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RraAS2 requires both scaffold domains of RNase ES for high-affinity binding and inhibitory action on the ribonucleolytic activity

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RraA is a protein inhibitor of RNase E (Rne), which catalyzes the endoribonucleolytic cleavage of a large proportion of RNAs in *Escherichia coli*. The antibiotic-producing bacterium *Streptomyces coelicolor* also contains homologs of RNase E and RraA, designated as RNase ES (Rns), RraAS1, and RraAS2, respectively. Here, we report that RraAS2 requires both scaffold domains of RNase ES for high-affinity binding and inhibitory action on the ribonucleolytic activity. Analyses of the steady-state level of RNase E substrates indicated that coexpression of RraAS2 in *E. coli* cells overproducing Rns effectively inhibits the ribonucleolytic activity of full-length RNase ES, but its inhibitory effects were moderate or undetectable on other truncated forms of Rns, in which the N- or/and C-terminal scaffold domain was deleted. In addition, RraAS2 more efficiently inhibited the *in vitro* ribonucleolytic activity of RNase ES than that of a truncated form containing the catalytic domain only. Coimmunoprecipitation and *in vivo* cross-linking experiments further showed necessity of both scaffold domains of RNase ES for high-affinity binding of RraAS2 to the enzyme, resulting in decreased RNA-binding capacity of RNase ES. Our results indicate that RraAS2 is a protein inhibitor of RNase ES and provide clues to how this inhibitor affects the ribonucleolytic activity of RNase ES.

Keywords: *Streptomyces coelicolor*, RNA stability, RNase ES, RraAS2

Introduction

The endoribonuclease RNase E (Rne) plays a pivotal role in the decay and processing of numerous RNAs in *Escherichia coli* (Ghora and Apirion, 1978; Jain and Belasco, 1995; Hagege and Cohen, 1997; Ow *et al*., 2000; Lee *et al*., 2002; Li and Deutscher, 2002; Lee and Cohen, 2003; Gao *et al*., 2006). RNase E, encoded by the *rne* gene, has functionally distinct halves. The highly conserved N-terminal domain (amino acid residues 1–529) possesses the hydrolytic endoribonuclease activity that is essential for cell viability (Kido *et al*., 1996; McDowall and Cohen, 1996). The unstructured C-terminal domain (530–1061) provides a scaffold for binding of multiple proteins, resulting in the RNA degradosome complex (Callaghan *et al*., 2004).

The cellular level and enzymatic activity of RNase E are coordinately controlled depending on cellular physiological conditions in *E. coli* via several mechanisms. RNase E cleaves the 5′ untranslated region (UTR) of its own transcript to maintain its relatively stable level (Mudd and Higgins, 1993; Jain and Belasco, 1995; Sousa *et al*., 2001). In addition, protein inhibitors of RNase E activity, RraA and RraB, bind to separate sites within the C-terminal domain of Rne and affect the composition of the degradosome (Ghora and Apirion, 1978; Lee *et al*., 2003; Gao *et al*., 2006). A recent study revealed that in addition to the ability of RraA homologs to inhibit the ribonucleolytic activity of RNase E-like enzymes, those found in some bacterial species have a divalent-metal-ion-dependent 4-hydroxy-4-methyl-2-oxoglutarate/4-carboxy-4-hydroxy-2-oxoadipate aldolase activity (Mazurkewich *et al*., 2014). It has also been shown that RraA can bind to and inhibit ATP-dependent DEAD-box RNA helicases such as RhlB and SrmB (Lee *et al*., 2003; Gao *et al*., 2006; Gorna *et al*., 2010). Nonetheless, the sites of the RraA molecule involved in the interaction with the RNase E have yet to be elucidated.

The antibiotic-producing bacterium *Streptomyces coelicolor* contains genes that encode orthologs of *E. coli* RNase E as well as two RraA-like proteins, designated as RNase ES (Rns), RraAS1, and RraAS2, respectively (Lee and Cohen, 2003; Ahn *et al*., 2008). RNase ES is a 1340-amino acid protein whose central portion (amino acids 563–973) has a 58.0% amino acid sequence similarity with the N-terminal domain of RNase E. Although RNase ES has an N-terminal extension from the catalytic region that is dissimilar to *E. coli* RNase E, the segments in both termini of RNase ES (amino acid positions 1–562 and 974–1340) bear arginine-rich, proline-rich, and discrete putative acidic regions that are similar to the C-terminal domain of RNase E (Hagege and Cohen, 1997; Lee and Cohen, 2003). RraAS1 and RraAS2 show 41.6% and 36.0% amino acid sequence similarity, respectively, with RraA. These RraA-like proteins from *S. coelicolor* have lower homology with RraA and are ~60 amino acid residues longer than previously characterized RraA orthologs: *Vibrio vulnificus* RraAV1 and RraAV2 (Lee *et al*., 2009, 2011; Kim *et al*., 2016).

