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Direct Analysis of the Transcription of *Escherichia coli mpB* Gene Harbored in a Multicopy Plasmid during Bacterial Growth

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Abstract: To examine the growth-phase dependent control of *Escherichia coli mpB* gene we used a combination of Northern analysis for RNA determination and Southern analysis for plasmid DNA determination. The relative amounts of metabolically unstable transcript derived from the internally deleted *mpB* gene harbored on a multicopy plasmid as well as the relative plasmid contents were measured by Northern analysis and Southern analysis, respectively, of total nucleic acids from *E. coli* cells containing the plasmid. The relative transcription activity of the *mpB* was represented by a ratio of the relative amount of the transcript to that of the plasmid DNA during bacterial growth. The *mpB* transcription increased rapidly with time during exponential growth, but started to decrease before the transition period of an exponential growing cell culture into the stationary phase. Although the expression pattern was similar to the changes of β-galactosidase activity expressed from the lysogenic strain carrying the chromosomal *mpB-lacZ* fusion which were shown in a previous work, the present data appears to represent a more actual growth-phase control of the *mpB* transcription than the previous data by the β-galactosidase assay. In addition the present method described for a direct analysis of both RNA and plasmid DNA provides a rapid and efficient method that can applied to an examination of transcription control by using a multicopy plasmid.
Key words: *Escherichia coli*, growth-phase control, *mpB* gene

Expression of the *Escherichia coli mpB* gene encoding M1 RNA, the RNA component of RNase P which is a tRNA processing enzyme (Robertson *et al.*, 1972), is controlled over different growth conditions (Lee *et al.*, 1991; Jeon *et al.*, 1993; Eun *et al.*, 1994). Previously, to examine growth-phase dependent control of the *E. coli mpB* gene we have reported the construction and use of an *E. coli* lysogenic strain where the λ prophage carried the *lacZ* gene fused to the *mpB* promoter (Eun *et al.*, 1994). In this construct, activity of β-galactosidase synthesized from the *mpB* promoter increases with growth time during exponential growth, but decreases at the transition period of an exponentially growing culture into the stationary culture and finally remains constant at the stationary phase. Although this study has shown that the *mpB* gene transcription is regulated in a growth-phase dependent manner, it is difficult to examine the regulation in detail using this construct because β-galactosidase activity does not simply indicate the *mpB* transcription activity. This problem may be resolved by directly observing changes of transcription from the *mpB* gene during growth. For this purpose, a plasmid carrying the *mpB* gene with a deletion in the M1 RNA structural gene is useful because it generates truncated M1 RNA transcripts which are metabolically unstable with half lifes of 2~3 min and consequently the amounts of the transcripts are directly related to *mpB* transcription (Lee *et al.*, 1991; Kim and Lee, unpublished results). Furthermore it canbeeasily differentiated with intact M1 RNA by size. However, the use of the plasmid in this analysis needs a compensation of the plasmid copy number because it is also changed during growth. The purpose of this study is to re-examine the growth-phase dependent control of the *mpB* transcription by directly analyzing the truncated M1 RNA transcribed from the plasmid carrying the internally deleted *mpB* gene. We used a direct phenol-extraction for purification of total nucleic acids from *E. coli* carrying the plasmid and applied the total nucleic acids to both Northern analysis for determination of the RNA contents and Southern analysis for determination of the plasmid contents.

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Materials and Methods

Plasmid construction

Plasmid pLMd23 was a donor of the *rnpB* gene with the internal deletion in the M1 RNA structural gene (Lee *et al.*, 1991). The *rnpB* gene in pLMd23 contains the sequence between positions −269 and +1285 but has a deletion of the internal M1 RNA sequence between position +57 and +330. The 750 bp *Bam*HI-*Stu*I DNA fragment of pLMd23 carrying the internally deleted *rnpB* gene was ligated into *Bam*HI-*Eco*RV sites of pACYC184 (Chang and Cohen, 1978) to generate plasmid pAMd23.

