14 were purified and introduced into the *Eco*RI site of pBR322 and pBR328. B7 fragment (3) was introduced into the *Bam*HI site of pBR322. The arrows show the direction in which the promoters are inserted. Abbreviations: E, *Eco*RI site. B, *Bam*HI site. Ori, origin of replication. Amp^r, Cm^r and Tc^r, for ampicillin, chloramphenicol and tetracycline resistance respectively. kb, as in Fig. 1.

Fig. 1 shows a physical map of the replication origin region of the *B.* subtilis chromosome. Two *rrn* operons (*rrn*O and *rrn*A) have been found in this region (5). These *rrn* operons have been cloned in lambda charon vector (13). Promoter containing fragments E19 and E14 were subcloned and used for this study.

E19 or E14 were inserted at the *Eco*RI site of pBR322 and pBR328 (Fig. 2). In pBR322 we could only insert in one direction towards the Tc^r (resistance to Tc) gene. Insertion of B7, a larger fragment containing promoters (3), at the *Bam*HI site resulted in the same situation, no insertion occurred towards Amp^r (resistance to Amp) gene direction. We obtained the same results selecting by either Amp or Tc, indicating the failure in inserting the fragment towards Amp^r gene is not due to direct inhibition of expression of this marker. In pBR328 we were able to insert E19 or E14 in both directions although the inserted plasmids were unstable and rapidly lost from the cells. Table 2 shows that pBR328 alone is maintained stably in the cell after the growth in the absence of Amp or Tc for 10 generations, but when it has E19 or E14 it becomes unstable and this effect is more conspicuous when it carries E19.

Insertion of *B. subtilis rrn* promoters in

Table 2. Stability of pBR322 or pBR328 containing E19 or E14 in *E. coli.*

Inserted Fragment	Percentage of segregation			
	Time 0		After 10 generations	
	pBR322	pBR328	pBR322	pBR328
None	0	0	0	0
E19 (a)	–	57	–	89
E19 (b)	0	42	7.5	57
E14 (a)	–	12	–	25
E14 (b)	5	3	7.5	18

Percentage of segregation was determined as described in *MATERIALS AND METHODS.* (a) and (b) as in Table 1.

pMS102′.

pMS102′ is a composite plasmid able to replicate in *E. coli* and *B. subtilis* (3). Studying *rrn* promoter containing fragments in this plasmid, a completely different situation ocurred, thus we could not clone E19 or E14 as a single insert in either direction (Fig. 3). We found inserts of E19 or E14 in both directions always accompanied by an extra-fragment of DNA of unknown origin and molecular weight similar to the inserted fragment (a and b in Fig. 3). We also found a clone with three inserts containing two E14 fragments with the direction of promoters converging into the accompanying fragment (c in Fig. 3). This plasmid is unstable, but plasmids carrying one molecule of E19 or E14 accompanied by the unknown fragment are quite stable (Table 3).

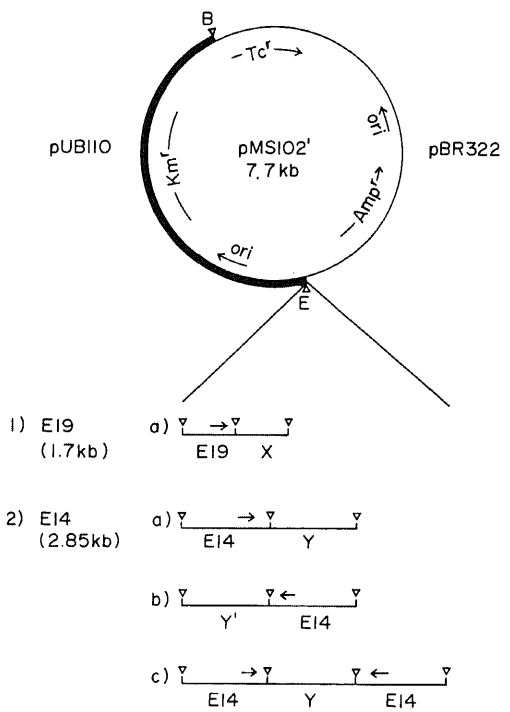

Fig. 3. Insertion of promoter regions of *B. subtilis* *rrn* genes in pMS102′. pMS102′ is a composite plasmid containing a pBR322 portion and a pUB110 portion (3). E19 and E14 were inserted at the *Eco*RI site of pMS102′. They could not be inserted as a single insert in either direction (a) or (b), they were always accompanied by an unknown DNA fragment (X, Y or Y′) which made cloning feasible. (c), two E14 fragments with Y DNA between them. Km^r, for kanamycin resistance. Abbreviations as in Fig. 2.

