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RNase E Polypeptides Lacking a Carboxyl-Terminal Half Suppress a *mukB* Mutation in *Escherichia coli*

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We have isolated suppressor mutants that suppress temperature-sensitive colony formation and anucleate cell production of a *mukB* mutation. A linkage group (*smbB*) of the suppressor mutations is located in the *rne*/*ams*/*hmp* gene encoding the processing endoribonuclease RNase E. All of the *rne* (*smbB*) mutants code for truncated RNase E polypeptides lacking a carboxyl-terminal half. The amount of MukB protein was higher in these *rne* mutants than that in the *rne*$^+$ strain. These *rne* mutants grew nearly normally in the *mukB*$^+$ genetic background. The copy number of plasmid pBR322 in these *rne* mutants was lower than that in the *rne*$^+$ isogenic strain. The results suggest that these *rne* mutations increase the half-lives of *mukB* mRNA and RNAI of pBR322, the antisense RNA regulating ColE1-type plasmid replication. We have demonstrated that the wild-type RNase E protein bound to polynucleotide phosphorylase (PNPase) but a truncated RNase E polypeptide lacking the C-terminal half did not. We conclude that the C-terminal half of RNase E is not essential for viability but plays an important role for binding with PNPase. RNase E and PNPase of the multiprotein complex presumably cooperate for effective processing and turnover of specific substrates, such as mRNAs and other RNAs in vivo.

RNase E was first defined as a processing endoribonuclease that catalyzes the maturation of 5S rRNA (2, 3, 17). Mutations in the *rne*/*ams* gene have a stabilizing effect on the bulk of mRNAs (4, 37, 44). This enzyme also cleaves RNAI (27, 33, 52), the antisense RNA regulating ColE1-type plasmid replication (53), and is involved in the processing and turnover of many bacterial and bacteriophage mRNAs (14, 16, 23, 28–30, 35, 42, 46) (for a review, see reference 11). The *Escherichia coli rne*/*ams*/*hmp* gene codes for the RNase E protein of 1,061 amino acids (8). The RNase E protein migrates as a 170- to 180-kDa polypeptide in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7, 12, 35). Taraseviciene et al. (50) concluded that the RNA binding domain and catalytic functions overlap in the N-terminal half of the protein. The more precise mapping reported that, while the catalytic function of RNase E is in the N-terminal half, the RNA binding domain is located in the C-terminal half and does not overlap with the catalytic domain (31). It would be more accurate to state that the N-terminal half of Rne (residues 1 to 498) has the catalytic function of RNase E (31, 50) while a nonoverlapping RNA binding activity has been mapped near the start of the C-terminal half (50). RNase E, polynucleotide phosphorylase (PNPase), and 3′-to-5′ exonucleases were found in the same multiprotein complex in *E. coli* cell extracts (6, 45).

The MukB protein of *E. coli* has an important role in proper partitioning of the replicated sister chromosomes into two daughter cells (40, 41, 57, 59, 60). The MukB protein consists of 1,484 amino acid residues (57). MukB has been suggested to be a motor protein essential for chromosome partitioning (41) (for reviews, see references 18 and 19). We described previously the *mukB106* mutant (41, 57), which shows temperature-sensitive colony formation and anucleate cell production at permissive temperature.

In this work, we have isolated extragenic suppressor mutants which suppressed both temperature-sensitive colony formation and anucleate cell production of the *mukB106* mutation. Results reveal that a linkage group (*smbB*) of the extragenic suppressors is located in the *rne*/*ams*/*hmp* gene coding for RNase E. These suppressor mutations coded for truncated RNase E polypeptides lacking a C-terminal half. Furthermore, we have found that the truncated RNase E polypeptides failed to interact with PNPase, in contrast with the wild-type RNase E protein. We discuss the roles of RNase E and PNPase of the multiprotein complex in processing and turnover of mRNA molecules and other RNA molecules.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. Bacterial strains and plasmids used are shown in Table 1 and Fig. 1, respectively. Plasmids carrying the chromosomal DNA segments were constructed with vectors pHSG439 (5), pHSG576 (49), pHSG399 (49), pACYC177 (9), and pACYC184 (9). M13 phage mp18 (61) was used for DNA sequencing.

Media. L medium contained 1% tryptone, 0.5% yeast extract, and 0.5% NaCl (pH 7.4). E medium (54) was supplemented with 0.5% glucose and 50 μg each of L-tryptophan and L-histidine (or 0.4% Casamino Acids) per ml. M9 medium (34) was supplemented with 0.5% glucose and 0.4% Casamino Acids or required amino acids (50 μg/ml each). P medium contained 1% Polypeptone and 0.5% NaCl (pH 7.4). To prepare agar plates, 1.4% (wt/vol) agar was added to media. To isolate phages carrying the chromosomal DNA segments, P agar plates were overlaid with P soft agar (P medium containing 0.7% agar) supplemented with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μg/ml) and IPTG (isopropyl-β-D-thiogalactopyranoside) (0.1 mM). To select antibiotic-resistant transformants, antibiotics were added to media as follows: ampicillin, 30 μg/ml; tetracycline, 15 μg/ml; kanamycin, 25 μg/ml; and chloramphenicol, 20 μg/ml.

DNA cloning and sequencing. A cosmid library of the wild-type chromosome DNA segments of strain W3110 was prepared with the cosmid vector pHSG439 (20). Plasmid DNA was extracted with the Qiagen kit. DNA fragments for subcloning were purified from agarose gels with the Gene Clean kit (Bio 101, Inc., La Jolla, Calif.). DNA fragments were subcloned into M13mp18 and sequenced by the dideoxy-chain termination method (48) using Sequenase version II (United States Biochemical Corp.). Restriction enzymes and DNA modifica-

