

Effects of Overexpression of C5 Protein on *rnpB* Gene Expression in *Escherichia coli*

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Escherichia coli RNase P is composed of a large RNA subunit (M1 RNA) and a small protein subunit (C5 protein). Since both subunits are assembled in a 1:1 ratio, expression of M1 RNA and C5 protein should be coordinately regulated for RNase P to be efficiently synthesized in the cell. However, it is not known yet how the coordination occurs. In this study, we investigated how overexpression of C5 protein affects expression of the *rnpB* gene encoding M1 RNA, using a lysogenic strain, which carries an *rnpB-lacZ* transcription fusion. Primer extension analysis of *rnpB-lacZ* fusion transcripts showed that the overexpression of C5 protein increased the amount of the fusion transcripts, suggesting that *rnpB* expression increases with the increase of intracellular level of C5 protein.

Key Words: M1 RNA, C5 Protein, RNase P, Coordinate regulation

Introduction

The ribonucleoprotein enzyme RNase P was initially characterized as a tRNA-processing enzyme that removes extraneous 5' sequences from precursor tRNAs to generate mature 5' termini.¹ In addition to assisting with tRNA processing, RNase P also contributes to the processing of other non-tRNA substrates (e.g., 4.5S RNA and tmRNA) and assists with decay of several *Escherichia coli* mRNAs.²⁻⁶ *Escherichia coli* RNase P holoenzyme is composed of two subunits, including a large RNA subunit (M1 RNA, 377 nucleotides) and a small basic protein (C5 protein, 119 amino acids), which are encoded by *rnpB* and *rnpA*, respectively.^{7,8} While the M1 RNA can perform catalytic reactions in the absence of C5 protein *in vitro*,⁹ both components are essential *in vivo*.¹⁰ C5 protein stabilizes the catalytically active conformation of M1 RNA^{11,12} and modulates the substrate specificity of the RNase P reaction.¹³⁻¹⁷ C5 protein also functions as a metabolic stabilizer of M1 RNA in the cell.¹⁸ In RNase P holoenzyme, M1 RNA and C5 protein are assembled in a 1:1 ratio. Therefore, there should be a coordinate regulation between both components for efficient synthesis of RNase P holoenzyme in the cell. However, such a coordinate regulation has not been yet known.

In this study, we set to determine whether or not M1 RNA biosynthesis would be regulated by C5 protein. To do this, we examined using a lysogenic strain with an *rnpB-lacZ* transcription fusion how *rnpB* expression was changed by overexpression of C5 protein. We found that the overexpression of C5 protein increased the intracellular level of *rnpB-lacZ* fusion transcripts.

Experimental Section

Bacterial strain and plasmids. Strain MC1000 (λ RZ*rnpB-lacZ*), which carries the *lacZ* gene fused to the *rnpB* promoter,¹⁹ was used for analysis of *rnpB* transcription. The C5 protein-expressing plasmid used was pACTC5, which was previously constructed by the insertion of the *Ava*I-*Pvu*II DNA fragment of pSSC5 containing *lacI^f* gene and the *tac*

promoter-controlled *rnpA* gene into pACYC184.²⁰

Western blot analysis. The lysogenic cells containing plasmids were grown in LB containing ampicillin (50 μ g/mL) and tetracycline (25 μ g/mL) at 30 °C to an A₆₀₀ of about 0.5, and IPTG was added into the culture, and the culture was further incubated for 1 h. Cells were centrifuged down at 10,000 g for 10 min. After harvesting the cells, the cell pellet was suspended in cold phosphate buffered saline (pH 7.2) containing 50 mM Tris-HCl, pH 7.5, 120 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF, 200 μ M sodium orthovanadate, 1 mM PMSF, and protease inhibitor (CompleteTM; Roche) and sonicated. The soluble fraction was resolved on a 15% SDS-polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane (Amersham). Protein bands were immunostained with an anti C5 protein antibody, and were visualized by using an Enhanced Chemi-Luminescence Kit (Amersham).