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We recently characterized the ability of these *S. coelicolor* RraA homologs to inhibit the ribonucleolytic activity of RNase ES and the mechanism of their action. In this report, we present experimental results from studies on RraAS2; those from RraAS1 studies will be reported elsewhere.

Materials and Methods

Strains and plasmids

The construction of *rne*-deleted *E. coli* strains that express full-length Rns (KSL2005) or its truncated form (KSL2006, KSL2007, or KSL2008) has been described previously (Lee and Cohen, 2003). The pKAN6B-RraAS2 and pKAN6B-RraAS2-Myc vectors were constructed by ligating polymerase chain reaction (PCR) products (DNA fragments) digested with restriction enzymes *Nde*I and *Xba*I into the same site of pKAN6B (Yeom and Lee, 2006). We synthesized PCR products containing coding regions of RraAS2 and RraAS2-Myc using primers RraAS2-NdeI-F1 (5′-AATTCATATGTCAC TCCCCCGTCCC-3′) and RraAS2-XbaI-R (5′-ATTATCT AGATCACCGCTTCGAGGCGCGCCAC-3′) for RraAS2 and primers RraAS2-NdeI-F1 and RraAS2-XbaI-R (5′-GC TCTAGATCACAGGTCCTCCTCTGAGATCAGCTTCT GCTCCCGCTTCGAGGCGCGCCACT-3′) for RraAS2-Myc, with the genomic DNA from *S. coelicolor* as a template. pET15b-rraAS2 was constructed by ligating PCR products digested with *Nde*I and *Bam*HI restriction enzymes into the pET15b vector. We synthesized PCR fragments containing coding regions of RraAS2 using primers RraAS2-NdeI-F2 (5′-GGAATTCCATATGTCACTCCCCCGTCCCGC-3′) and RraAS2-BamHI-R (5′-CGGGATCCTCACCGCTTCGAGG C-3′) for RraAS2 with the genomic DNA from *S. coelicolor* as a template.

Measurement of the plasmid copy number

The procedure for measuring a plasmid copy number has been described previously (Lee and Cohen, 2003). Briefly, KSL2005–2008 cells were cultured in the Luria-Bertani (LB) medium containing 10 μM isopropylthiogalactoside (IPTG). Then, 1 mM IPTG and 0.2% arabinose were added when the cell culture reached optical density at 600 nm (OD_{600}) of 0.1. The cells were further grown to OD_{600} of 1.0 and harvested to obtain plasmid DNA. The plasmids were digested with restriction enzymes *Afl*II and *Hin*dIII, corresponding to the unique site in pRNES101-104 as well as pKAN6B and pKAN6B-RraAS2. The digested plasmid DNA was analyzed by electrophoresis in a 0.9% agarose gel and stained with ethidium bromide. The plasmid copy number was calculated relative to concurrently present pSC101 derivatives (pRNES 101-104), whose replication is independent of Rns, by measuring the molar ratio of the pSC101 derivatives to the ColE1-type plasmid (pKAN6B and pKAN6B-RraAS2).

RNA preparation and reverse transcriptase (RT) PCR

The procedure for RT-PCR analysis was described previously (Kim *et al*., 2016). The primers were ftsZ 5′RT (5′-CCATA TGTTTGAACCAATGGAA-3′) and ftsZ 3′RT (5′-TTAAT CAGCTTGCTTACG-3′) for *ftsZ*.

Protein expression and purification

These procedures have been described elsewhere (Lee and Cohen, 2003). Hexahistidine-tagged Rns, M-Rns, and RraAS2 were purified from KSL2005, KSL2008, and BL21 (DE3) cells harboring pET15b-rraAS2.

Immunoprecipitation

KSL2005–2008 cells were cultured in the LB medium containing 10 μM IPTG. Next, 1 mM IPTG and 0.2% arabinose were added when the cell culture reached OD_{600} of 0.1. The cells were further grown to OD_{600} of 1.0 and harvested for immunoprecipitation experiments. The cells were resuspended in lysis buffer (1% NP-40, 150 mM NaCl, 20 mM Tris-HCl, pH 7.5) and lysed by means of a French Press. The RraAS2-Myc protein and its associated proteins were separated from the lysate using ProG beads (Pierce), and washed with 1× PBS-T buffer (PBS containing 0.05% Tween 20). Then, the proteins were eluted from the ProG beads in protein loading dye by heating for 10 min at 100°C. RraAS2 was detected by means of a monoclonal antibody against Myc (1: 200 dilution).

