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Article

Implications of *Streptomyces coelicolor* RraAS1 as an activator of ribonuclease activity of *Escherichia coli* RNase E

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Streptomyces coelicolor RraAS1의 *Eschechia coli* RNase E의 RNA 분해작용에 대한 활성제로서 기능 암시

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ABSTRACT: RNase E (Rne) is an essential enzyme involved in the processing and degradation of a large portion of RNAs in *Escherichia coli*. The enzymatic activity of RNase E is controlled by regulators of ribonuclease activity, namely, RraA and RraB. Gram-positive bacterium *Streptomyces coelicolor* also contains homologs of Rne and RraA, designated as RNase ES (Rns), RraAS1, and RraAS2. In the present study, we investigated the effect of *S. coelicolor* RraAS1 on the ribonucleolytic activity of RNase E in *E. coli*. Coexpression of RraAS1 with Rne resulted in the decreased levels of *rpsO*, *ftsZ*, and *rnhB* mRNAs, which are RNase E substrates, and augmented the toxic effect of Rne overexpression on cell growth. These *in vivo* effects appeared to be induced by the binding of RraAS1 to Rne, as indicated by the results of co-immunoprecipitation analysis. These results suggested that RraAS1 induces ribonucleolytic activity of RNase E in *E. coli*.

Key words: *Streptomyces coelicolor*, RNase E, RNase ES, RraA, RraAS1

Bacterial RNA degradation and processing are controlled by numerous factors including RNA structural determinants, RNA-binding factors, and ribonucleases. RNase E (Rne), an endoribonuclease, plays a major role in the degradation and processing of RNA transcripts in *Escherichia coli* (Ghora and Apirion, 1978; Lee *et al*., 2002, 2003). Rne is a large protein containing 1,061 amino acids and comprises two distinct halves, i.e., N-terminal and C-terminal halves. The conserved N-terminal half of Rne contains its catalytic activity, which is essential for cell viability, and the unstructured C-terminal half of Rne serves as a scaffold region for the assembly of a multi-protein complex, called degradosome (Kido *et al*., 1996; Callaghan *et al*., 2004).

Rne autoregulates its cellular concentration by cleaving the 5' UTR of its own transcript when its activity exceeds the cellular need (Mudd and Higgins, 1993; Jain and Belasco, 1995). In addition, protein inhibitors, regulators of ribonuclease activity A and B (RraA and RraB, respectively) (Lee *et al*., 2003; Gao *et al*., 2006) control the activity of Rne. RraA and RraB inhibit RNase E-induced endoribonucleolytic cleavage of selective group of transcripts by interacting with different regions of its C-terminal domain (Lee *et al*., 2003; Gao *et al*., 2006). A recent study showed that L4 ribosomal protein can also control the ribonucleolytic activity of RNase E (Singh *et al*., 2009). In addition, recent studies have shown that RraA modulates the RNA-binding and helicase activities by interacting with a DEAD box helicase (Gorna *et al*., 2010; Pietras *et al*., 2013).

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Previous studies showed that *Streptomyces coelicolor* endoribonuclease RNase ES (Rns), which shows RNase E-like activity, functionally complements Rne in *E. coli* (Hagege and Cohen, 1997; Lee and Cohen, 2003). Rns contains 1,340 amino acid proteins. The central portion of Rns (amino acids 563–973) shows 58.0% amino acid sequence similarity with the N-terminal catalytic region of *E. coli* Rne. Segments in both the termini of Rns (amino acids 1–562 and 974–1,340), which interact with the components of the degradosome (Lee and Cohen, 2003; Kim *et al*., 2007), contain motifs that are similar to those in the C-terminal half of *E. coli* Rne. *E. coli* RraA and RraB have been shown to interact with the scaffold domains of Rns and inhibit its enzymatic activity both *in vivo* and *in vitro* (Yeom *et al*., 2008b).