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TABLE 1. Bacterial strains

Strain	Genotype	Source, reference, or construction
W3110	Prototroph	24
YK1100	*trpC9941*	55
AZ5040	Δ*mukB*::*kan smbA40*	58
GC7528	*hsdR trpE61 trpA62 tna-5* Δ*mukB*::*kan*	41
PB103	*dadR trpE61 trpA62 tna-5*	13
GC7471	PB103 except for *zcb*::Tn*10*	41
SH392	*met gal hsdR sfiC supE44*	22
SH3208	*his* Δ*trpE5* (λ)	20
SH3367	*his* Δ*trpE5 mukB106* (λ)	41
SH6067	W3110 except for Δ*mukB2*::*kan*[a]	This laboratory
SH9019	*his* Δ*trpE5 mukB33 zcb*::Tn*10*	57
CH1828	*araD139* Δ(*araABC-leu*)*7697* Δ(*lac*)*X74 galU galK hsdR rpsL150 thi rne-1*(Ts) *zce-726*::Tn*10*	37
TL112	HfrC *phoA8 glpD3 glpR2 relA1 spoT1 pit-10 fhuA22 ompF627 fadL701* (λ) *glpK1 plsB26 gyrA zce-726*::Tn*10*	26
BZ4	*his* Δ*trpE5 mukB106 smbB104* (λ)	This study
BZ5	*his* Δ*trpE5 mukB106 smbB105* (λ)	This study
BZ31	*his* Δ*trpE5 mukB106 smbB131* (λ)	This study
BZ70	*his* Δ*trpE5 mukB106 smbB170* (λ)	This study
BZ91	*his* Δ*trpE5 mukB106 smbB191* (λ)	This study
BZ99	*his* Δ*trpE5 mukB106 smbB199* (λ)	This study
BZ188	BZ4 except for Δ*mukB*::*kan*	P1/GC7528→BZ4
BZ189	BZ5 except for Δ*mukB*::*kan*	P1/GC7528→BZ5
BZ215	BZ31 except for Δ*mukB*::*kan*	P1/GC7528→BZ31
BZ427	BZ70 except for Δ*mukB*::*kan*	P1/GC7528→BZ70
BZ429	BZ91 except for Δ*mukB*::*kan*	P1/GC7528→BZ91
BZ431	BZ99 except for Δ*mukB*::*kan*	P1/GC7528→BZ99
BZ433	BZ188 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ188
BZ435	BZ189 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ189
BZ437	BZ215 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ215
BZ439	BZ427 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ427
BZ441	BZ429 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ429
BZ443	BZ431 except for *zcb*::Tn*10 mukB33*	P1/SH9019→BZ431
BZ450	BZ188 except for *zcb*::Tn*10 mukB*$^+$	P1/AZ5180→BZ188
BZ452	BZ189 except for *zcb*::Tn*10 mukB*$^+$	P1/AZ5180→BZ189
BZ249	BZ215 except for *zcb*::Tn*10 mukB*$^+$	P1/AZ5180→BZ215
BZ453	BZ431 except for *zcb*::Tn*10 mukB*$^+$	P1/AZ5180→BZ431

[a] SH6067 has the Δ*mukB2*::*kan* mutation, in which the 3.6-kb *Xho*I-*Hpa*I segment of the *mukB* gene is replaced by the 1.3-kb *kan* segment of pACYC177.

tion enzymes were purchased from Takara Shuzo, Boehringer Mannheim Biochemicals, and New England Biolabs.

Microscopic observation of cells and nucleoids. Cells were grown in L medium or M9 medium containing glucose, Casamino Acids, and L-tryptophan. Collected cells were fixed, stained with DAPI (4′,6-diamidino-2-phenylindole), and observed by Hiraga's fluo-phase combined method (20).

P1 transduction. P1*vir*-mediated transduction was performed (34). Transductants were selected at 25, 30, or 37°C overnight or for 2 days on L agar plates containing 20 mM sodium citrate. When necessary, antibiotics were supplemented at the following final concentrations: tetracycline, 15 μg/ml; kanamycin, 30 μg/ml; and chloramphenicol, 30 μg/ml.

Immunoblotting. Bacterial strains SH3367, BZ4, BZ5, BZ31, BZ70, BZ91, and BZ99 were grown in M9 medium containing glucose, Casamino Acids, and L-tryptophan at 30°C. When turbidity of the culture (5 ml) reached 60 Klett units, cells were collected and washed once with 0.84% NaCl solution. The washed cells were suspended in 20 μl of TE buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA) added to 5 μl of 5× SDS-PAGE loading buffer (0.3 M Tris-HCl [pH 6.8], 10% SDS, 50% glycerol, 0.05% bromphenol blue, and 25% β-mercaptoethanol) and boiled for 5 min. Samples were analyzed by SDS–8% PAGE. Proteins were electrotransferred onto a nitrocellulose filter as described elsewhere (47). The blots were treated with anti-RNase E (anti-170) rabbit antibody (gift from Vick Norris) in phosphate-buffered saline–Tween 20 (0.1%) and then incubated with a goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Amersham). The filters were analyzed by the immunoluminescence method using the ECL Western blotting (immunoblotting) detection reagents (Amersham). X-ray film was exposed to luminescence and then developed.

Immunoprecipitation. Immunoprecipitation was carried out by the method described by Carpousis et al. (6), with minor modifications. The following buffers were prepared immediately before use: lysozyme-EDTA buffer, 50 mM Tris-HCl (pH 7.5)–100 mM NaCl–5% glycerol–3 mM EDTA–1 mM dithiothreitol–10 mg of lysozyme per ml–1 mM phenylmethylsulfonyl fluoride; DNase-Triton buffer, 50 mM Tris-HCl (pH 7.5)–100 mM NaCl–5% glycerol–1 mM dithiothreitol–30 mM magnesium acetate–3% Triton X-100–1 mM phenylmethylsulfonyl fluoride–20 μg of DNase I per ml; and buffer A, 10 mM Tris-HCl (pH 7.5)–5% glycerol–1 mM EDTA–0.1 mM dithiothreitol–0.1 mM phenylmethylsulfonyl fluoride. Each of the above buffers also contained 2 μg of aprotinin and 0.8 μg each of leupeptin and pepstatin A per ml. SH3367 and BZ31 strains were grown at 30°C in L medium. When the turbidity of the culture (200 ml) reached 80 Klett units, the cells were collected by centrifugation and washed twice with 50 ml of 50 mM Tris-HCl (pH 7.5). The following steps were performed between 0 and 4°C. The cell pellet was suspended in 16 ml of lysozyme-EDTA buffer. The cell suspension was kept at 4°C for 20 min and treated with three cycles of freeze-thawing. Then, 8 ml of DNase-Triton buffer (room temperature) was added, and the suspension was placed on ice for 20 min. Six milliliters of 5 M NH_4Cl was added slowly with stirring. The lysate was stirred for another 30 min and then centrifuged at 17,000 × *g* for 60 min. The supernatant was precipitated with ammonium sulfate (40% saturation), and the pellet was suspended in 2 ml of buffer A containing 50 mM NaCl, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. In subsequent steps, buffer A containing 300 mM NaCl was used throughout. Anti-RNase E or anti-PNPase antibody was added to 0.4 ml of the sample and kept for 2 h at 4°C. Protein A-agarose was added, and the reaction continued for another hour with gentle mixing. The agarose was precipitated by centrifugation, washed five times with phosphate buffer, suspended in SDS-PAGE loading buffer, and boiled for 5 min. The sample was analyzed by SDS-PAGE and the immunoluminescence method using anti-RNase E rabbit antibody or anti-PNPase rabbit antibody (gift from Beatrice Py and C. F. Higgins).