In vivo cross-linking and purification of cross-linked Rns and RNA complexes

These procedures have been described previously (Lim and Lee, 2015). KSL2005 cells harboring pKAN6B or pKAN6B-RraAS2-myc were cultured to OD_{600} of 0.1 in the presence of 10 μM IPTG, and then 1 mM IPTG and 0.2% arabinose were added to the cultures. The cells were further grown to OD_{600} of 1.0 and harvested for *in vitro* cross-linking experiments. The cells were resuspended in ice-cold PBS, and 37% formaldehyde was added to the final concentration of 1% with incubation for 10 min with gentle rotation at room temperature. The cross-linking reaction was quenched by addition of 1 M glycine (pH 7.0) to the final concentration of 0.25 M with incubation for 5 min at room temperature. Next, we washed the cells with ice-cold PBS twice. The cells were resuspended in lysis buffer (0.5% NP-40, 0.5% Tween 20, 50 mM KCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride [PMSF], 10 mM Tris-HCl, pH 7.9) and lysed by means of a French Press. The Rns-His protein and its associated complex were separated from the lysate using Ni-NTA agarose beads (Qiagen). The beads were then washed four times with lysis buffer, and the proteins were eluted with PBS containing 400 mM imidazole. Rns was detected using an anti-His monoclonal antibody (Qiagen). To isolate cross-linked RNA, 20 μg of proteinase K was added with incubation at 42°C for 30 min for digestion. The formaldehyde cross-links were reversed by incubation at 70°C with gentle rotation for 45 min. The cross-linked RNA was purified using the TRIzol Reagent (Ambion).

An *in vitro* cleavage assay

The procedures for radiolabeling of BR10 + hpT and the *in vitro* RNase E cleavage assay have been described previously (Kim *et al*., 2016). Briefly, BR10 + hpT was labeled at the 5′ end with [γ-^{32}P]ATP using T4 polynucleotide kinase (TaKaRa), and the labeled products (p-BR10 + hpT) were purified on