Preparation of total cellular RNA. Lysogenic cells containing pACYC184 or pACTC5 were grown overnight in LB containing ampicillin (50 mg/mL) or tetracycline (25 μ g/mL) at 30 °C, diluted 1:100 in the same medium and grown to an A₆₀₀ of approximately 0.5 at 30 °C. Expression of C5 protein was induced via the addition of 0.01 mM IPTG, followed by 10-min incubation. For RNA stability assays, rifampicin (150 μ g/mL) was added to the cultures to stop transcription. Cultures were then analyzed at different time intervals. Total cellular RNA was prepared by hot phenol extraction, as described previously.²¹

Primer extension analysis. *rnpB-lacZ* fusion transcripts were analyzed by primer extension with primer R-cat (5'-TTGTCCTACTCAAGCTTGGCTGCAGGTCGA-3'), which is complementary to the CAT sequence. The primer was labeled with T4 polynucleotide kinase and [γ -³²P]-ATP at the 5' end. For the primer-annealing reaction, 0.6 pmole of the labeled primer was mixed with about 30 μ g total RNA in 10 μ L AMV RT buffer (Promega). The primer/RNA mixture was incubated at 65 °C and allowed to cool to 25 °C. After annealing, 3 μ L of 5 \times AMV RT buffer (Promega), 1.5 μ L of 20 mM dNTP mix, 1 μ L of RNasin (40 U, Promega), 2 μ L of AMV RT (40 U, Promega), and 7.5 μ L of RNase free water were added. The reaction mixture was incubated at 42 °C for 1 h

and the extension products were electrophoresed on a 5% polyacrylamide sequencing gel.

Results and Discussion

In order to determine if C5 protein would affect synthesis of M1 RNA, we examined the change of *rnpB* expression when cellular levels of C5 protein were increased. To regulate the intracellular level of C5 protein, plasmid pACYC5, an IPTG-inducible plasmid for expression of C5 protein, was used. The change of *rnpB* transcription was monitored by using a lysogenic strain, MC1000 (λ RZ*rnpB-lacZ*), which carries a *rnpB-lacZ* transcription fusion. The *rnpB-lacZ* fusion included *rnpB* gene sequence between -319 and +168 (where the *rnpB* transcription start is +1), a translation initiation signal with a part of the N-terminal region sequence of the chloramphenicol acetyltransferase (CAT) gene, and the *lacZ* coding sequence (Fig. 1). We introduced pACTC5 into the lysogenic strain. Then, the induced levels of C5 protein at various concentrations of IPTG were tested by western blot analysis (Fig. 2). The cellular levels of C5 protein were increased in an IPTG dose-dependent manner. Since we found that C5 protein was highly expressed even at 0.01 mM IPTG, that IPTG concentration was used for the protein induction in cells containing pACTC5 for analysis of *rnpB* transcription.

To analyze the change of intracellular levels of *rnpB-lacZ* fusion transcripts by overexpression of C5 protein, we performed primer extension analysis after inducing C5 protein. Total RNAs from cells containing pACTC5 that were grown in the absence or presence of 0.01 mM IPTG were used with a primer complementary to the N-terminal CAT region for primer extension analysis (Fig. 1). The amounts of primer extension products increased by about 50% with the IPTG induction (Fig. 3), suggesting that C5 protein increases *rnpB* transcription. However, the increase of *rnpB-lacZ* fusion transcripts was much less than that of C5 protein. We reasoned that C5 protein expressed from pACTC5 in the absence of IPTG might have been enough to increase the *rnpB* transcription. We observed the basal level expression of C5 protein from pACTC5 without the IPTG treatment. Furthermore, cells carrying pACTC5 produced *rnpB-lacZ* transcripts 14-fold more than did those carrying the control vector pACYC184. These results suggest that the increase of *rnpB* transcription by C5 protein in the cell is very effective.

One may argue that the increase of *rnpB-lacZ* transcripts by overexpression of C5 protein might have resulted from the increased stability of the transcripts by C5 protein. Since C5 protein is essential for maintaining the metabolic stability of



Figure 1. Schematic representation of the *rnpB-lacZ* fusion in strain MC1000 (λ RZ*rnpB-lacZ*). The CAT DNA fragment was introduced to provide the fusion with the S/D sequence and the translation start codon. The *rnpB-lacZ* transcript contains the 5' sequence of M1 RNA from nucleotides 1 to 168.

