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Complementation of an RNase P RNA (*rnpB*) Gene Deletion in *Escherichia coli* by Homologous Genes from Distantly Related Eubacteria

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We report the construction of a strain of *Escherichia coli* in which the only functional gene for the RNA moiety of RNase P (*rnpB*) resides on a plasmid that is temperature sensitive for replication. The chromosomal RNase P RNA gene was replaced with a chloramphenicol acetyltransferase gene. The conditionally lethal phenotype of this strain was suppressed by plasmids that carry RNase P RNA genes from some distantly related eubacteria, including *Alcaligenes eutrophus*, *Bacillus subtilis*, and *Chromatium vinosum*. Thus, the *rnpB* genes from these organisms are capable of functioning as the sole source of RNase P RNA in *E. coli*. The *rnpB* genes of some other organisms (*Agrobacterium tumefaciens*, *Pseudomonas fluorescens*, *Bacillus brevis*, *Bacillus megaterium*, and *Bacillus stearothermophilus*) could not replace the *E. coli* gene. The significance of these findings as they relate to RNase P RNA structure and function and the utility of the described strain for genetic studies are discussed.

The mature 5′ ends of tRNAs are formed by the action of the endoribonuclease RNase P (see references 2 and 22 for reviews). The products of two genes are required for RNase P activity in the eubacteria *Bacillus subtilis* and *Escherichia coli*. One of these (*rnpA*) codes for a 14-kDa (119-amino-acid) protein, whereas the other (*rnpB*) directs the synthesis of a 130-kDa (ca. 400-nucleotide) RNA molecule. In vitro, at high ionic strength, the RNase P RNA catalyzes the precise endonucleolytic cleavage of tRNA precursors in the absence of the RNase P protein (11). The RNase P protein is thought to screen electrostatic repulsion associated with enzyme and substrate RNAs in vivo (25).

Because the catalytic moiety of RNase P is composed of RNA, its structure and mechanism of action are of particular interest. So far, most information regarding structure-function relationships in RNase P RNA has come from biochemical studies in vitro. One reason that genetic studies have lagged behind in vitro studies is the lack of an appropriate genetic background in which to study RNase P RNA mutants. Since the *rnpB* gene is essential for viability, only conditionally lethal mutations can be isolated. A few thermosensitive *E. coli* RNase P mutants have been identified and studied to various degrees. The best characterized and most widely used are the A49 (27) and ts709 (21) strains, which bear mutations in the genes for the RNase P protein and RNA, respectively. However, both of these mutations are leaky at the restrictive temperature, and tRNA precursors accumulate in cells at the permissive temperature (20). Moreover, these mutants have peculiar complementation properties that are possibly a consequence of the persistence of the defective gene products (20, 28).

We report here the construction of a strain of *E. coli* in which the *rnpB* gene is eliminated from cells in response to a temperature shift. We show that the conditionally lethal phenotype of this *rnpB* gene deletion can be complemented by a plasmid-borne *E.coli rnpB* gene. Moreover, the *rnpB* genes from some distantly related eubacteria are also capable of complementing the deletion and therefore of functioning as the sole source of RNase P RNA in *E. coli*. This result indicates that some domains of the *E. coli* RNase P RNA structure are dispensable in vivo as well as in vitro.