The appearance of the unknown fragment is not due to recombination events in *E. coli* since identical results were obtained when the experiment was repeated using a recA mutant *E. coli* cell. Therefore these fragments must be inserted during construction of plasmids using cloned E19 or E14 fragment in lambda charon vector. As described below, they are derived from *E. coli* chromosome most likely present in lambda preparation.

Experiments with pBR322 and pMS102′ show that E14 fragment is as effective as E19 in suppression of plasmid replication. To confirm the effect being due to promoters of *rrn*A in E14, the fragment was divided into two halves, one 1.0 kilobase pairs (kb) containing the promoters and the other 1.8 kb without them. The 1.8 kb fragment could be inserted into pMS102′ easily without any accompanying fragment, while no plasmid was cloned with an intact 1.0 kb fragment.

Identification of the origin of the accompanying fragments.

Only one clone with the insertion of E19 and an extra fragment (named X) was isolated while many clones were obtained containing E14 with extra fragment. Therefore we concentrated on the analysis of an extra fragment

Table 3. Stability of pMS102′ carrying E19 or E14 in *E. coli*

Inserted Fragment	Percentage of segregation	
	Time 0	After 10 generations
None	0	0
E19+X (a)	28	39
E14+Y (a)	0	4
E14+Y′ (b)	3	6
2 [E14]+Y (c)	41	74

The percentage of segregation was determined as described in *MATERIALS AND METHODS*. (a), (b), (c), X, Y and Y′ as in Table 1.

coinserted with E14. Analysis of 15 clones by several restriction enzymes showed surprisingly that there were only two different types of fragments. They were named Y and Y′ (Fig. 3). To determine the origin of these fragments we performed Southern hybridization experiments using three cloned DNA (E14, Y′ fragment and pMS102′) as probes. E14 and pMS102′ probes did not hybridize to fragments X, Y or Y′. The Y′ probe hybridized to fragment Y (pLAR11) as well as to Y′ (pLAR33), indicating that both fragments are related (Fig. 4), it also hybridized to the chromosome of *E. coli* digested with *Eco*RI, strongly at the same position as Y′ (2.85 kb) and weakly at other positions of higher molecular weight. Y′ did not hybridize to *B. subtilis* DNA, E14, pNO2003 and pMS102′. In Fig. 4 we can also see some hybridization to pMS102′ because Y′ probe has some contamination of pMS102′. However pMS102′ probe did not hybridize to Y and Y′. We conclude that Y and Y′ are related fragments coming from *E. coli* chromosome. Most likely some *E. coli* chromosomal DNA was present in our preparations of lambda DNA and consequently when we digested them with *Eco*RI, the *E. coli* DNA was also cut and fragments comigrating with E19 and E14 were also extracted.

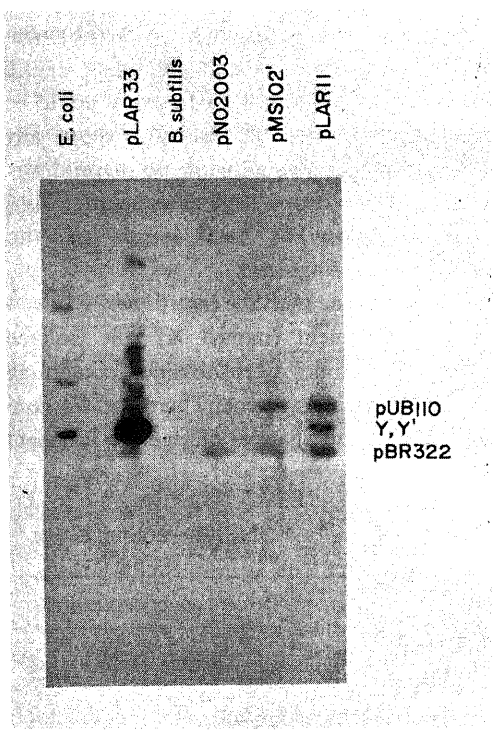

Fig. 4. Hybridization of Y′ DNA fragment labeled with ^{32}P to several DNA. Hybridization was performed as described in *MATERIALS AND METHODS*. *E. coli* and *B. subtilis* whole chromosomal DNA were digested with *Eco*RI. pLAR33, pMS102′ and pLAR11 were digested with *Eco*RI and *Pvu*II. pNO2003 was digested with *Eco*RI and *Ava*I. pBR322 and pUB110 are two fragments of digested pMS102′. Y and Y′ are described in the text.