S. coelicolor contains homologs of *E. coli* RraA-like proteins, designated RraAS1 and RraAS2 (41.6% and 36.0% amino acid sequence similarity, respectively, with RraA) (Ahn *et al*., 2008). RraAS2 has been shown to inhibit RNase E activity. However, this inhibitory effect on RNase E activity does not restore proper processing and decay of Rne substrates, which is required for the normal growth of Rne-overproducing cells (Ahn *et al*., 2008). In the present study, we investigated whether RraAS1 can modulate the ribonucleolytic activity of *E. coli* RNase E *in vivo* by using a genetic system involving *rne*-knockout *E. coli* cell, whose viability was maintained through exogenous *rne* expression from a plasmid (Lee *et al*., 2002).

Materials and Methods

Strains and plasmids

The construction of *rne*-deleted *E. coli* strains that express full-length RNase E from pLAC-RNE2 (KSL2003) or express N-terminal Rne from pNRNE5 (KSL2002) have been previously described (Lee *et al*., 2002). pKAN6B-RraAS1 and pKAN6B-RraAS1-myc plasmids were constructed by ligating polymerase chain reaction (PCR) DNA digested with *Nde*I and *Xba*I restriction enzymes into the same sites in pKAN6B. DNA fragments containing the coding regions of RraAS1 and RraAS1-myc were amplified using primers RraAs1-Nde1-F (5′-GGAA TTCCATATGTTCATTGCTGCGGCGAC-3′) and RraAs1-Xba1-R (5′-GCTCTAGATCATCGGGCCACCACCGCGC-3′) for RraAS1 and primers RraAs1-Nde1-F and RraAS1-XbaI-R (5′-GCTCTAGATCACAGGTCCTCCTCTGAGATCAGCTTC TGCTCCATTCGGGCCACCACCGCGCG-3′) for RraAS1-Myc, using genomic DNA of *S. coelicolor* as the template.

RNA extraction and reverse transcription-PCR

Reverse transcription-PCR (RT-PCR) was performed as described previously (Yeom *et al*., 2008b; Yeom and Lee, 2006). The following primers were used for RT-PCR: rpsO 5′ RT (5′-GTACACTGGGATCGCTGAATT-3′) and rpsO 3′ RT (5′-GGCCCCCTTTTCTGAAACTCG-3′) for *rpsO*, ftsZ RT 5′ (5′-CCATATGTTTGAACCAATGGAA-3′) and ftsZ RT 3′ (5′-TTAATCAGCTTGCTTACG-3′) for *ftsZ*, rnhB RT 5′ (5′ -CCATATGATCGAATTTGTTTAT-3′) and rnhB RT 3′ (5′ -TCAGGACGCAAGTCCCAG-3′) for *rnhB*, and bdm 5′ RT (5′-ATGTTTACTTATTATCAGGCAG-3′) and bdm 3′ RT (5′ -TTAAAGCGTAGGGTGCTGGCCAC-3′) for *bdm*.

Co-immunoprecipitation

KSL2003 cells harboring pKAN6B-RraAS1-myc were cultured in LB medium containing 10 μM isopropylthiogalactoside (IPTG). Next, 1 mM IPTG and 0.2% arabinose were added to the cell culture when its optical density at 600 nm (OD_{600}) reached 0.1. The cells were grown further until OD_{600} of the culture reached 0.8, after which they were harvested for performing immunoprecipitation analyses. The cells were suspended in lysis buffer (1× TBS-T, 10 μg/ml lysozyme and 1 mM PMSF) and were sonicated. RraAS1-Myc and its associated complex or proteins were immunoprecipitated from cell lysates by using proG beads (Pierce), and were washed with 5 X TBS-T buffer and TDW. The immunoprecipitated proteins were eluted from the proG beads by heating the beads at 100°C for 10 min in a protein loading dye. Next, the samples were analyzed by performing western blotting.

Western blotting

The procedure for western blotting has been previously described (Yeom and Lee, 2006). Briefly, proteins were electrophoresed on an 8% SDS-polyacrylamide gel and were electrophoretically transferred onto nitrocellulose membranes (Protran, 0.45 μm; Whatman). The proteins were detected using

a monoclonal antibody against c-Myc (dilution, 1:1,000) and polyclonal antibodies against His-tag (dilution, 1:1,000) and ribosomal protein S1 (dilution, 1:20,000). The primary antibodies were detected using anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary antibody (400 μg/ml; diluted to 1:5,000 in TBS containing 0.1% BSA and 0.1% Tween 20; Santa Cruz Biotechnology) as the secondary antibody (1:5,000 dilution in TBS). The proteins were visualized using Western Blotting Luminol Reagent (Santa Cruz Biotechnology).