MATERIALS AND METHODS

Plasmid constructions. pDW160 was created by ligating a 3.4-kb *Ssp*I-*Xho*I fragment of pPM103 (17), a derivative of pSC101 temperature sensitive for replication, with the 5-kb *Ssp*I-*Xho*I fragment of pDW159, which includes the *E. coli rnpB* gene and a Kan^r determinant. pDW159 was constructed by inserting a 1.1-kb *Kpn*I-*Stu*I fragment of pLN2 (20) that includes the *E. coli rnpB* gene into *Nru*I-digested pDW155, after the sticky ends (*Kpn*I) were blunted with T4 DNA polymerase and deoxynucleoside triphosphates. The insert is oriented so that the Kan^r and *rnpB* genes are transcribed in the same direction. pDW155 was constructed by deleting a 1.7-kb *Eco*RI fragment (containing the *lacI* gene) from pZC1 (4). pDW161 was constructed by inserting a 1.4-kb *Hin*cII-*Sma*I fragment of pDW154 encoding chloramphenicol resistance into *Sma*I-*Bss*HII-digested pLN2 (Fig. 1), after the sticky ends (*Bss*HII) were blunted with T4 DNA polymerase and deoxynucleoside triphosphates. The insert is oriented so that the Cam^r gene is transcribed in the same direction as was the *rnpB* gene it replaced. pDW154 was constructed by inserting a 1.4-kb *Hha*I fragment of pBR328 (30) that includes a Cam^r gene into *Eco*RV-digested Bluescript KS$^+$ (1, 29), after the *Hha*I ends were blunted with T4 DNA polymerase. The insert is situated such that the Cam^r and β-lactamase (Amp^r) genes are transcribed in opposite directions. pDW3 was constructed by inserting a 0.9-kb *Hin*dIII-*Pst*I fragment of *B. subtilis* DNA that includes the *rnpB* gene (24) into the polylinker region of pDW16 (D. S. Waugh, Ph.D. thesis, Indiana University, Bloomington, 1989). pDW10 contains the *B. subtilis rnpB* gene cloned adjacent to a bacteriophage T7 promoter in pUC19 (Waugh, Ph.D. thesis, 1989). The *rnpB* gene is

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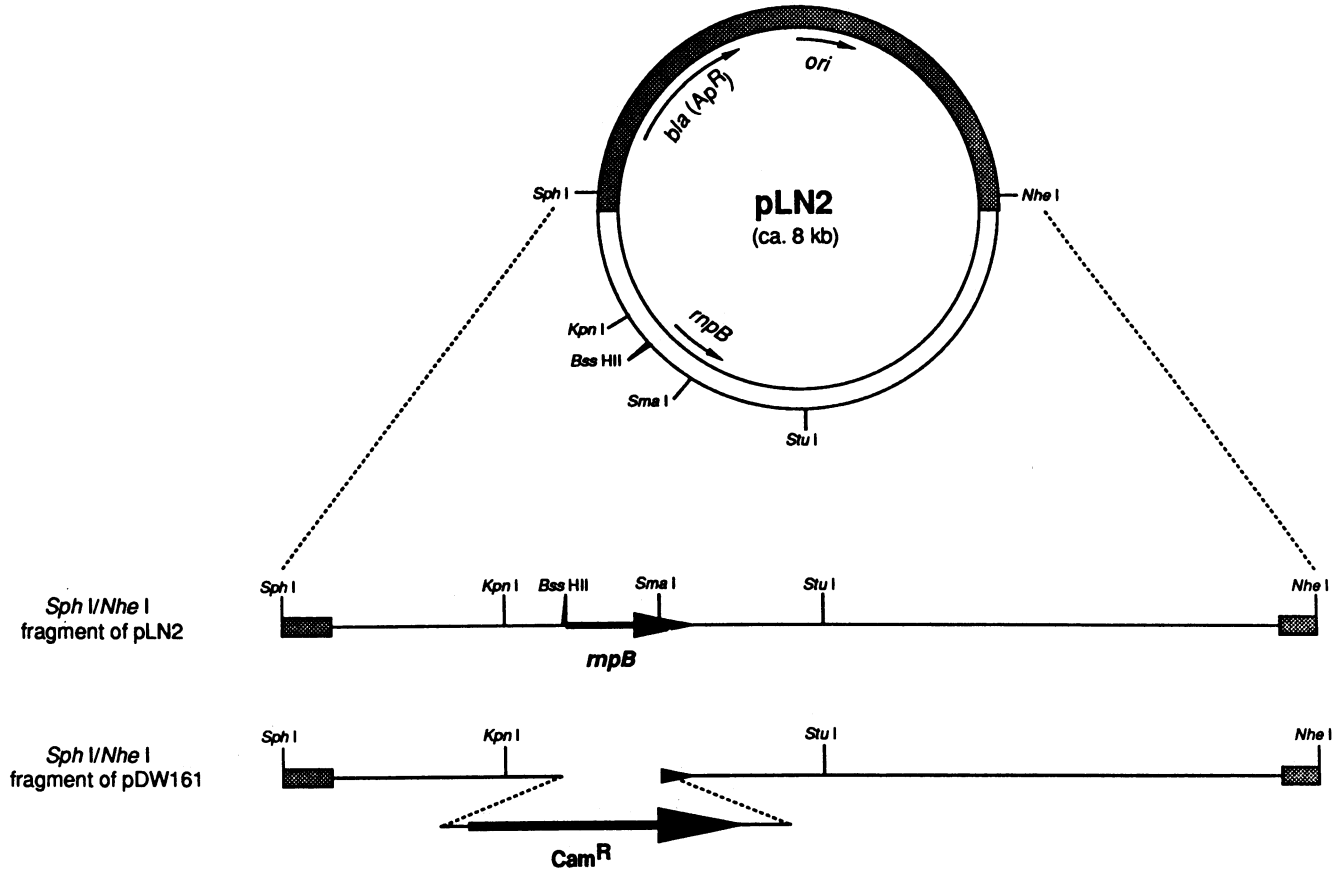