Analysis of the amount of MukB protein. Cells were grown at 30°C in M9 medium supplemented with 0.5% glucose and 18 amino acids (50 μg/ml each) without methionine and cysteine. When turbidity of the culture (5 ml) reached 60 Klett units (with a green filter), 1 ml of the culture was removed, labeled with the EXPRE^{35}S^{35}S protein labeling mix (37 TBq/mmol; Dupont) for 30 min at 30°C,

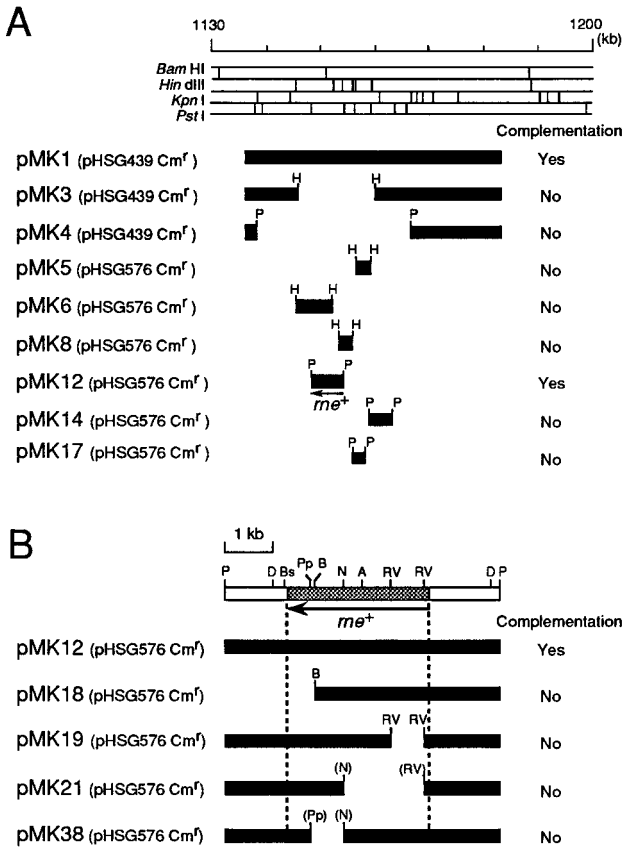

FIG. 1. Subcloning of the wild-type gene that complements the *smb-131* mutation suppressing temperature sensitivity of the *mukB106* mutation. Solid bars represent chromosomal DNA segments carried by the indicated plasmids. Vector plasmids and their selective markers are shown in parentheses. These cosmids or plasmids were introduced into the BZ31 strain (*mukB106 smb-131*) and tested for colony formation at 44°C on L agar medium containing chloramphenicol (30 μg/ml). Complementation was defined as inability to grow at 44°C. The scales in panels A and B are different. A, *Afl*II; B, *Bam*HI; Bs, *Bst*BI; D, *Dra*I; H, *Hin*dIII; N, *Nru*I; Pp, *Ppu*MI; P, *Pst*I; RV, *Eco*RV; Sp, *Sph*I.

and chased with 50 μg of unlabeled methionine and cysteine per ml for 2 min. Labeled samples were analyzed by SDS–5% PAGE. Radioactivity was quantified with image analyzer BAS2000 (Fuji Film).

Construction of mini-F plasmids carrying DNA segments of the *mukFEB* operon. The 8.5-kb *Bam*HI DNA segment from pAX850 (60), which carries the *smtA*, *mukF*, *mukE*, and *mukB* genes, was inserted at the *Bam*HI site of the mini-F plasmid pXX704 (39), yielding pMK51. The 8.5-kb *Bam*HI DNA segment from pKX732 (57), which carries the *mukB106* mutation, was similarly inserted into pXX704, yielding pMK52.

Assay of β-lactamase. Bacterial cells of strains harboring plasmid pBR322 (*bla* gene conferring ampicillin resistance) were grown exponentially at 37°C in L medium, collected by centrifugation, and assayed for β-lactamase activity with nitrocefin (compound 87/312; Glaxio-Allenburys Research) as a substrate (43).

Measurement of the amount of plasmid DNA. Plasmid DNA was extracted from bacterial cells, at the same density, carrying pBR322 or pHSG576 grown in L medium at 37°C. Plasmid DNA was digested with *Eco*RI, and the fluorescence intensities of DNA bands stained with ethidium bromide were analyzed with an image analyzer.

Nucleotide sequence accession number. Our DNA sequence of the *rne* gene has been registered in GenBank under accession number U49647.

RESULTS

Isolation of temperature-resistant suppressor mutants carrying the *mukB106* mutation. The *mukB106* mutant (SH3367) was unable to form colonies at 44°C on L agar medium; the colony forming ability at 44°C was only 10^{-5} of that at 30°C. This mutant has a missense mutation causing amino acid substitution Ser-33→Phe in the MukB protein (57). Temperature-resistant suppressor mutants that suppressed temperature sensitivity of the *mukB106* mutation were independently isolated at 44°C on L agar medium. One hundred and twenty mutants were able to grow at 44°C. To exclude intragenic revertants of the *mukB* gene, these temperature-resistant mutants were tested for cotransduction frequency by P1*vir* phage with the *zcb*::Tn*10* marker of strain AZ5167 (*mukB106 zcb*::Tn*10*). The *zcb*::Tn*10* marker is closely linked to the *mukB106* mutation. It was therefore expected that, when a suppressor mutation was located in or near the *mukB* gene, 30 to 50% of tetracycline-resistant transductants would be temperature sensitive. When a suppressor was located in another locus far from the *mukB* gene, all tetracycline-resistant transductants were temperature resistant. Seventy-four of 120 were extragenic suppressor mutants. These extragenic suppressor mutations suppress both anucleate cell production and temperature-sensitive colony formation of *mukB106.*

Cloning of the wild-type DNA segment complementing the *smb-131* suppressor mutation. One of the extragenic suppressors, the BZ31 strain (*mukB106 smbB131*), was first analyzed. To clone the wild-type DNA segment complementing the *smbB131* suppressor mutation, BZ31 cells were infected with a cosmid library carrying chromosome segments of the wild-type W3110 strain, and chloramphenicol-resistant (Cm^r) colonies were isolated at 30°C. Four hundred Cm^r colonies were tested for colony forming ability at 44°C on L agar medium containing chloramphenicol. Eight clones among them failed to form colonies at 44°C. Cosmid DNAs from these clones were analyzed by using restriction enzymes. At least two clones carried a common DNA segment. We used one of them, cosmid pMK1 (Fig. 1), for further analyses. To subclone the DNA segment complementing the *smbB131* suppressor mutation, pMK1 was digested with various restriction enzymes and DNA fragments were cloned into pHSG439, pHSG576, or pACYC184. The resulting plasmids were introduced into BZ31 cells and examined for temperature sensitivity, as shown in Fig. 1. We found that the BZ31 strain harboring pMK12 was unable to grow at 44°C, suggesting that pMK12 complements the *smbB131* mutation (Fig. 1). Plasmid pMK12 carries the 5.8-kb *Pst*I DNA segment which contains the *rne*/*ams*/*hmp* gene encoding RNase E (6, 12, 51) but not other genes. This indicated that the wild-type *rne* gene complements the *smbB131* mutation. Deletion derivatives pMK18, pMK19, pMK21, and pMK38 derived from pMK1 lost the ability to complement the *smbB131* suppressor mutation (Fig. 1B), supporting the above conclusion.