Results

Effects of RraAS1 coexpression on the growth of *E. coli* cells overexpressing Rne proteins

To investigate whether RraAS1 can regulate the ribonucleolytic activity of RNase E, we used *E. coli* strain KSL2003 lacking chromosomal *rne* and expressing exogenous *rne* from a plasmid under the control of an IPTG-inducible *lac*UV5 promoter (Lee *et al*., 2002). Addition of 10 μM IPTG induces the synthesis of C-terminally hexahistidine-tagged full-length Rne for the growth and survival of this strain (Lee *et al*., 2002; Yeom *et al*., 2008a).

The growth of KSL2003 cells was reduced by overexpression of Rne in the presence of 1 mM IPTG (Fig. 1A), which was similar to that observed previously (Yeom and Lee, 2006). To determine the effect of *S. coelicolor* RraAS1 on *E. coli* RNase E activity, we introduced a compatible kanamycin resistance (Km^r) plasmid expressing RraAS1 under the control of an arabinose-inducible promoter (pKAN6B-RraAS1) into KSL2003 cells. Our results showed that growth of KSL2003 cells over-expressing both Rne and RraAS1 in the presence of 1 mM IPTG and 0.2% arabinose was more inhibited than that of KSL2003 cells harboring an empty vector (pKAN6B). Propagation of toxic effect of RraAS1 on the growth of KSL2003 cells overexpressing Rne is not likely to result from RraAS1 overexpression itself because the growth of KSL2002 cells overexpressing both N-Rne and RraAS1 was similar to that of KSL2002 cells harboring the empty vector (pKAN6B) (Fig. 1B). Together, these results indicated that RraAS1 coexpression augmented the toxic effect of Rne overexpression on the growth of *E. coli* cells.

Physical interactions between RNase E and RraAS1

To test whether Rne interacts with RraAS1, C-terminally Myc-tagged RraAS1 (RraAS1-Myc) was overexpressed in KSL2002 and KSL2003 cells, and immunoprecipitated using an antibody against the Myc-tag. Coexpression of RraAS1-Myc in KSL2003 cells overexpressing Rne in the presence of 1 mM IPTG showed a growth pattern similar to that of KSL2003 cells coexpressing untagged RraAS1 and Rne (Fig. 1A), indicating that RraAS1-Myc is as active as RraAS1 in KSL2003 cells. As shown in Fig. 2, Rne but not N-Rne co-immunoprecipitated