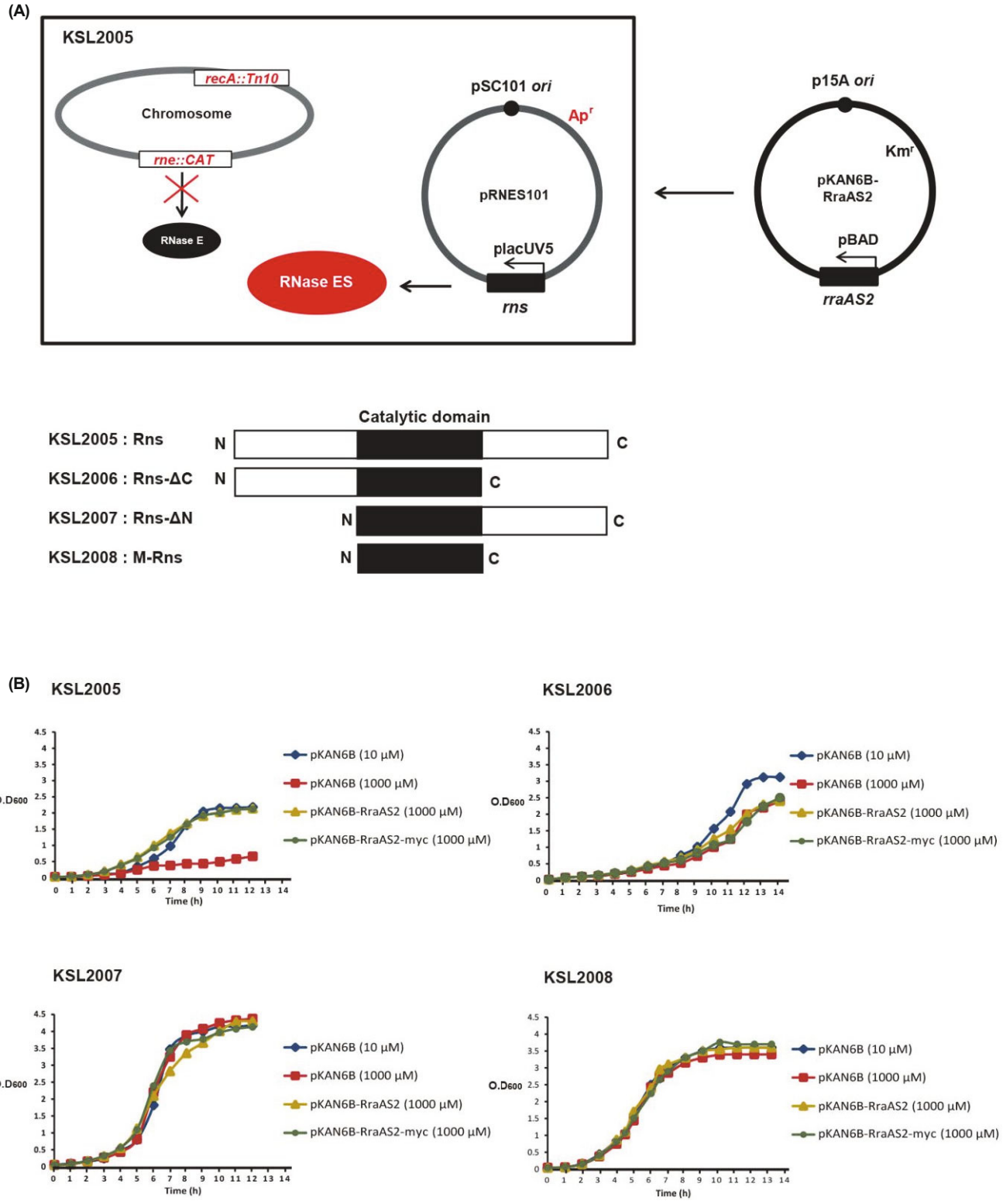

Fig. 1. Effects of RNase ES overproduction and coexpression of RraAS2 on *E. coli* growth. (A) Coexpression of full-length Rns or its derivatives and the RraAS2 expression procedure. Promoters for plasmids (pBAD, placUV5), the replication origin of plasmids (pSC101 *ori*, p15A *ori*), and antibiotic markers (Ap^r, Km^r) are indicated. (B) Effects of coexpression of RraAS2 in *E. coli* cells overproducing RNase ES on growth. The cultures of KSL2005-2008 cells harboring pKAN6B or pKAN6B-RraAS2 were grown in the LB medium containing 10 μM IPTG. Then, 0.2% arabinose and either no additional IPTG or 1 mM IPTG was added to the cultures with cell density (OD_{600}) of 0.1, and growth was monitored by analysis of cell density at time intervals. 10 μM and 1,000 μM in parentheses indicate IPTG concentrations.

MicroSpin™ G25 columns (GE Healthcare). Approximately 0.5 pmol of p-BR10 + hpT was preincubated with 0.2 pmol of purified Rns or 0.3 pmol of M-Rns proteins at varying concentrations of RraAS2 on ice for 10 min in 20 μl of 200 mM Tris-HCl buffer (pH 8.0) containing 1 M NaCl, 1 mM DTT, 50 mM $MgCl_2$, and 50% (v/v) glycerol. The cleavage reaction proceeded at 37°C for 2 h. The reaction products were separated using electrophoresis in a 12% denaturing polyacrylamide gel.

Results

Effects of coexpression of RraAS2 on the growth of *E. coli* cells overproducing Rns proteins

To resolve the question whether *S. coelicolor* RraAS2 can regulate the ribonucleolytic activity of RNase ES, we used *E. coli* strains with chromosomally deleted *rne* and harboring pRNES101 (KSL2005), pRNES102 (KSL2006), pRNES103 (KSL2007), or pRNES104 (KSL2008), which direct the synthesis of Rns, Rns-ΔC, Rns-ΔN, or M-Rns, respectively, under the control of an IPTG-inducible *lacUV5* promoter (Lee and Cohen, 2003) (Fig. 1A). Addition of 10 μM IPTG allowed for the synthesis of Rns or truncated forms of Rns that support normal cell growth of the *rne*-deleted *E. coli* cells, as shown elsewhere (Lee and Cohen, 2003). Expression levels of Rns proteins and their size have been also shown previously (Lee and Cohen, 2003; Yeom *et al*., 2008). The growth of KSL2005 cells was strongly retarded by overexpression of Rns in the presence of 1 mM IPTG, whereas overexpression of truncated derivatives (Rns-ΔN and M-Rns) did not significantly affect the bacterial growth. Overexpression of Rns-ΔC moderately inhibited the growth of KSL2006 cells. These results suggested that the degree of *in vivo* ribonucleolytic activity of the above proteins might have been different, as has been reported for RNase E; elevated RNase E activity by overexpression or hyperactive mutations inhibited normal growth of *E. coli* cells whereas overexpression of N-Rne did not (Lee *et al*., 2002; Lee and Cohen, 2003; Go *et al*., 2011). Next, to test the effects of RraAS2 on the ribonucleolytic activity of Rns proteins, a compatible Km^r plasmid expressing RraAS2 under the control of an arabinose-inducible promoter (pKAN6B-RraAS2) was introduced into KSL2005–2008 cells. KSL2005 cells overproducing both proteins (Rns and RraAS2) in the presence of 1 mM IPTG and 0.2% arabinose were able to grow at rates similar to those of the KSL2005 cells harboring an empty vector (pKAN6B) when grown in the medium containing 10 μM IPTG and 0.2% arabinose (Fig. 1B). Coexpression of RraAS2 and truncated forms of Rns in KSL2006–2008 cells did not significantly affect the growth rate of these cells (Fig. 1B). These results implied that RraAS2 effectively modulated the ribonucleolytic activity of full-length Rns to support their normal growth.