Mapping of the *smbB131* mutation by transduction with phage P1*vir*. To confirm that the *smbB131* mutation is located in the *rne* gene, we tested whether the *smbB131* mutation is closely linked with the *zce-726*::Tn*10* marker (24.3 min) that is closely linked with the *rne* gene. Phage P1*vir* grown on strain TL112 carrying the *zce-726*::Tn*10* marker was used to infect BZ31 cells, and tetracycline-resistant (Tc^r) transductants were selected at 30°C and then tested for colony forming ability at 44°C. Six clones of 10 showed temperature-sensitive growth, indicating that the *smbB131* mutation was closely linked with the *zce-726*::Tn*10* marker (Table 2). This result supported the conclusion that the *smbB131* mutation is located in the *rne* gene.

We surveyed other extragenic suppressor mutants by P1*vir* transduction and found that suppressor mutations in BZ4, BZ5, BZ70, BZ91, and BZ99 were also linked with the *zce-726*::Tn*10* marker. Moreover, these suppressor mutations were complemented by pMK12 but not by the deletion derivatives pMK18, pMK19, pMK21, and pMK38. The six suppres-

TABLE 2. Transduction with phage P1 and complementation of *smb* mutations by various plasmids

Strain	Chromosomal genes	Frequency of cotransduction[a]	Growth at 44°C[b]				
			pHSG576	pMK12	pMK18	pMK19	pMK38
SH3208	*mukB*$^+$ *rne*$^+$ (*smbB*$^+$)		+	+	+	+	+
CH1828	*mukB*$^+$ *rne-1*		−	+	+	−	+
SH3367	*mukB106 rne*$^+$ (*smbB*$^+$)		−	−	−	−	−
BZ4	*mukB106 smbB104*	8/9	+	−	+	+	+
BZ5	*mukB106 smbB105*	8/10	+	−	+	+	+
BZ31	*mukB106 smbB131*	4/8	+	−	+	+	+
BZ70	*mukB106 smbB170*	5/7	+	−	+	+	+
BZ91	*mukB106 smbB191*	5/10	+	−	+	+	+
BZ99	*mukB106 smbB199*	7/10	+	−	+	+	+

[a] Ratio of *rne*$^+$ (*smbB*$^+$) clones to tetracycline-resistant transductants. Phage P1*vir* grown on strain TL112 (*zce-726*::Tn*10 rne*$^+$) was used to infect the indicated strain.
[b] Colony formation on L agar medium containing chloramphenicol (30 μg/ml).

sor mutants including BZ31 have a mutation in the *rne* gene, as summarized in Table 2.

A *mukB*-null mutation, Δ*mukB*::*kan*, was introduced into these *smbB* mutants by P1 transduction to replace *mukB106* (Table 1). The resulting transductants were temperature sensitive. This indicated that the *smbB* mutations failed to suppress the temperature-sensitive colony formation of the *mukB*-null mutants. In contrast, previously described *smbA* (*pyrH*) mutations located at 4 min (1, 58) are able to suppress the defect of *mukB*-null mutants. Furthermore, when another missense mutation, *mukB33* (a substitution, Asp-1201→Asn [57]), was introduced in the *smbB* mutants by P1 transduction to replace Δ*mukB*::*kan* (Table 1), the *smbB* mutations suppressed temperature-sensitive colony formation and anucleate cell production of *mukB33*.

Construction of plasmids carrying *smbB* mutations. The 5.8-kb *Pst*I *rne* segments of strains BZ5, BZ31, BZ91, and BZ99 were cloned into pACYC177. To select the plasmid carrying the *Pst*I segment, the temperature-sensitive *rne-1* mutant CH1828 was used as the host. Temperature-resistant and kanamycin-resistant transformants were selected. The resulting plasmids pMK31, pMK32, pMK33, and pMK35 carried the *smbB131*, *smbB105*, *smbB191*, and *smbB199* mutations, respectively.

Analysis of the *smbB131* mutation site within the *rne* gene. Plasmid pMK43 was constructed by ligation of the *Dra*I-*Afl*II segment of pMK41 carrying the wild-type *rne* gene and the *Afl*II-*Sca*I segment of pMK31 carrying the *smbB131* gene (Fig. 2A). The pMK43 plasmid was introduced into BZ31 cells at 30°C. The resulting Tc^r transformants with pMK43 were able to form colonies at 44°C on L agar medium containing tetracycline. Another plasmid, pMK47, was constructed by ligation of the *Pst*I-*Afl*II DNA segment from pMK31 and the *Afl*II-*Sca*I DNA segment from pMK40 (Fig. 2B). When pMK47 was introduced into BZ31 cells, transformants were unable to form colonies at 44°C on L agar medium containing kanamycin. These results indicated that the *smbB131* mutation was located in the 3′-terminal half of the *rne* gene, that is, within the segment from the *Afl*II site to the 3′ terminus.

Sequencing of the *smbB* suppressor mutations. The 1.6-kb *Afl*II-*Bst*BI fragments containing the *smbB* mutations were isolated from pMK31, pMK32, pMK33, and pMK35 and sequenced. We found a deletion of two nucleotides, G and T at positions 2295 and 2296 (numbered as in references 7 and 8),