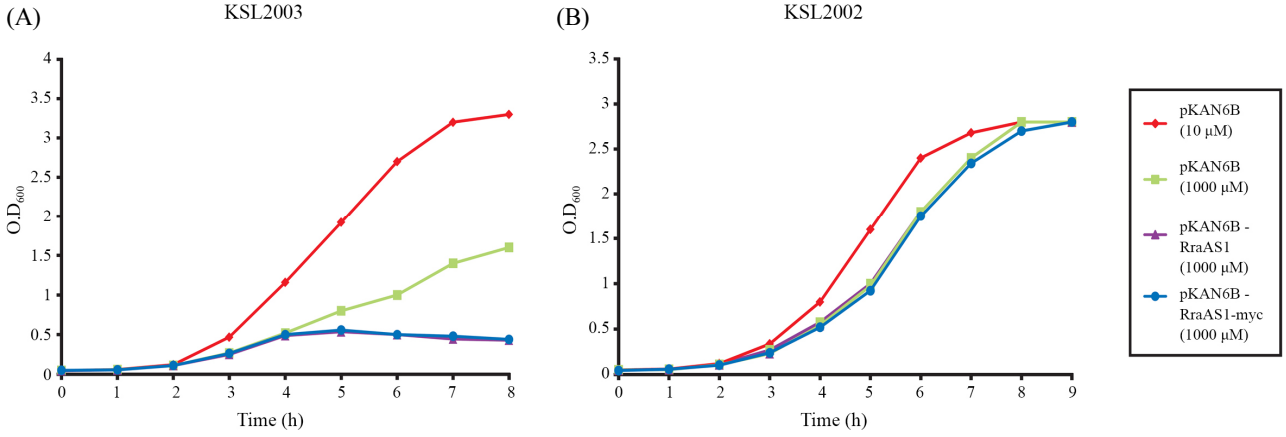

Fig. 1. Effects of RraAS1 coexpression on the growth of KSL2002 and KSL2003 cells. Effects of RraAS1 coexpression on the growth of *E. coli* cells overexpressing Rne (A) or the N-terminal region of Rne (B). KSL2002 or KSL2003 cells harboring pKAN6B or pKAN6B-RraAS1 were grown in LB medium containing 10 μM IPTG and 0.2% arabinose, and no additional IPTG (KSL2003 + pKAN6B + 10 μM IPTG) or 1,000 μM IPTG (KSL2003 + pKAN6B + 1,000 μM IPTG and KSL2003 + pKAN6-RraAS1 + 1,000 μM IPTG) were added when OD_{600} reached 0.1. The growth of the cells was monitored by analyzing cell density (OD_{600}) at indicated time intervals.

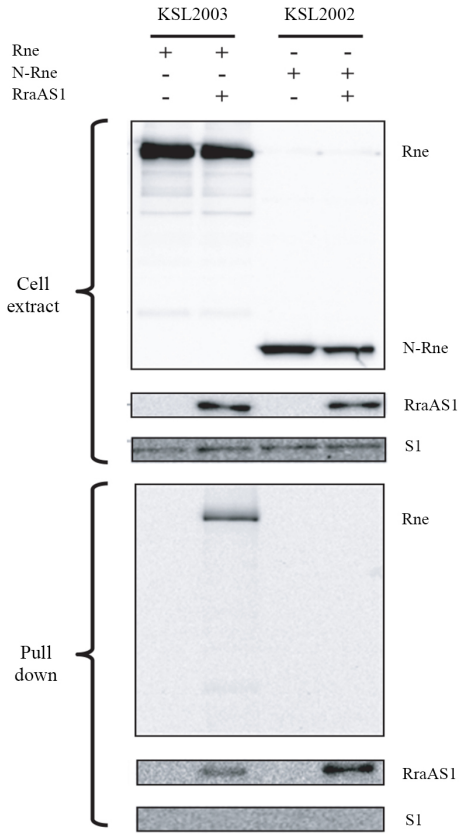

Fig. 2. Interaction between Rne and RraAS1. C-terminally Myc-tagged RraAS1 was expressed in KSL2002 or KSL2003 cells and was immunoprecipitated with a monoclonal antibody against the Myc-tag. Immunoprecipitated proteins were analyzed by performing Western blotting with polyclonal antibodies to His-tag for Rne proteins.