Growth and total nucleic acids preparation

E. coli strain CF898 (F, *thy1*, *proE60*, *argE3*, *proA2*, *thr1*, *leu6*, *mtl1*, *xyl5*, *ara14*, *galK2*, *lacY1*, *str31*, *nalA*, *cdd*, *supE44*), which is a derivative of CF748 (Metzger *et al.*, 1988) was transformed with pAMd23. After the transformed cell CF898/pAMd23 was grown overnight in LB medium containing chloramphenicol (100 μg/ml), diluted 1:5000 in 100 ml of the same medium and continued to be grown with shaking at 37℃. Aliquots of the cell culture were taken at intervals for determination of cell growth and isolation of total nucleic acids. The cultures of 0.7 ml were directly extracted with the same volume of phenol saturated with TE buffer. The aqueous phase was re-extracted with the phenol two times more, extracted with chloroform, ethanol-precipitated, and resuspended in water.

RNA analysis

Total nucleic acids were fractionated in a 5% polyacrylamide gel containing 7 M urea in 1×TBE buffer, pH 8.0. After electrophoresis, the gel and a nylon membrane (Hybond-N^+; Amersham, Amersham, UK) were equilibrated in 0.5×TBE buffer for 15 min, and RNA was electrotransferred to the membrane with a Hoefer Semi-Phor Semi-Dry transfer unit. The hybridization was carried out in the presence of 50% formamide, 5×SSPE, 10% dextran sulfate, 1% SDS, and 0.25 mg/ml ssDNA at 42℃ for 12 h. After hybridization, the membrane was washed for 10 min in 2×SSPE containing 0.5% SDS and then for 1 h in 0.2×SSPE containing 0.5% SDS at 52℃. The antisense RNA probe was prepared by T7 RNA polymerase using the *Hin*dIII-treated pLMd23 as a template and labeled with [γ-^{32}P]ATP by the 5′ end labeling method (Sambrook *et al.*, 1989). Relative amounts of RNA species were estimated by analyzing the filter with a Molecular Dynamics Phosphorimager, Image Quant Version 3.3.

Plasmid DNA analysis

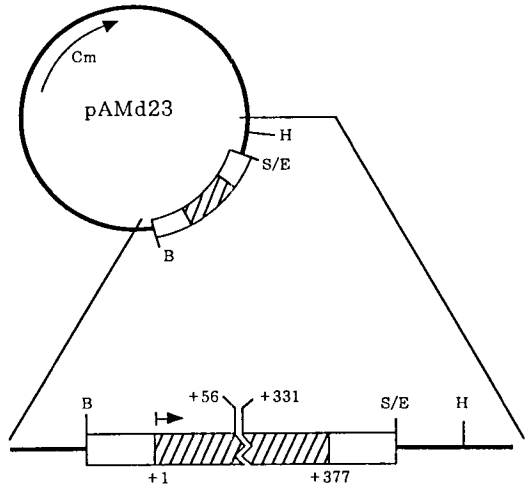

Fig. 1. Plasmid map of plasmid pAMd23. The rectangle indicates the *rnpB* gene and the numbers refer to positions in the *rnpB* gene. The structural sequence of M1 RNA is hatched. Restriction sites are marked to represent their relative locations in the plasmid. The internal deletion points of the M1 RNA sequence are indicated by wavy lines. B: *Bam*HI; E: *Eco*RV; H: *Hin*dIII; S: *Stu*I. Cm stands for the chloramphenicol resistance gene.

Relative plasmid copy number was estimated by Southern hybridization of the same total nucleic acids used for RNA analysis. For the analysis, total nucleic acids were treated with *Hin*dIII in the presence of DNase-free RNase, electrophoresed in a 1% agarose gel and transferred to a nylon membrane (Hybond-N^+; Amersham) by the capillary action as described by Sambrook *et al.* (1989). The hybridization with the antisense RNA probe was carried out by the same method as described in RNA analysis.

Results and Discussion

In order to examine growth-phase dependent control of the *E. coli rnpB* transcription, we directly analyzed the truncated transcripts derived from plasmid pAMd23 carrying the internally deleted *rnpB* gene (Fig. 1). Plasmid pAMd23 contains the *rnpB* DNA sequence between positions −270 and +845 with the internal deletion of an M1 RNA structural sequence between positions +57 and +300 (Lee *et al.*, 1991). The internally deleted *rnpB* gene is expected to generate two major truncated transcripts. One of them is a primary transcript of 143 nucleotides that is initiated from *rnpB* P-1 promoter and terminated at T1 (Lee *et al.*, 1989a and 1989b). The other is its processed product of 111 nucleotides at 3′ end (Gurevitz *et al.*, 1983). These RNAs are metabolically unstable with half lives of 2~3 min so that their intracellular contents can be regarded as a relative transcription activity (Lee *et al.*, 1991; Kim and Lee, unpublished results). First we isolated total