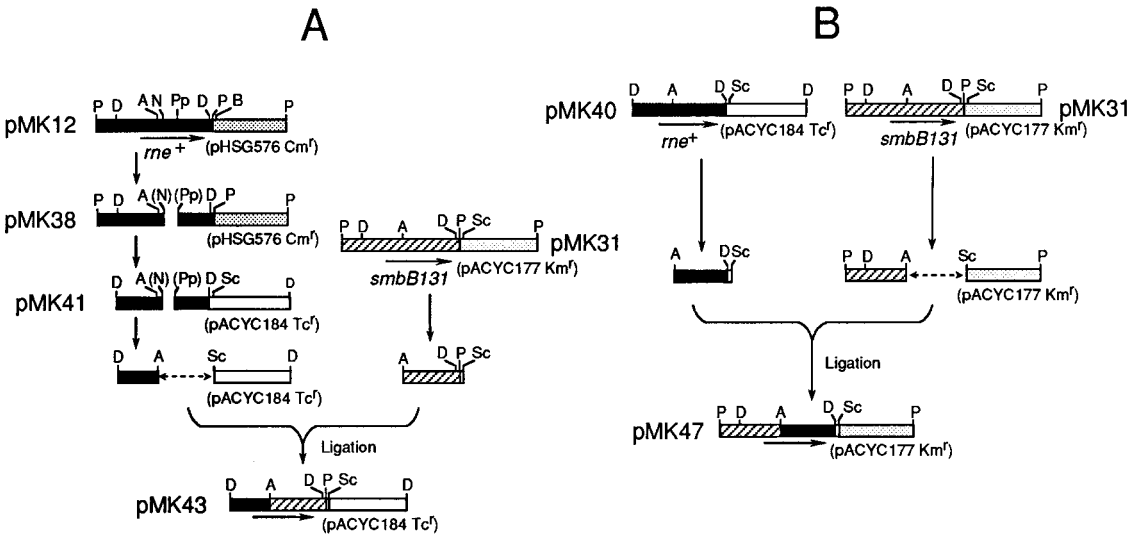

FIG. 2. Construction of the hybrid DNA of *rne*$^+$ and *smbB* genes. (A) Plasmid pMK12 carrying the *rne*$^+$ gene was digested with *Nru*I and *Ppu*MI. The 3′ end of the *Nru*I-*Ppu*MI fragment (8.7 kb) was treated with T4 DNA polymerase and ligated, yielding pMK38. Plasmid pMK41 was constructed by cloning the *Dra*I fragment (3.9 kb) of pMK38 into the *Dra*I site of pACYC184. The *Afl*II-*Sca*I fragment (3.1 kb) of pMK31 was cloned into the *Afl*II-*Sca*I site of pMK41, yielding pMK43. (B) Plasmid pMK40 was constructed by cloning the *Dra*I fragment (4.6 kb) containing the *rne*$^+$ gene of pMK12 into the *Dra*I site of pACYC184. The *Afl*III-*Sca*I fragment (2.0 kb) of pMK40 was cloned into the *Afl*II-*Sca*I site of pMK31, yielding pMK47. A, *Afl*II; B, *Bam*HI; D, *Dra*I; N, *Nru*I; Pp, *Ppu*MI; P, *Pst*I; Sc, *Sca*I.

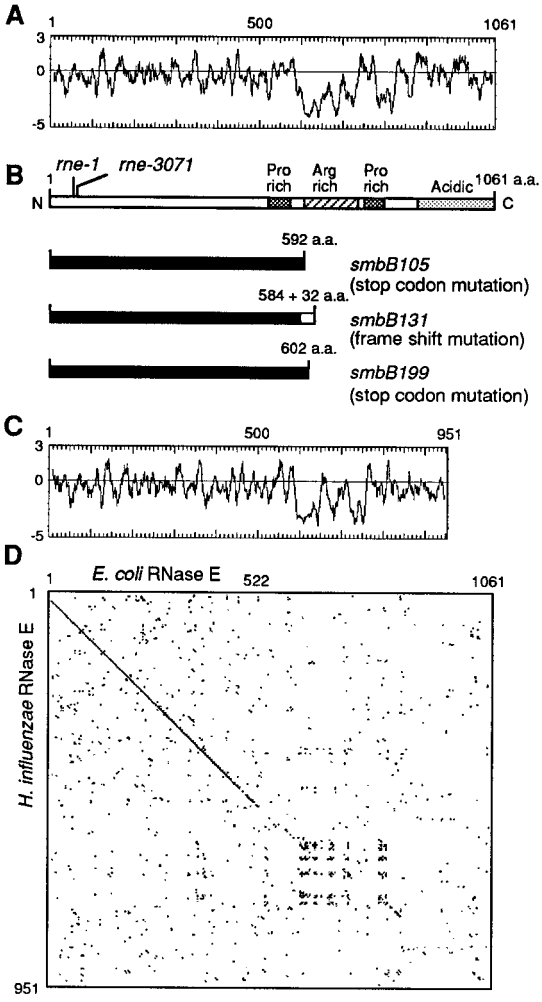

FIG. 3. (A) Hydrophobicity plot of RNase E protein (RNase E) of *E. coli* using the Kyte-Doolittle program of Genetyx-Mac. The amino acid sequence of RNase E was deduced from the DNA sequence determined in this study (see the text). (B) Locations of the *rne-1* and *rne-3071* mutations and proline-rich, arginine-rich, and acidic regions (7, 8). Solid bars represent truncated RNase E polypeptides encoded by *smbB* mutants. The open region in *smbB131* represents additional 32 amino acids (a.a.) encoded by downstream nucleotide sequences beyond the frameshift mutation. (C) Hydrophobicity plot of RNase E protein (RNase E) of *H. influenzae*. (D) Harr plot of the *E. coli* RNase E protein and the *H. influenzae* RNase E protein using HarrPlot Program 1 of Genetyx-Mac (unit size to compare, 5; dot plot score, 3.00).

in the *smbB131* mutant. The deletion results in the frameshift translation beyond the deletion; therefore, the *smbB131* mutant was predicted to encode a truncated polypeptide of 616 amino acid residues followed by additional 32 residues beyond the deletion. In the *smbB105* mutant, C-2317 is substituted by T, creating a stop codon, TAA. The *smbB105* mutant was predicted to encode a truncated polypeptide of 592 amino acids. In the *smbB199* mutant, G-2347 is substituted by T, creating a stop codon, TAA. The *smbB199* mutant was predicted to encode a truncated polypeptide of 602 amino acids. The *smbB191* mutant was found to have a 153-bp deletion from A-2337 to A-2488, resulting in the frameshift translation in a fused DNA segment beyond the deletion. The *smbB191* mutated gene was predicted to encode a truncated polypeptide of 602 residues followed by another 5 residues. All the *smbB* mutants sequenced thus encode truncated RNase E proteins lacking a C-terminal half (Fig. 3B).

During the sequencing of the 1.6-kb *Afl*II-*Bst*BI segment containing the 3′-terminal half of the *rne* gene, we found some nucleotide sequences different from the *rne* sequence described by Casarégola et al. (7, 8): A-2187 to T (no amino acid change), CG at positions 2230 and 2231 to GC (Arg-564 to Ala), G-2892 to C (Lys-784 to Asn), and GC at positions 3317 and 3318 to CG (no amino acid change). On the basis of our results, the *rne* gene was predicted to encode a polypeptide of 1,061 amino acids with an estimated M_r of 118,110.