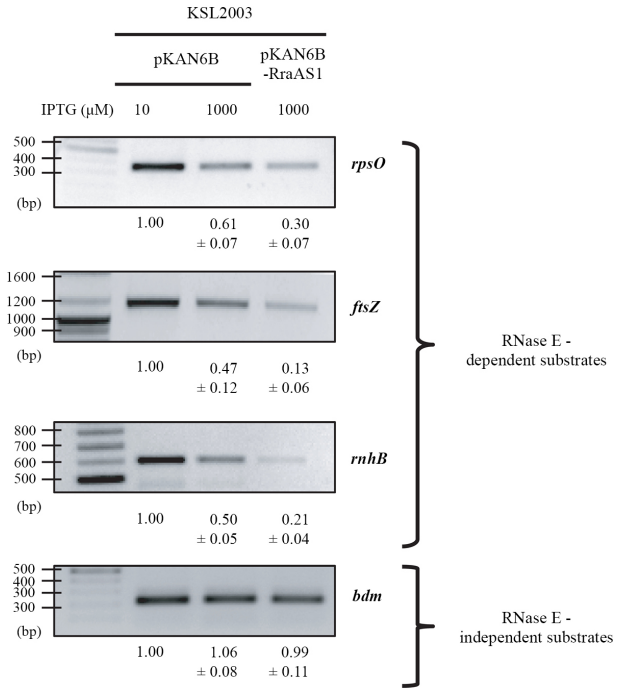

Fig. 3. Effects of RraAS1 coexpression on the levels of *rpsO*, *ftsZ*, and *rnhB* mRNAs in KSL2003 cells. Total RNAs were isolated from the cultures used in the growth curve of KSL2003 cells (Fig. 1A) and used for RT-PCR analysis. The steady-state levels of *bdm* mRNA were used as a non RNase E substrate. RT-PCR products were electrophoresed in a 1.0% agarose gel and intensity of bands was measured. The experiment was repeated at least three times, and standard errors of mean were designated as "± numbers".

with RraAS1-Myc. These data indicate that the C-terminal scaffold domain of Rne is required for high affinity binding of RraAS1-Myc to Rne.

Effects of RraAS1 coexpression on the ribonucleolytic activity of RNase E *in vivo*

To investigate whether RraAS1 can regulate the ribonucleolytic activity of RNase E *in vivo*, we analyzed steady-state levels of three RNase E substrates, *rpsO*, *ftsZ*, and *rnhB* mRNAs, in KSL2003 cells. Induced overexpression of Rne in KSL2003 cells resulted in decreased abundance of these mRNAs by approximately 30–50% than that in KSL2003 cells expressing Rne in the presence of 10 μM IPTG (Fig. 3). The degree of decrease in the levels of these mRNAs was further extended by approximately 70–90% when RraAS1 was coexpressed in KSL2003 cells overexpressing Rne in the presence of 1 mM IPTG. The levels of *bdm* mRNA, which is an RNase III substrate (Sim *et al*., 2010), were not significantly changed by RraAS1 coexpression and/or Rne overexpression. These results indicated that coexpression of RraAS1 enhanced a rapid degradation of RNase E substrates.

Discussion

We investigated whether RraAS1, a *S. coelicolor* homolog of RraA, can regulate the ribonucleolytic activity of RNase E in *E. coli*. Our results indicated that, unlike other RraA homologs including RraAS2 (Ahn *et al*., 2008) and RraAV1 (Lee *et al*., 2009), a *Vibrio vulnificus* RraA homolog, which exerts similar inhibitory effects on RNase E as RraA, RraAS1 appeared to activate RNase E activity in *E. coli*. This mode of RraAS1 action on RNase E activity is likely to be mediated by its interaction with the scaffold domain of Rne and probably

explains why the toxic effect of Rne overexpression on the growth of KSL2003 cells was propagated when RraAS1 was coexpressed. These results were unexpected because RraA and RraAV1, which were examined for their interaction with Rne and shown for the requirement of the C-terminal scaffold-domain of Rne for their high-affinity binding, showed inhibitory effect on RNase E activity in *E. coli* (Lee *et al*., 2003, 2009). Therefore, further studies should be performed to determine molecular mechanisms underlying this unique effect of RraAS1 on Rne and RraAS1-mediated RNA metabolism in *S. coelicolor*.

적 요

RNase E는 대장균(*Escherichia coli*)에서 수많은 RNA의 가공 및 분해에 관여하는 필수적인 효소이다. RNase E의 효소 활성은 RraA와 RraB에 의해 조절된다. 그람양성균인 *Streptomyces coelicolor*는 RNase ES, RraAS1, RraAS2라고 명명되는 RNase E와 RraA의 동족체를 가지고 있다. 이 연구에서는 *S. coelicolor* 유래의 RraAS1이 *E. coli*에서 RNase E의 효소활성을 저해하는지 연구하였다. 대장균에서 RraAS1의 발현은 RNase E의 과발현에 의해 감소된 세포생장을 더욱 저하시켰으며, RNase E의 기질인 *rpsO*, *ftsZ*, *rnhB* mRNA의 양을 감소시키는 것을 확인 하였다. 이러한 RraAS1의 효과는 공동면역침전실험을 수행한 결과에서 유추할 수 있듯이, Rne 단백질과 RraAS1의 결합으로 유도되는 것으로 보인다. 이러한 결과는 RraAS1이 대장균에서 RNase E의 리보핵산 가수분해 활성을 유도함을 시사한다.

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