It is amazing that two unique fragments related to each other are selected from a small amount of contaminant DNA in E19 or E14 preparation. These fragments must contain special structures which counteract the inhibition of plasmid replication by promoters. An obvious candidate for such function is the terminator signal for transcription.

Effect of terminators on the plasmid carrying *rrn* promoters.

In order to verify the assumption that the accompanying fragments may act as terminators of transcription, we placed known terminators instead of fragments Y and Y′. Our strategy was first to modify the *Eco*RI site upstream from the promoters and then to remove fragments Y and Y′ by *Eco*RI digestion (Fig. 5). At the remaining *Eco*RI site we inserted a fragment containing one of the following terminators, *rrn*O terminator, *rrn*A terminator or lambda t_{R1}/t_0 terminators. No stable transformant was obtained when the parental plasmid containing E14 alone was introduced into *E. coli*. In contrast, a large number of stable transformants were obtained when the terminator containing fragment was inserted adjacent to the E14 fragment (Table 4). Analysis of the direction of insertion of the terminator containing fragment revealed that, unexpectedly in all cases, the direction of the terminator is opposite to that of transcription from the promoter. All the transformants so far analysed contain intact promoter region

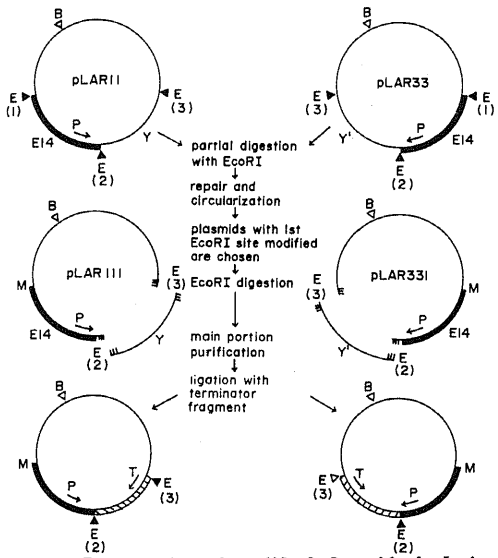

Fig. 5. Preparation of modified plasmids, isolation of terminators of transcription and construction of terminator containing plasmids. Partial digestion in order to produce one cut per molecule was carried out with *Eco*RI. The DNA was repaired with T4 DNA polymerase and fractionated in 0.8% low melting agarose. DNA bands which migrated at the same position as pLAR11 and pLAR33 linear full length molecules were extracted. The DNA was ligated with T4 DNA ligase as described (15) and *E. coli* competent cells were transformed as in (16). Plasmids from the transformants were isolated by an alkaline extraction procedure (19). Plasmids with the first *Eco*RI site modified (upstream from the promoters) were selected (pLAR111 and pLAR331). pNO1010 and pMS102′-B6 were digested with *Hind*III, and a 1.3 kbp (E4′) and 2.0 kbp fragment (E3′) containing terminators of *rrn*O and *rrn*A respectively, were isolated from 0.8% low melting agarose. Plasmid pLOp-1 was digested wish *Bgl*II and a 0.65 kbp fragment containing terminators (t_{R1} and t_0) was isolated in 1.5% low melting agarose. The DNA was purified and repaired with T4 DNA polymerase. *Eco*RI linkers were phosphorylated with polynucleotide kinase and ligated to the blunt ends of the fragments. Plasmids pLAR111 and pLAR-331 were digested with *Eco*RI and fractionated in 0.8% of low melting agarose. The main portion of the plasmids was purified and the 5′ ends were dephosphorylated. Terminator containing fragments were ligated at the *Eco*RI site of the main portion of pLAR111 and pLAR331. The ligation mixture was used to transform *E. coli* C600 competent cells. Abbreviations: P, promoters. T, terminator. Y and Y′, are described in the text. B and E as in Fig. 2. M, *Eco*RI site modified. The arrow shows the direction of the insertion of the terminator and the promoters.

and a terminator (Table 4). They seem to be as stable as those containing X, Y or Y′. Apparently the terminator in the opposite direction is more effective to counteract the inhibitory effect of the strong promoters.