Detection of truncated RNase E polypeptides by immunoblotting. The mutated *rne* gene products were detected by immunoblotting using anti-RNase E antibody. The RNase E protein of the wild-type strain migrated as a 170-kDa protein in SDS-PAGE, while truncated RNase E polypeptides of six *smbB* mutants rapidly migrated as around 50- to 60-kDa polypeptides (Fig. 4). These results indicated that all of the *smbB* mutants encode truncated RNase E polypeptides.

Complementation of the temperature-sensitive lethal *rne-1* mutation by plasmids encoding truncated RNase E polypeptides. The *rne-1* mutant is unable to grow at 42°C (4, 7, 25). The mutation in the *rne-1* allele was mapped at Gly-60 by McDowall et al. (32). The temperature-sensitive growth of the *rne-1* mutant was overcome by plasmids pMK18 and pMK38 (Fig. 1B) carrying the 5′-terminal half of the *rne* gene and, also, by the *smbB* mutant plasmids pMK31, pMK32, pMK33, and pMK35, which coded for truncated RNase E polypeptides lacking a C-terminal half.

Effects of *smbB* mutations on the copy number of plasmid pBR322. RNase E is involved in rapid degradation of the bulk mRNA (4, 36) and RNAI of ColE1-type plasmids (for a review, see reference 11). If the *smbB* mutants should affect degradation of RNAI, the copy number of plasmid pBR322 would be different from that in the rne^+ strain. The activities of β-lactamase expressed from plasmids are generally proportional to the copy number of the plasmid. We therefore analyzed β-lactamase activities in *smbB* mutant and rne^+ strains ($mukB^+$ genetic background). β-Lactamase activity was 0.21 in the rne^+ strain. In contrast, the activities were 0.11, 0.09, 0.07, and 0.09 in *smbB104*, *smbB105*, *smbB131*, and *smbB199* strains, respectively. These results suggested that the copy numbers of pBR322 in the *smbB* mutants are 30 to 50% of that of the rne^+ strain. Furthermore, we analyzed the amount of plasmid DNA using agarose gel electrophoresis. A pSC101 derivative, pHSG576, was used as an internal control. As shown in Table 3, pBR322/pHSG576 ratios in the $smbB^+$ and *smbB131* strains were 3.3 and 2.4, respectively. All the above results indicated that the copy numbers of pBR322 in the *smbB* mutants were reduced relative to that of the isogenic rne^+ strain. These *smbB* mutations presumably influence the stabil-

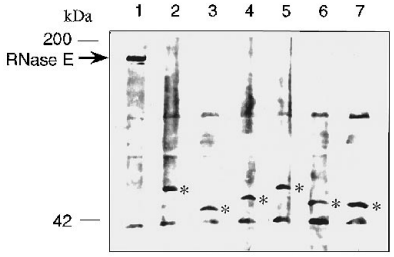

FIG. 4. Western blot of RNase E polypeptides in the rne^+ strain and *smbB* mutant strains. Lane 1, SH3367 (rne^+); lane 2, BZ4 (*smbB104*); lane 3, BZ5 (*smbB105*); lane 4, BZ31 (*smbB131*); lane 5, BZ70 (*smbB171*); lane 6, BZ91 (*smbB191*); lane 7, BZ99 (*smbB199*). Arrow, wild-type RNase E polypeptide; asterisks, truncated RNase E polypeptides.

TABLE 3. Amounts of pBR322 and pSC101 derivative pHSG576 DNAs in *smbB*$^+$ and *smbB131* mutant strains

Strain	Amt of plasmid DNA[a]		pBR322/pHSG576 DNA ratio
	pBR322	pHSG576	
SH3208(pBR322)(pHSG576)	237 ± 10	71 ± 0.4	3.3
BZ249(pBR322)(pHSG576)	193 ± 14	80 ± 0.4	2.4

[a] Arbitrary units.

ity of RNAI. *smbB* mutants with the *mukB*$^+$ genetic background (BZ249, BZ450, BZ452, and BZ453 [Table 1]) grew at a doubling time of 25 min at 37°C in L medium, the same as that for an isogenic *rne*$^+$ strain (SH3208). Cell shape of the mutants was also normal. These results demonstrate that the C-terminal half of RNase E is not essential for viability.

Effects of *smbB* mutations on the amount of MukB protein. The above results prompted us to test the possibility that mRNA of the *mukFEB* operon is stabilized by the *smbB* mutations and the increased amount of the MukB106 protein allows cell growth of the *mukB106* mutant even at 44°C. The amounts of MukB106 in *smbB* mutants and the *rne*$^+$ (*smbB*$^+$) strain were measured as follows. Exponentially growing cells were labeled with a mixture of [^{35}S]methionine and [^{35}S]cysteine; then, the cell lysate was analyzed by SDS-PAGE. Under the experimental conditions, MukB106 (or MukB) and RNase E were clearly separated from each other and from other proteins, as shown in Fig. 5A. The amount of radiolabeled MukB106 protein measured with an imaging analyzer was normalized as the ratio to the amount of the radiolabeled β′ subunit of RNA polymerase (Fig. 5B). Relative amounts of MukB106 in these *smbB* mutants were 1.5- to 2.3-fold higher than that of MukB106 in the *rne*$^+$ strain (Table 4). Figure 5D shows proteins in a *mukB*-null mutant (AZ5040) as a control to identify the MukB band. Bands of MukB and Rne were furthermore confirmed by Western blotting using anti-MukB and anti-Rne antibodies (data not shown).

Suppression of temperature-sensitive colony formation of the *mukB106* mutant by overproduction of the MukB106 protein. The effect of overproduction of the mutant MukB106 protein expressed from the plasmid on temperature-sensitive colony formation by these mutants at 44°C was tested as follows. The mini-F plasmid pMK52 carrying the *mukB106* gene was introduced into a *mukB106* mutant strain (SH3367) and a *mukB*-null mutant strain (SH6067). The resulting strains SH3367(pMK52) and SH6067(pMK52) were able to form colonies at 44°C on L agar medium. To measure the amount of the MukB106 protein, cells of these strains were examined as above. As shown in Table 5, amounts of the radiolabeled MukB106 protein in these strains were three- to fourfold higher than that in the plasmid-free strain SH3367. These results indicated that an increase in the amount of MukB106 suppressed temperature-sensitive colony formation. Similar overproduction of the MukB protein was observed also in SH3367(pMK51) and SH6067(pMK51), which harbored plasmid pMK51 carrying the wild-type *mukB* gene (Table 5).