Effects of coexpression of RraAS2 on the ribonucleolytic activity of RNase ES *in vivo*

To test whether RraAS2 could modulate the ribonucleolytic activity of Rns proteins *in vivo*, two of the known RNase E substrates in *E. coli* were analyzed at their steady-state level. The *in vivo* activity of Rns proteins toward RNA I, an antisense repressor of ColE1-type plasmid replication, was assessed by measuring the relative copy number of a ColE1-origin plasmid (pKAN6B or pKAN6B-RraAS2) to a pSC101-origin plasmid (pRNES101–104) in *E. coli* KSL2005–2008 cells when RraAS2 was conditionally coexpressed by addition of 0.2% arabinose. Induced expression of Rns, Rns-ΔC, or Rns-ΔN in the presence of 1 mM IPTG in KSL2005–2007 cells increased the copy number of the ColE1-type plasmid pKAN6B 1.5- to 2.4-fold relative to the copy number observed in cells expressing Rns in the presence of 10 μM IPTG. In contrast, no significant change in the copy number of the ColE1-type plasmid pKAN6B was detected in KSL2008 cells. When RraAS2 was coexpressed in the presence of 0.2% arabinose, the increased ColE1-type plasmid copy number in KSL2005 cells overexpressing Rns in the presence of 1 mM IPTG was reduced to a level that optimally supported the growth of these cells in the presence of 10 μM IPTG. On the other hand, the inhibitory activity of RraAS2 on other truncated Rns derivatives was not detected in terms of the ColE1-type plasmid copy number. These results indicated that RraAS2 effectively inhibited the ribonucleolytic activity of full-length Rns, not that of truncated forms, on RNA I *in vivo* (Fig. 2A).

We further studied the ribonucleolytic activity of Rns proteins on another RNase E substrate, *ftsZ* mRNA (Lee *et al*., 2009), when RraAS2 was coexpressed. The abundance of *ftsZ* mRNA was measured using semiquantitative RT-PCR. The results showed that coexpression of RraAS2 efficiently inhibited Rns action on *ftsZ* mRNA in KSL2005 cells, resulting in increased abundance of *ftsZ* mRNA *in vivo*; however, no significant changes were observed in KSL2006–2008 cells when RraAS2 was coexpressed (Fig. 2B). We observed that truncated forms of Rns protein have different levels of ribonucleolytic activity as well as substrate specificity compared to the full-length Rns as indicated by variations in the copy number of ColE1-type plasmid and the abundance of *ftsZ* mRNA when Rns proteins were overexpressed in KSL2005–2008 strains.

Physical interactions between Rns and RraAS2

To identify the basis for the observed different inhibitory effects of RraAS2 on Rns and on its truncated derivatives, physical interactions between these proteins were examined in immunoprecipitation experiments. C-terminally Myc-tagged RraAS2 (RraAS2-Myc) was coexpressed in KSL2005–2008 cells and immunoprecipitated with a monoclonal antibody against Myc-tag. Coexpression of RraAS2-Myc from pKAN6B-RraAS2-myc in these cells overproducing Rns proteins in the presence of 1 mM IPTG resulted in growth patterns similar to those coexpressing untagged RraAS2 protein from pKAN6B-RraAS2, indicating that RraAS2-Myc is as functional as RraAS2 *in vivo* (Fig. 1B). Immunoprecipitated RraAS2-Myc was analyzed for Rns, Rns-ΔC, Rns-ΔN, or M-Rns by western blot analysis. We found that RraAS2 was abundantly coprecipitated with full-length Rns, whereas Rns-ΔC, Rns-ΔN, or M-Rns were not detected (Fig. 2C). These results showed that the segments in both termini of Rns are required for high-affinity binding of RraAS2 to the Rns protein; this finding