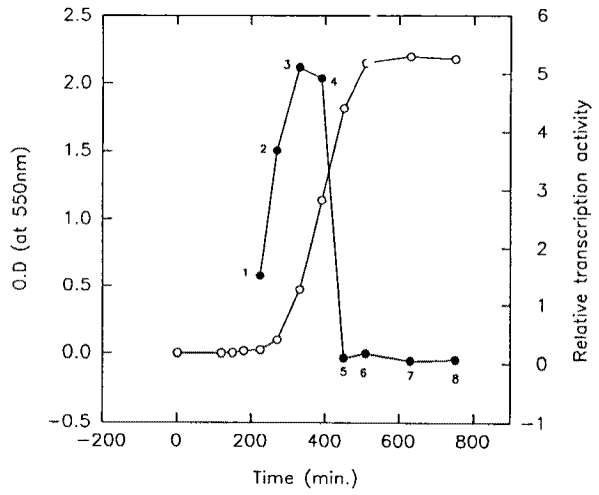

Fig. 2. The growth curves and the *mpB* transcription activity. Aliquots of the culture taken for preparation of total nucleic acids were also used for determination of absorbance at 550 nm (A_{550}). Growth is represented with open circles (○). A relative transcription activity (an arbitrary unit), which is a ratio of a sum of relative amounts of the truncated M1 RNA transcripts to that of the relative plasmid content, is represented with filled circles (●).

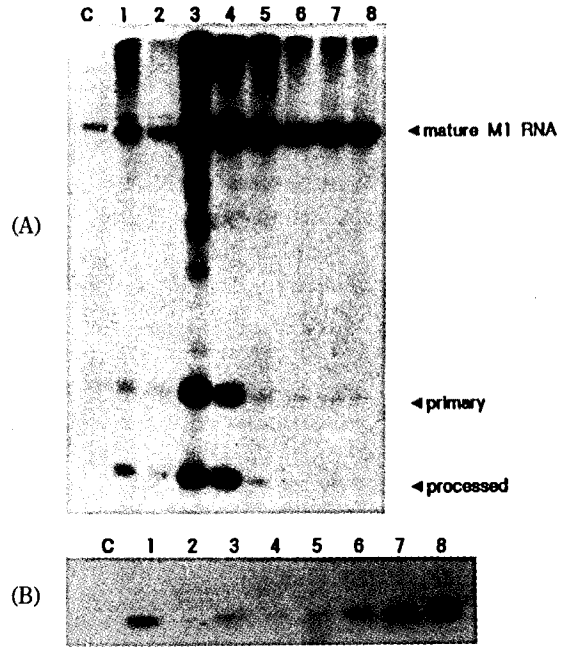

Fig. 3. RNA and plasmid DNA analysis of bacterial cells at different growth phases. Cells containing pAMd23 at the growth phases indicated as numbers in Fig. 2 were analyzed for RNA and plasmid DNA contents. Lanes 1 and 2, total nucleic acids prepared from 1×10^6 cells; lanes 3 to 8, total nucleic acids from 2×10^7 cells; lane C, a 1:20 dilution of total nucleic acids loaded in lane 3 as a control to confirm a linearity between the signals and the amount of total nucleic acids loaded in the lanes. (A) Northern analysis of total nucleic acids. Aliquots of the culture were taken at indicated times after 1:5000 dilution of the overnight culture of *E. coli* cells containing pAMd23 into fresh media. During growth total nucleic acids were prepared from cells, analyzed in 5% polyacrylamide gel containing 7 M urea and subjected to Northern analysis. The primary transcript from the internally deleted *mpB* gene and its processed product were indicated by arrows. (B) Southern analysis of total nucleic acids from cells. The same total nucleic acids prepared and used for Northern analysis of panel A were digested with *Hin*dIII, electrophoresed in a 1% agarose gel and subjected to Southern analysis. The amounts of total nucleic acids used were identical with those for Northern analysis.