Coimmunoprecipitation of RNase E and PNPase. RNase E and PNPase are copurified as the same multiprotein complex (6, 45). We performed immunoprecipitation experiments with cell extracts of the *smbB131* strain BZ31 and the *smbB*$^+$ strain SH3367 to examine if the C-terminal half of RNase E is required for binding with PNPase. The cell extract was treated with anti-RNase E antibody, and the precipitate was analyzed for PNPase by Western blotting using anti-PNPase antibody. PNPase was coprecipitated in the extract of SH3367 (Fig. 6B, lane 1) but not in the extract of BZ31 (Fig. 6B, lane 2). The results indicated that PNPase was precipitated together with the wild-type RNase E protein but not with the truncated RNase E polypeptide. As a control, when anti-PNPase antibody was used for immunoprecipitation, the amounts of precipitated PNPase in SH3367 and BZ31 were essentially the same (Fig. 6A, lanes 1 and 2). When anti-PNPase antibody was used for immunoprecipitation, the wild-type RNase E protein was precipitated together with PNPase (Fig. 6D, lane 1) but the truncated RNase E polypeptide from BZ31 was not (Fig. 6D, lane 2). In control experiments, when anti-RNase E antibody was used for immunoprecipitation, the wild-type RNase E protein from SH3367 was detected in the precipitate (Fig. 6C, lane 1) and the truncated RNase E polypeptide from BZ31 was also detected in the precipitate (Fig. 6C, lane 2). These results indicated that the C-terminal half of RNase E is crucial for binding with PNPase.

The band of the wild-type RNase E protein was faint (Fig. 6C, lane 1) in comparison with the truncated RNase E protein

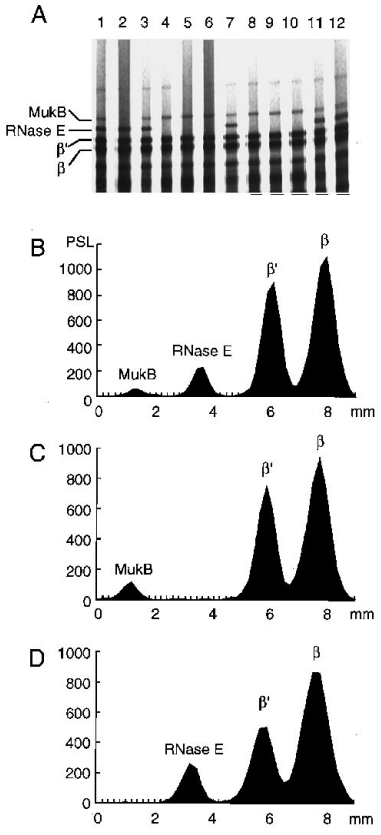

FIG. 5. Amounts of MukB and MukB106 proteins relative to the amounts of the β′ subunit of RNA polymerase in the *rne*$^+$ strain and *smbB* mutant strains. The growing cells were labeled with the mixture of [^{35}S]methionine and [^{35}S]cysteine. Labeled proteins were analyzed as described in Materials and Methods. (A) Autoradiographic pattern of total cellular proteins after SDS-PAGE. Lane 1, SH3208 (*mukB*$^+$); lane 2, AZ5040 (Δ*mukB*::*kan*); lane 3, SH3367 (*mukB106*); lane 4, BZ4 (*mukB106 smbB104*); lane 5, BZ5 (*mukB106 smbB105*); lane 6, BZ31 (*mukB106 smbB131*); lane 7, SH3367 (*mukB106*); lane 8, BZ70 (*mukB106 smbB170*); lane 9, BZ91 (*mukB106 smbB191*); lane 10, BZ99 (*mukB106 smbB199*); lane 11, SH3367 (*mukB106*); lane 12, SH3208 (*mukB*$^+$). (B, C and D) Histograms of radiolabeled proteins assayed by using an imaging analyzer. (B) SH3367 (*mukB106*); (C) BZ31 (*mukB106 smbB131*); (D) AZ5040 (Δ*mukB*::*kan*). Horizontal axis, position on the gel; vertical axis, relative radioactivity. The positions of MukB, RNase E, and β and β′ of RNA polymerase are indicated.

TABLE 4. Amounts of MukB and MukB106 proteins in *rne*$^+$ (*smbB*$^+$) strains and *smbB* mutant strains

Strain	Genotype	Relative amt of MukB or MukB106[a]
SH3208	*mukB*$^+$ *rne*$^+$ (*smbB*$^+$)	4.1 ± 0.2
AZ5058	Δ*mukB*::*kan rne*$^+$ (*smbB*$^+$)	0
SH3367	*mukB106 rne*$^+$ (*smbB*$^+$)	5.3 ± 0.2
BZ4	*mukB106 smbB104*	9.6 ± 0.3
BZ5	*mukB106 smbB105*	10.7 ± 0.5
BZ31	*mukB106 smbB131*	12.1 ± 0.5
BZ70	*mukB106 smbB170*	7.8 ± 0.4
BZ91	*mukB106 smbB191*	9.2 ± 0.4
BZ99	*mukB106 smbB199*	8.3 ± 0.4

[a] Ratio (percentage) of the radiolabeled MukB or MukB106 protein to the radiolabeled β′ subunit of RNA polymerase at 30°C.

(Fig. 6C, lane 2). This would be due to low efficiency of electrophoretic transfer of the large RNase E protein from the gel to a membrane.

DISCUSSION

We have found in this work that the *smbB* mutations are located within the *rne* gene. All of the *smbB* mutated genes encode truncated RNase E polypeptides lacking a C-terminal half. The *smbB* mutations overcame both temperature-sensitive colony formation and anucleate cell production of the *mukB106* mutation. These *smbB* mutations cause overproduction of the MukB106 mutant protein, and excess amounts of MukB106 overcome the defect of the *mukB106* mutation. The *smbB* mutants with the *mukB*$^+$ genetic background are able to grow normally. Our results with the *smbB* mutants clearly demonstrate that the C-terminal half of RNase E is not essential for viability. The results are inconsistent with an earlier report of Wang and Cohen (56) that a plasmid, pFMK35, encoding a truncated Rne lacking a 3′-terminal segment (beyond the *Bam*HI site) does not complement an *rne*-null mutation. This plasmid, however, did complement temperature sensitivity of the *rne-1* mutation, as described by Babitzke and Kushner (4). The pFMK35 plasmid encodes the catalytic segment but not the C-terminal end of Rne (4). It is likely that, when Wang and Cohen (56) used the plasmid for the complementation test, the plasmid had a spontaneous mutation defective in RNase E activity. Because overexpression of Rne protein results in slow growth of cells (unpublished data), spontaneous mutations would tend to accumulate in cultures.