FIG. 1. Schematic representation of *Sph*I-*Nhe*I restriction fragments from pLN2 and pDW161. The two plasmids are identical except that the DNA between the *Bss*HII and *Sma*I sites in pLN2 was deleted and replaced by a Cam^r gene in pDW161 (see Materials and Methods). The *Sph*I-*Nhe*I fragment of pDW161, which was used to transform strain JC7623(pDW160) to Cam^r, includes approximately 1 kb of *E. coli* DNA upstream of the Cam^r gene and approximately 2 kb of *E. coli* DNA distal to the gene. The unshaded and shaded portions of the map represent *E. coli* and pBR322 sequences, respectively. Some of the unique restriction sites in pLN2 and pDW161 are indicated (both plasmids have two *Bss*HII sites that are separated by only 15 nucleotides).

oriented so that antisense *B. subtilis* RNase P RNA is produced by in vitro transcription with T7 RNA polymerase. pDHEco$^-$ was created by ligating a *Bam*HI-*Sna*BI fragment of pDW27 (Waugh, Ph.D. thesis, 1989) that includes the *E. coli rnpB* coding sequence with the large *Bam*HI-*Stu*I fragment of pDW19 (Waugh, Ph.D. thesis, 1989). The insert is oriented so that antisense *E. coli* RNase P RNA is produced by in vitro transcription with T7 RNA polymerase. pBBR1 was constructed by inserting a 3.0-kb *Pst*I-*Hin*dIII fragment of *Bacillus brevis* DNA that includes the *rnpB* gene (13) into *Pst*I-*Hin*dIII-cut pTZ18U (18). pBME1 was constructed by inserting a 2.0-kb *Pst*I fragment of *Bacillus megaterium* DNA that includes the *rnpB* gene (13) into *Pst*I-cut pTZ18U (18). pBST1 was constructed by inserting a 5.5-kb *Hin*dIII-*Sal*I fragment of *Bacillus stearothermophilus* DNA that includes the *rnpB* gene (13) into *Hin*dIII-*Sal*I-cut pTZ19U (18). pCVI1 was constructed by inserting a 2.5-kb *Hin*dIII-*Eco*RI fragment of *Chromatium vinosum* DNA that includes the *rnpB* gene (13) into *Hin*dIII-*Eco*RI-cut Bluescript KS$^+$ (1, 29). pATU1 was constructed by inserting a 2.4-kb *Pst*I-*Hin*dIII fragment of *Agrobacterium tumefaciens* DNA (strain A348) that includes the *rnpB* gene (13) into *Pst*I-*Hin*dIII-cut pTZ18U (18). pPFL1 was constructed by inserting a 3.0-kb *Nco*I-*Sal*I fragment of *Pseudomonas fluorescens* DNA that includes the *rnpB* gene (13) into *Nco*I-*Sal*I-cut pDW20 (Waugh, Ph.D. thesis, 1989). pAEU1 was constructed by inserting a 2.5-kb *Pst*I fragment of *Alcaligenes eutrophus* DNA (strain 335) that includes the *rnpB* gene (13) into *Pst*I-cut Bluescript KS$^+$ (1, 29). pDW27 contains the *E. coli rnpB* gene cloned adjacent to a bacteriophage T7 promoter in pUC19 (31). All restriction fragments were purified by elution from polyacrylamide or agarose gels as described by Maniatis et al. (16) before their inclusion in subsequent reactions.