Discussion

We have inserted *B. subtilis rrn* promoters into several plasmids and studied their replication in *E. coli* cells. At the *Eco*RI or *Bam*HI site of pBR322 these promoters are inserted only in one direction and the plasmid is stably maintained in the cell. In pBR328 the promoters are inserted in either direction but the cells easily lose the plasmid. At the *Eco*RI site of pMS102′, a fragment containing promoters can not be inserted in either direction, unlesss they are accompanied with fragment X, Y, Y' or known terminators of transcription. All these clones are stable.

In the cases where promoters of *rrn*O fail to be inserted, a fragment containing *rrn*A promoters (E14) also fail. Therefore the inhibitory effect of promoters is not restricted to that of *rrn*O but seems to be common to all promoters of rRNA operon.

There are some mechanisms to explain the fact that strong promoters are not clonable, such as: (a) overproduction of a protein harmful for the cell, (b) untoward effects on the expression of downstream genes (20), (c) direct effect on replication.

Overproduction of a protein harmful for the cell is unlikely in the case of pBR vectors since these plasmids can be expressed and maintained at high copy number in the cell. In the case of pBR322 it is known that it does not code for any harmful protein for the cell. However in the case of pUB110, the effect of its products in *E. coli* is not known, therefore, we can not rule out completely overproduction of a harmful protein coded in pUB110.

Untoward effects on the expression of downstream genes may be ruled out because the insertion of *rrn* promoters is independent of the selected genetic marker (Amp^r or Tc^r). The other possibility is that high transcription

from *rrn* promoters towards Amp^r gene may interfere with primer RNA synthesis for initiation of replication. However we may rule it out because it is known that other transcripts upstream from the origin can be used as primers for the replication of pBR322 (21).

Direct effect on replication is favourable. Even though we do not have direct proof, some interference against replication may occur because high transcription from strong promoters could cause a change in comformation that may affect replication. It is known that during transcription the RNA polymerase unwinds the DNA (22, 23), therefore if there is unwinding in one part of the helix in a closed duplex DNA circle, this must cause an overwinding of the DNA elsewhere in the molecule (24). When promoters are highly transcribed and there is no terminator downstream from and in the vicinity of the promoters, a large portion of DNA is kept opened resulting in a conformational change in the whole molecule of the circular plasmid. This change in structure may affect the replication of the plasmid. The length of the transcript up to a suitable terminator may also be important. The strong promoters would not be clonable, unless they have a suitable terminator at an appropriate place so that a change in structure will not affect plasmid replication. In fact we could insert *rrn* promoters into *Eco*RI sites of pMS102′ together with known terminators of transcription. Unexpectedly these terminators enter in the reverse orientation. A similar case has been reported recently (8). When the terminator sequences are in the reversed direction, the dyad symmetry is conserved (Fig. 6). Such structures may still cause the RNA polymerase to pause (26) and stop transcription. By unknown reasons it seems that terminators in the reverse orientation are better for the cloning of strong promoters.

If strong promoters can be inserted into plasmids only with a suitable terminator downstream and within an appropriate distance from the promoter as discussed above, pBR322 should have suitable terminators close to Tc^r gene, but not to Amp^r gene. Insertion of Cm^r gene in pBR322 should create the signal in both directions. This possibility is now under investigation.

Conclusion

The inhibitory effect of *B. subtilis rrn* promoters on plasmid replication in *E. coli* is not confined to that of *rrn*O promoters but seems to be common to all promoters of rRNA operons.

The insertion of *rrn* promoters into the

Table 4. Cloning of terminator containing fragments in pLAR331 and pLAR111

Inserted Fragment	*rrn*O terminator (1.3 kb)		*rrn*A terminator (2.0 kb)		λ tR_1/t_0 terminators (0.65 kb)		None	
Vector[1]	A	B	A	B	A	B	A	B
Yield of clones with insert (%)	93	92	100	60	75	75		
Yield of clones with intact promoters (%)[2]	100	100	100	100	100	100		
Number of transformants/μg vector[3]	5000	2500	2000	1000	850	375	unstable[4]	unstable[4]

[1] : *Eco*RI digested pLAR111 (A) or pLAR331 (B) (without fragments Y and Y′) was purified and used to insert terminators. [2] : Intact promoters mean the conservation of some restriction enzyme sites within the promoter region. The yield of clones with insert is taken as 100%. [3] : The selected marker is Amp (30 μg/ml). [4] : When the vectors (A) or (B) were selfcircularized and used to transform *E. coli* C600 (*rec*$^+$) cells, some transformants appeared. When these transformants were streaked on LB+Amp plates most of them did not grow. The few that did grow, quickly lysed in liquid medium.