nucleic acids from cells containing pAMd23 grown in LB medium at different stages of growth. Growth of the *E. coli* cells containing the plasmid entered exponential growth phase after 4 h and shifted to stationary phase after 8 h following a 1:5000 dilution of the overnight culture (Fig. 2). Then the total nucleic acids were subjected to both Northern analysis for determination of the RNA contents and Southern analysis for determination of the plasmid contents. Northern analysis showed two hybridization signals: the upper band corresponded to the primary transcript and the lower band to the processed RNA (Fig. 3A). The hybridization signal of each band was quantitated. For Southern analysis, the total nucleic acids were digested with *Eco*RI and the hybridization signal in the 1200 bp *Eco*RI fragment of pAMd23 containing the *mpB* gene was also quantitated by a phosphorimage analyzer (Fig. 3B). However, it should be noted that the quantitated values of Northern and Southern signals showed only the relative contents of the transcripts and plasmid DNA within the cells, respectively. Therefore, the relative transcription activity of the *mpB* promoter was represented as a ratio of the relative amount of the transcript to that of the plasmid DNA in the total nucleic acids isolated from the cells growing at a specific stage of growth. Since it is also possible that the rate of processing of the primary transcripts to its processed product is changed during growth for the transcription activity of the *mpB* promoter, we used a ratio of the sum of the Northern signals of both transcripts to the Southern signal of the plasmid DNA (Fig. 2). As shown in Fig. 2, the *mpB* transcription increased with growing time during exponential growth, but started to decrease before the transition period of an exponential growing culture into the stationary phase and remained constant at the low level in the stationary phase. This expression pattern was similar to the changes of β-galactosidase activity expressed from the lysogenic strain carrying the chromosomal *mpB-lacZ* fusion (Eun *et al.*, 1994). Therefore, the present data confirmed the previous results by directly observing the *mpB* transcription that *mpB* transcription is regulated in a growth-phase-dependent manner. However the observed time points at which the maximum of the transcription activity occurred were different in two assay systems, i.e., RNA analysis used in this study and β-galactosidase analysis used

in the previous study. The data of this study shows that transcription reached the the maximum at the mid-exponential phase and then rapidly decreased while the previous data of β-galactosidase analysis revealed that maximum activity was exhibited after the mid-exponential phase and sustained for about 2 h before it decreased. Since the RNA assay system represents more actual *rnpB* transcription than the β-galactosidase assay using the *rnpB-lacZ* fusion, this slower response is due likely to accumulation of β-galactosidase synthesized from the *rnpB-lacZ* fusion during growth. Since the growth-phase dependent control of the *rnpB* transcription is still operating under an artificial condition of high gene dosage provided by the multicopy plasmid, any cellular factors involved in this control might not be limited factors in cells. Although the mechanism of this growth phase-dependent control of the *rnpB* transcription is not known, the change of the transcription during growth seems to result from the intrinsic nature of the *rnpB* promoter. Furthermore the stage of growth at which the *rnpB* transcription activity decreases is correlated with the growth stage where RNA polymerase σ^{70} factor is replaced by σ^{38}, the *rpoS* gene product known to be induced upon entry to stationary phase (Tanaka *et al.*, 1993; Gentry *et al.*, 1993). Therefore, the *rnpB* promoter appears to be one of promoters recognized only by RNA polymerase holoenzyme containing σ^{70} (Tanaka *et al.*, 1993). The *rnpB* transcription activity at different stages of growth is also correlated to expression of Fis, a nucleoid-associated protein which is high during early exponential phase and low during late exponential phase and stationary phase (Ball *et al.*, 1992; Nilsson *et al.*, 1992; Ninnemann *et al.*, 1992; Thompson *et al.*, 1987). The consensus sequence for Fis binding (Lazarus *et al.*, 1993) appears in the upstream sequence of the *rnpB* gene. There are five sites for Fis binding in upstream sequence of *rnpB* gene up to position −270 with respect to the transcription starting point. It remains to be demonstrated, however, whether the *rnpB* transcription is correlated to Fis. Finally a combination of Northern analysis for RNA determination and Southern analysis for plasmid DNA determination used in this study provides a rapid and efficient method that can contribute to an examination of putative control elements for a growth-phase dependent control of the *rnpB* transcription using a multicopy plasmid which carries the internally deleted *rnpB* gene because alteration of the putative control elements is easily manipulated in the plasmid.

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