Effects of Signal Peptide Peptidase A on Growth Inhibition by Overexpression of C5 Protein in *Escherichia coli*

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RNase P was initially identified as a tRNA-processing enzyme in *Escherichia coli*.[1] This enzyme generates the mature 5' termini by removing extra 5′ sequences from precursor tRNAs. RNase P is also known to be involved in processing of other non-tRNA substrates, such as 4.5S RNA and tmRNA, and in the degradation of several mRNAs.[2-5] *E. coli* RNase P is composed of two subunits, M1 RNA and C5 protein. M1 RNA can carry out the catalytic reaction in the absence of C5 protein *in vitro*,[6] whereas both subunits are required *in vivo* and for the efficient *in vitro* reaction.[7] C5 protein has functions of stabilizing the catalytically active conformation of M1 RNA[8] and modulating substrate specificity in the RNase P reaction.[9-11]

Overexpression of C5 protein can be induced by IPTG in cells containing a C5 protein-expression plasmid.[12] When C5 protein was overexpressed at the exponential phase, the cells stopped their growth (Fig. 1). However, if C5 protein was overexpressed as an MBP fusion protein,[13] the growth defect by overexpression of C5 protein was not observed.[14] It has been shown that MBP-C5 fusion protein can equally function as a cofactor for RNase P reaction. Therefore, C5 protein may be involved in some other metabolism than as a cofactor in RNase P reaction.[13] Since the MBP domain was fused to the N-terminus of C5 protein for the construction of MBP-C5 fusion protein, it is likely that the N-terminus of C5 protein participates in such metabolism. In this study, an experiment to search for genes that could restore the growth defect of C5 protein-overexpressing cells was set as an initial step for examining of the mechanism involved in the growth inhibition.

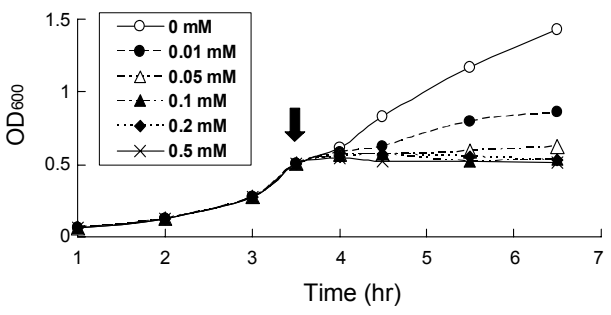

Figure 1. Cell growth inhibition by overexpression of C5 protein. Growth characteristics of JM109 cells containing pACTC5 in the presence of varying concentrations of IPTG were monitored by measuring OD_{600} values.

For this purpose, an experiment was designed in which an *E. coli* genomic library was introduced into C5 protein-overexpressing cells. Plasmid pACTC5, a derivative of the pACYC184 vector, was used as a C5 protein-overexpressing plasmid.[15] First, the minimum concentration of IPTG that inhibits growth of cells containing pACTC5 was determined (Fig. 1). Above 0.1 mM IPTG concentration the cell growth completely stopped. *E. coli* cells containing pACTC5 was transformed with a pBR322-based *E. coli* genomic library and clones that were able to survive in the presence of 0.1 mM IPTG were screened. Clone 6 was identified to restore the growth defect by C5 protein-overexpression (Fig. 2). The restriction mapping of clone 6 revealed that the size of insert DNA was about 4 kb. Its sequencing analysis showed that it contained the full sequences of *ydjA* and *sppA*. Since there is no open reading frame other than *sppA* and *ydjA* in the cloned DNA, either of the two genes seemed to be responsible for the growth restoration by overexpression of C5 protein. To test which gene was accountable for the growth restoration, subcloning experiments were performed. The *Sal*I/*Hin*dIII fragment carrying both *sppA* and *ydjA* from clone 6 was subcloned into pGEM3Zf(+) to generate pGHS. And the 1651-bp *Eco*RI fragment carrying *ydjA* but lacking the sequence encoding the C-terminal domain of *sppA* was subcloned to generate pGEE. Cells containing pGHS survived under condition of overexpression of C5 protein, but the presence of pGEE could not let cells survive under condition of overexpression of C5 protein. These results suggest that *sppA* is responsible for the restoration of the growth defect of C5 protein-overexpressing cells. The *sppA* gene encodes signal