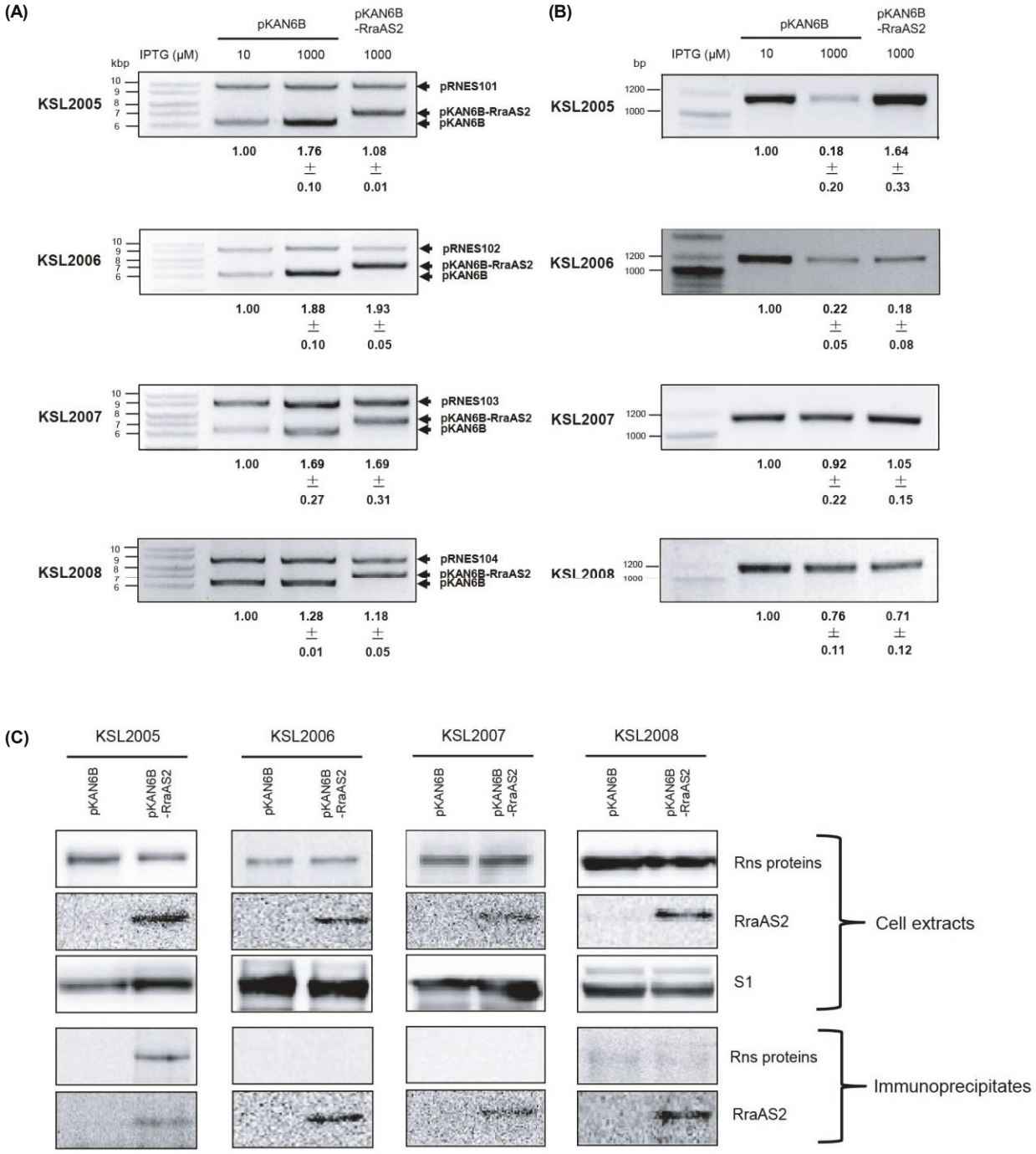

Fig. 2. Effects of coexpressed RraAS2 on the ribonucleolytic activity of RNase ES *in vivo*. (A) Effects of coexpressed RraAS2 on the copy number of ColE1-type plasmids. Plasmid DNA was isolated from the cultures used in Fig. 1B. Plasmids were digested with restriction enzymes *Afl*II and *Hin*dIII, subjected to electrophoresis in a 0.9% agarose gel, and stained with ethidium bromide. The plasmid copy number was calculated by measuring the molar ratio of the concurrently present pSC101 derivatives (pRNES101–104), which is independent of Rne, to ColE1-type plasmids (pKAN6B or pKAN6B-RraAS2). Densitometric analyses of the bands corresponding to each plasmid were based on conversion to actual ratios after normalizing the values to the size of ColE1-type plasmids and are shown at the bottom of the gel. (B) Effects of coexpressed RraAS2 on the steady-state level of *ftsZ* mRNA. Total RNA was isolated from KSL2005–2008 cells grown to OD_{600} of 1.0 in the same way as described in Fig. 1B, and RT-PCR was performed. The relative abundance of each mRNA is shown at the bottom of the gels. The experiments were performed at least three times, and the standard error of the mean is used to indicate the range of the assay results in the graph. (C) Interactions of Rns proteins with RraAS2. The cultures of KSL2005–2008 cells harboring pKAN6B or pKAN6B-RraAS2 were grown in the same way as described in Fig. 1B. Myc-tagged RraAS2 was purified using ProG beads, and then copurified Rns or its derivatives were identified by western blot analysis using an anti-Rns polyclonal antibody. A monoclonal antibody against Myc-tag was used to detect RraAS2-Myc. An anti-S1 polyclonal antibody was used to detect ribosomal protein S1, which was used as an internal standard to evaluate the amount of the cell extract loaded in each lane.