*Eco*RI site of pMS102′ is possible due to the presence of *E. coli* DNA fragments (X, Y, or Y′) always located downstream from the promoters, suggesting the presence of structures that act as terminators to counteract the promoters. The fact that fragments X, Y or Y′ are selected from small amounts of *E. coli* DNA present in our preparations suggests that the *rrn* promoter containing plasmids would serve as good terminator searching vectors.

Terminators of transcription containing a dyad symmetry sequence inserted in an orientation opposite to their natural one and downstream from the promoters seem to be quite efficient for the cloning of strong promoters.

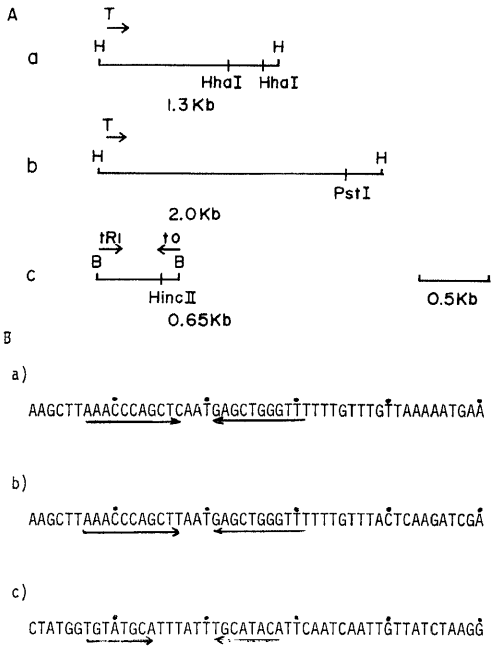

Fig. 6. Strucure of terminators of transcription. A) Fragments containing *rrn*O terminator (a) and *rrn*A terminator (b) were extracted from a *Hind*III digest of pNO1010 and pMS102′-B6 respectively. In both cases the terminator was located just after the *Hind*III site (H), distal to 5S rRNA gene (see Fig. 1). The orientation of the terminators is shown by the arrows. Lambda t_{R1}/t_o terminators were contained in a 0.65 kbp fragment which was isolated from a *Bgl*II digest of pLO-p1. These terminators have opposite directions as indicated by the arrows. The restriction sites shown in the figure were used to determine the orientation of the terminators. B) (a) DNA sequence of *rrn*O terminator region (13). (b) DNA sequence of *rrn*A terminator region (13). (c) DNA sequence of lambda t_{R1} terminator region (25). The dyad symmetry is underlined. kb, as in Fig. 1.

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リボゾームRNAプロモーターのプラスミド複製に対する作用：金沢大学がん研究所生物物理部　マリア デル ピラール アギナガ（指導教官　吉川　寛）

抄　録　枯草菌染色体の複製開始領域に存在するリボゾームRNAオペロンのプロモーターによるプラスミド複製に対する阻害作用を大腸菌を宿主として研究した．このプロモーターを持つプラスミドは枯草菌の中では全く複製せず，従って阻害機構を調べることができない．

我々はこの研究において，異種の大腸菌の中でもプロモーターは挿入する位置と方向によってプラスミドの複製を阻害することを発見した．大腸菌プラスミドのpBRをベクターとすると，挿入部位周辺の遺伝子構成に依存して，一方又は両方向に挿入できる．pBRとStaphylococcus aureus由来のプラスミドpUB110との雑種ベクターの場合には，プロモーター単独では挿入できず，常にプロモーターの下流にDNA断片の挿入を伴っていた．このDNAは大腸菌染色体に由来するもので，既知の転写終結配列を含むDNAによって置換できることが判明した．その結果，終結配列の探索に有用なベクターを初めて作成することに成功した．リボゾームRNAプロモーターによる複製阻害の機構についても考察した．