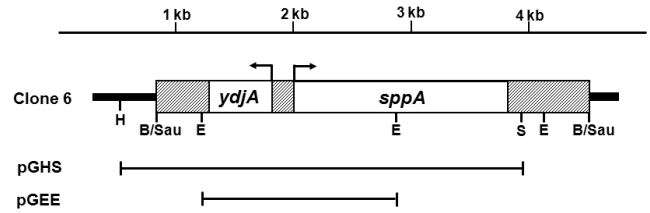

Figure 2. Schematic representation of the DNA insert in clone 6 and its subcloned DNA regions. The solid bar and the rectangle show the vector sequence and the *E. coli* chromosomal insert DNA, respectively. The *sppA* and *ydjA* coding sequences are shown as the open rectangle. H, *Hin*dIII; E, *Eco*RI; S, *Sal*I; B/Sau, *Bam*HI-*Sal*3AI junction. The arrows indicate the translation initiation sites. The 3394-bp *Hin*dIII-*Sal*I and 1651-bp *Eco*RI fragments from clone 6 were subcloned into pGEM3Zf(+) to generate pGHS and pGEE, respectively.

Table 1. Restoration of C5 protein-derived growth inhibition by *sppA*

IPTG (mM) / Plasmid	Growth restoration[a]					
	0	0.05	0.08	0.1	0.12	0.15
pGEM3Z5(+)	++	–	–	–	–	–
pGHS	++	++	++	++	++	++
pGEE	++	±	±	–	–	–

[a]Growth restoration tests were performed by observing the colony formation by *E. coli* JM109 cells containing pACTC5 and one of pGEM3Zf(+) derivatives on LB/ampicillin/chromaphenicol medium plate at 37 °C, in the presence or varying concentrations of IPTG. If the colony size was comparable to that in the absence of IPTG, the growth restoration was classified as ++ (the colony diameter was about 2 mm); cells which showed no visible colony formation were classified as –. Barely growing cells were classified as ± (the colony diameter was less than 0.3 mm).

peptide peptidase A (SppA), a protease that degrades the lipoprotein signal peptide.[16,17] Therefore, it seems likely that SppA removes overexpressed C5 protein by cleaving them. Since SppA is localized in the membrane as a signal peptidase, overexpressed C5 protein would be also present in the membrane and possibly exert its toxic effect there on the cells. It is noteworthy that the N-terminal region of C5 protein is composed of mostly hydrophobic residues with three positive charged residues,[18] which could localize C5 protein to the membrane. If this is the case, overexpression of C5 protein may perturb the membrane function and thereby inhibit the cell growth. Localization of C5 protein in the cell suggested by this study would be an interesting subject because its localization is the place where tRNA processing occurs.

Experimental Section

Bacterial strain and plasmids. The *E. coli* K-12 strain JM109 was used for plasmid construction and growth analysis. The *E. coli* genomic library carrying about 4 to 6 kb DNA fragments in the pBR322 vector was kindly provided from Prof. Y. Lee. The C5 protein-expressing plasmid used was pACTC5, which carries a *tac* promoter-controlled *rnp*A gene in pACYC184.[15] The cloning vector used for subcloning was pGEM3Zf(+).

Analysis of bacterial cell growth. *E. coli* cells containing pACTC5 were grown overnight in LB media containing 10 μg/mL of tetracycline. The overnight culture was diluted (1:100) into fresh media, and grown at 37 °C to an OD_{600} of 0.5 at 37 °C, as previously described.[19] Then IPTG solution was added to the cell culture at varying concentrations, and the culture was further incubated. Cell growth progression was monitored by OD_{600} readings at different time points.

Screening of clones restoring the growth defect of C5 protein-overexpressing cells. The *E. coli* library was introduced into JM109 cells containing pACTC5 and spread on LB-agar plates containing 10 μg/mL tetracycline, 50 μg/mL of ampicillin, and 0.1 mM IPTG. Colonies were selected after 20 h of incubation at at 37 °C. The insert DNAs were mapped by restriction enzymes and one of them was sequenced.

Growth restoration assay. *E. coli* cells containing both pACTC5 and one of pGEM3Zf(+) derivatives were grown overnight in LB media containing 10 μg/mL of tetracycline and 50 μg/mL of ampicillin. The cells were serially diluted and spread on LB-agar plates containing 10 μg/mL tetracycline, 50 μg/mL of ampicillin, and varying concentrations of IPTG. Then the colony formation was observed after 20 h of incubation at 37 °C.

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