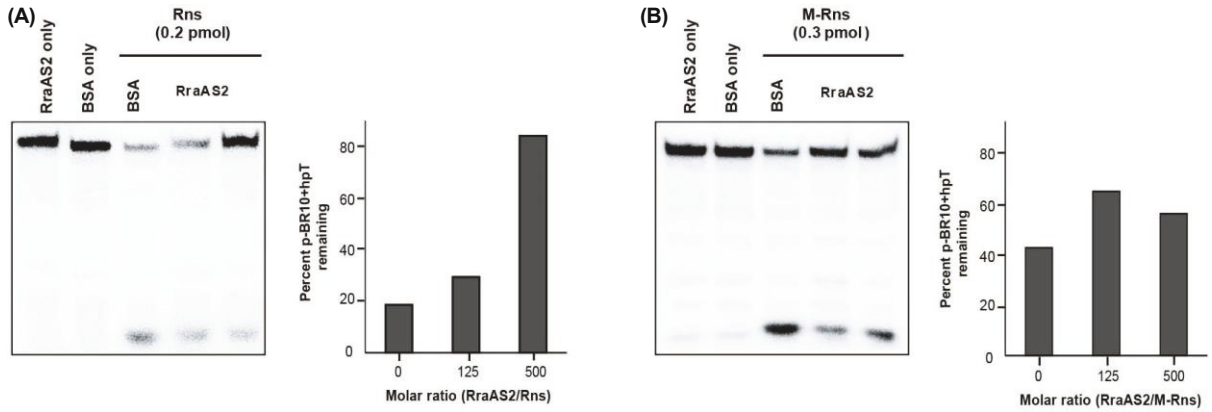

Fig. 3. Effects of RraAS2 on the ribonucleolytic activity of RNase ES *in vitro*. RraAS2 inhibition of p-BR10 + hpT cleavage by Rns *in vitro*. Two picomoles of 5′-end-labeled p-BR10 + hpT RNA was incubated with 0.2 pmol of Rns (A) or 0.3 pmol of M-Rns (B) with 400 pmol of BSA or varying concentrations of RraAS2 (as indicated in the graphs) in 20 μl of 1× cleavage buffer at 37°C for 2 h. The samples were mixed with an equal volume of loading buffer, and then denatured at 65°C for 5 min and loaded onto a 12% polyacrylamide gel containing 8 M urea. The percentage of uncleaved p-BR10 + hpT in the gel was quantitated using a phosphorimager and OptiQuant software.

explains why RraAS2 effectively inhibited the ribonucleolytic activity of full-length Rns, not of truncated forms, *in vivo*.

Inhibition of the catalytic activity of RNase ES by RraAS2 *in vitro*

To determine whether the inhibitory effect of RraAS2 on the ribonucleolytic activity of RNase ES *in vivo* results from direct action of RraAS2 on the enzyme, *in vitro* cleavage assays were performed. RraAS2, Rns, and M-Rns were affinity-purified and incubated with a 5′-^{32}P-end-labeled synthetic BR10 + hpT (p-BR10 + hpT) transcripts, which are a truncated form of RNA I that contains an RNase E cleavage sequence (Kim *et al*., 2016). RraAS2 strongly inhibited the cleavage of p-BR10 + hpT by the full-length Rns (Fig. 3A), whereas the inhibitory effect of RraAS2 on M-Rns was moderate (Fig. 3B). These results revealed that RraAS2 inhibited the ribonucleolytic activity of RNase ES via its direct action on the catalytic activity of the enzyme; this interaction required both the scaffold domains and the catalytic domain of Rns.