PNPase was precipitated together with the intact RNase E polypeptide but not with the truncated RNase E polypeptide of *smbB131* in immunoprecipitation utilizing anti-RNase E antibody. Conversely, the truncated RNase E polypeptide was not coprecipitated with PNPase in immunoprecipitation utilizing anti-PNPase antibody. The C-terminal half of RNase E plays an important role in formation of a complex with PNPase. Moreover, our results with the *smbB* mutants indicate that the RNase E polypeptides lacking a C-terminal half influence processing and turnover of RNAs in vivo. These results support the hypothesis that RNase E forms a protein complex together with PNPase in vivo and these nucleases in the complex act cooperatively and effectively in processing and turnover of RNAs (for a review, see reference 11). Our results suggest that mRNA is controlled via RNase E-PNPase association, which is dispensable for cell viability. It is also suggested that PNPase is important for mRNA stability.

The truncated RNase E polypeptides expressed from plasmids pMK18, pMK31, pMK32, pMK33, pMK35, and pMK38 complemented the temperature-sensitive lethality of the *rne-1* mutation, which causes the amino acid substitution Gly-60 to Ser in the N-terminal domain. This is consistent with reports that a purified truncated RNase E polypeptide containing residues 1 to 643 has the endoribonucleolytic activity of RNase E (50) and that the N-terminal half containing residues 1 to 498 of RNase E retains a catalytic function (31).

A highly hydrophilic arginine-rich region was found between residues Arg-603 and Arg-735 of RNase E (7, 10) (Fig. 3A and B). The *smbB105*, *smbB131*, and *smbB199* mutated genes code for truncated RNase E polypeptides of residues 1 to 592, 1 to 584, and 1 to 602, respectively, which lack the highly hydrophilic arginine-rich region (Fig. 3B). These *smbB* mutants are able to grow nearly normally; therefore, the highly hydrophilic arginine-rich region is not essential for viability.

A homolog of RNase E was found in *Haemophilus influenzae* (15). The *H. influenzae* RNase E protein (951 amino acid residues) is smaller than the *E. coli* RNase E protein (1,061 amino acid residues). The N-terminal domain of the *E. coli* polypeptide of residues 1 to 522 is highly homologous with the *H. influenzae* polypeptide region of residues 17 to 535 (80% identity, 90% similarity). The patterns of hydrophilicity of the N-terminal half are therefore similar in the two species (cf. Fig. 3A and C). However, sequence homology of the C-terminal half from residue 523 to terminal residue 1061 of the *E. coli* RNase E protein is low (Fig. 3D). Hydrophilic regions exist in the C-terminal half in both *E. coli* and *H. influenzae* RNase E proteins (cf. Fig. 3A and C). Proline-rich regions, an arginine-rich region, and an acidic region were also found in the C-terminal half in RNases E from both species (7, 15), though sequence homology is low in the C-terminal half.

In the *mukB*$^+$ strain SH3208, the ratio of the radiolabeled MukB protein to the radiolabeled β′ subunit of RNA polymerase was 4.1% (Table 3). On the basis of the number of methionine and cysteine residues of the proteins, it was calculated that the relative number of MukB molecules per cell is 5% of that of the β′ subunit molecules while the relative number of RNase E molecules per cell is 32% of that of the β′ subunit molecules in the strain. In exponentially growing cells of *E. coli*, the number of RNA polymerase molecules ranges between 1,000 and 3,000 molecules per chromosome (for a review, see reference 21). If the number of the β′ subunit molecules per cell is 3,000 for growth at 30°C, for MukB the number is 150 molecules per cell and for RNase E the number is 960 molecules per cell.

Casarégola et al. (7) proposed the possibility that Rne is a

TABLE 5. Amounts of the MukB and MukB106 proteins in *mukB106* mutants and *mukB*-null mutants harboring the *mukB106* mutated gene or the *mukB*$^+$ gene on a plasmid

Strain	*mukB*		Colony formation at 44°C	Relative amt of MukB or MukB106[a]
	Chromosome	Plasmid		
SH3367	*mukB106*	None	−	8 ± 0.4
SH3367(pMK51)	*mukB106*	*mukB*$^+$	+	30 ± 2
SH3367(pMK52)	*mukB106*	*mukB106*	+	24 ± 1
SH6067	Δ*mukB2*::*kan*	None	−	0
SH6067(pMK51)	Δ*mukB2*::*kan*	*mukB*$^+$	+	26 ± 2
SH6067(pMK52)	Δ*mukB2*::*kan*	*mukB106*	+	33 ± 9

[a] Ratio (percentage) of the radiolabeled MukB or MukB106 protein to the radiolabeled β′ subunit of RNA polymerase at 25°C. The amount of MukB plus MukB106 was measured in the case of SH3367(pMK51).

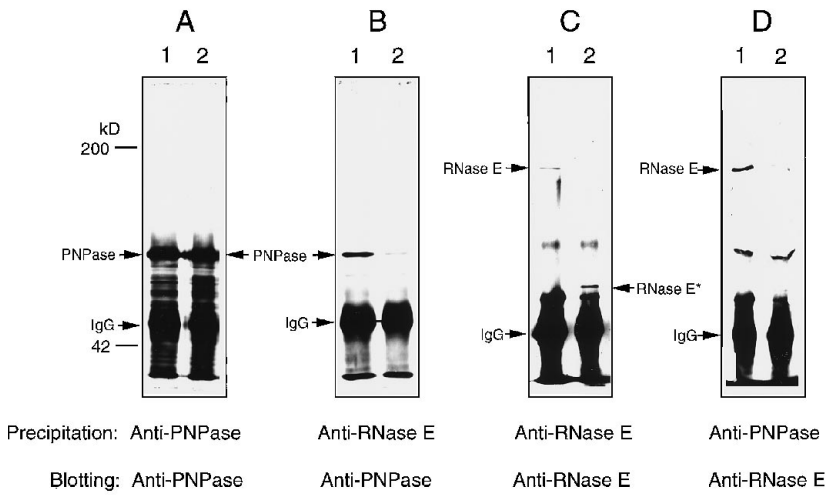

FIG. 6. Coimmunoprecipitation of RNase E and PNPase proteins. Cell extracts of the *rne*$^+$ strain SH3367 and the *smbB131* mutant strain BZ31 were treated with the indicated antibody for immunoprecipitation, and then the precipitates were analyzed by Western blotting using the indicated antibody. Lane 1, SH3367; lane 2, BZ31. RNase E*, truncated RNase E polypeptide of the *smbB131* mutant (panel C, lane 2). IgG, immunoglobulin G.

motor protein, and the possibility of interaction between Rne and MukB is worthy of consideration. However, so far, there is nothing suggesting this type of interaction between Rne and MukB.

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