M1 RNA,¹⁸ it is also a plausible explanation that C5 protein may bind to the *rnpB-lacZ* transcripts through the 5' M1 RNA sequence (+1 to +168) to stabilize the transcripts. We examined whether the metabolic stability of the *rnpB-lacZ* fusion transcripts would be affected by overexpression of C5 protein. We

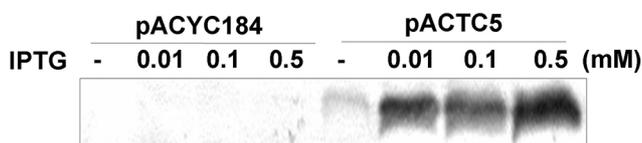


Figure 2. IPTG induction of C5 protein. MC1000 (λ RZ*rnpB-lacZ*) cells containing pACYC184 or pACTC5 were grown to A_{600} of 0.5 at 30 °C, and C5 protein was induced by adding various concentrations of IPTG. Total cellular proteins were electrophoresed on a 15% SDS polyacrylamide gel and electrotransferred to a nylon membrane. The cellular levels of C5 protein were analyzed by anti-C5 protein antibody.

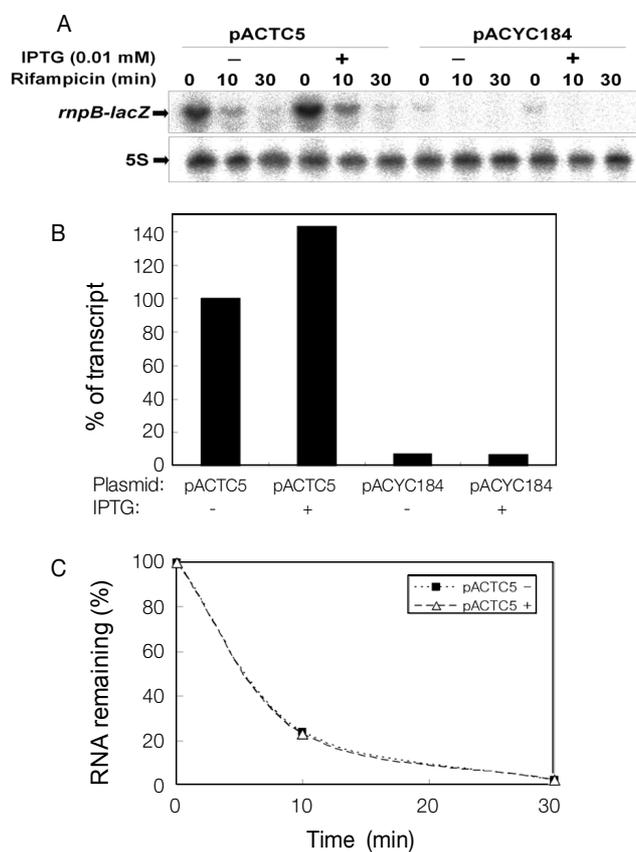


Figure 3. Primer extension analysis of *rnpB-lacZ* fusion transcripts. (A) MC1000 (λ RZ*rnpB-lacZ*) cells containing pACTC5 plasmids were grown to A_{600} of 0.5 at 30 °C, and 0.01 mM of IPTG was added in to the cultures to induce C5 protein. Total cellular RNAs were isolated from these cells. The levels of *rnpB-lacZ* fusion transcripts were measured by primer extension analysis using a primer complementary to the CAT region. The extended products were analyzed on a 5% polyacrylamide gel containing 7 M urea. (B) The relative *rnpB-lacZ* transcripts (%) are expressed relative to that in pACTC5-containing cells in the absence of IPTG after normalization to 5S RNA. (C) The remaining RNA (%) was calculated as a ratio of the amount of RNA at the indicated times to that at the zero time point after the addition of rifampicin in cells containing pACTC5 in the absence or presence of IPTG.

determined the half-lives of the fusion transcripts in cells carrying pACTC5 with or without the IPTG treatment and compared them. The IPTG induction did not change the half-life of the fusion transcript (Fig. 3C). Furthermore, the fusion transcript degraded with a half-life of about 5 min in the presence of excess C5 protein, which is about the same as the average half-life of mRNA.²² Therefore, it seems likely that the intracellular increase of C5 protein has nothing to do with the metabolic stability of the fusion transcript.

Given that expression of M1 RNA and C5 protein should be coordinately regulated for efficient synthesis of RNase P in the cell, transcriptional activation of *rnpB* by C5 protein shown in this study may play a key role in this coordinate regulation. This regulation pattern supplies the appropriate balance of M1 RNA needed against C5 protein for synthesis of RNase P. Furthermore, *rnpA* encoding C5 protein is the second gene of the *rpmH* operon,⁸ where the first *rpmH* gene encodes ribosomal protein L34, and consequently expression of C5 protein is linked to ribosome synthesis. Therefore, our results suggest that the expression of M1 RNA could be also controlled by the rate of ribosome synthesis.

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