Alterations in the RNA-binding capacity of RNase ES under the influence of RraAS2 *in vivo*

In order to determine the reason for the decreased ribonucleolytic activity of RNase ES during interaction with RraAS2, we analyzed the RNA bound to Rns by *in vivo* cross-linking with purification of Rns-RNA complexes. RraAS2 was coexpressed in KSL2005 cells, and Rns was purified with Ni-NTA agarose beads. The amount of *ftsZ* mRNA that was cross-linked to Rns was decreased by approximately two-fold when RraAS2 was coexpressed, but it was approximately four-fold more abundant in KSL2005 cells coexpressing RraAS2 as compared to the cells harboring pKAN6B (Fig. 4). These results indicated that RraAS2 binding resulted in the de-

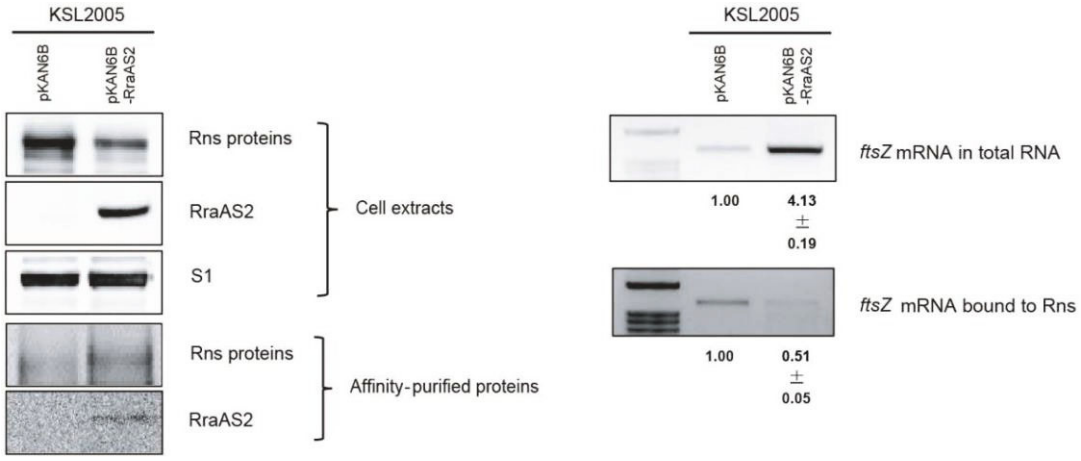

Fig. 4. Effects of RraAS2 on the RNA-binding capacity of RNase ES *in vitro*. KSL2005 cells harboring pKAN6B or pKAN6B-RraAS2 were grown in the LB medium with 10 μM IPTG. Then, 0.2% arabinose and 1 mM IPTG were added to the cultures at OD_{600} = 0.1. Culture samples were harvested in the log phase (OD_{600} = 1.0). Subsequently, the cells were cross-linked with formaldehyde and harvested to purify Rns by means of Ni-NTA agarose beads. The amount of purified Rns was analyzed by a western blot assay (left panel). RNA samples were isolated and analyzed by semiquantitative RT-PCR with *ftsZ*-specific primers (right panel).

creased RNA-binding capacity of Rns.

Discussion

RraA has global modulatory effects on transcript levels in *E. coli* as an inhibitor of RNase E (Lee *et al*., 2003). RraA homologs from other bacteria, including RraAS2 in *S. coelicolor* and RraAV1 in *V. vulnificus*, also exert the inhibitory effect on the ribonucleolytic activity of *E. coli* RNase E (Lee *et al*., 2003, 2009; Ahn *et al*., 2008). In addition, a recent study identified RraAV1 as a protein inhibitor of RNase EV: a *V. vulnificus* ortholog of RNase E (Kim *et al*., 2016). These studies are indicative of the conserved role of RraA homologs in modulation of the ribonucleolytic activity of RNase E-like enzymes. In this study, our findings showed that RraAS2 is an inhibitor of RNase ES activity; this result further supports the above notion.

All RraA homologs examined so far–including RraAS2 in this study–appear to require unstructured scaffold regions for their high-affinity binding to and inhibitory action on RNase E-like enzymes. Although it still remains unknown how RraA homologs inhibit the ribonucleolytic activity of RNase E-like enzymes, our study showed that RraAS2 binding to Rns led to a decrease in RNA-binding capacity of the enzyme. Further studies will reveal the molecular mechanism of RraAS2's interference with RNA-binding activity of Rns as well as its role in RNase ES-mediated RNA cleavage in *S. coelicolor*.

Acknowledgements

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