Bacterial strains. The following strains were used: JC7623 (obtained from the *E. coli* Genetic Stock Center), F$^-$ *thr-1 ara-14 leuB6*Δ(*gpt-proA*)*62 lacY1 sbcC201 tsx-33 supE44 galK2* λ$^-$ Rec$^-$ *sbcB15 hisG4 rfbD1 recB21 recC22 rpsL31* (Str^r) *kdgK51 xyl-5 mtl-1 argE3 thi-1*; ER1794 (obtained from E. Raleigh, New England BioLabs, Inc.), F$^-$ *fhuA*(*tonA2*) Δ(*lacZ*)r1 *supE44* λ$^-$ *trp-31* Rec$^-$ *his-1 rpsL104*(Str^r) *xyl-7 mtl-2 metB1* Δ(*mrr-hsdRMS-mcrB*)*10*::IS*10 mcrA6*; and JC10240 (obtained from the *E. coli* Genetic Stock Center), Hfr (point of origin: PO45 of Hfr KL16) *thr-300* λ$^-$ *recA56 srl-300*::Tn*10*(Tet^r) *relA1 rpsE2300*(Spc^r) *spoT1 ilv-318 thi-1*.

Transformation of JC7623(pDW160) with linear DNA and selection of Cam^r recombinants. JC7623 cells were first transformed to kanamycin resistance (50 μg/ml) with pDW160 as described by Chung et al. (7). The same procedure was used to prepare JC7623(pDW160) cells for trans-

formation with linear DNA, pDW161 DNA cleaved with *Nhe*I and *Sph*I (Fig. 1).

P1 transductions. P1 *vir* (W3110) was a gift from E. Raleigh (New England Biolabs). P1 transductions were carried out as described previously (19).

RNA extraction. Cell were grown in 30 ml of 2× YT broth (19) at various temperatures (as indicated in the figure legends) to an A_{600} of between 0.5 and 1.0. The cultures were chilled on ice, the cells were pelleted by centrifugation, and RNA was extracted as described elsewhere (6).

Gel electrophoresis and Northern (RNA) blot analysis. High-molecular-weight RNAs were resolved by electrophoresis through 5% polyacrylamide slab gels containing 8 M urea and TBE (90 mM Tris base, 90 mM boric acid, 2 mM EDTA), with 3.5% polyacrylamide stacking gels in 0.5× TBE. The gels were electroblotted to Hybond-N (Amersham) transfer membranes. The transfer was carried out in 50 mM sodium phosphate buffer (pH 6.5) at 4°C for 24 h (2 V/cm). The RNA was fixed on the membrane by exposure to a germicidal lamp (350 μW/cm^2, 2 min). The membrane was then sealed in a plastic bag with hybridization mix (5 ml/100 cm^2; 1× Denhardt solution [16], 5× SSC [16], 20 mM sodium phosphate [pH 7.6], 100 μg of polyadenylic acid per ml, 10% dextran sulfate [molecular weight, 5,000], 1 mM dithiothreitol, 0.05% sodium dodecyl sulfate) and incubated at 65°C. After 2 h, 10^7 cpm of ^{32}P-labeled probe was added. The bags were then sealed and incubated overnight (typically for 18 h) at 65°C. The hybridization solution was discarded, and the membranes were washed twice for 5 min each time with 2× SSC (20 ml/100 cm^2) at room temperature, once with 1× SSC for 10 min at 65°C, and once with 0.1× SSC at 65°C for 10 min. In some instances, the probes were then stripped from the membranes by boiling in 0.1% sodium dodecyl sulfate, and the membranes were reprobed by using the same procedure.

Synthesis of radiolabeled probes for Northern blot analysis. To prepare the *E. coli* RNase P RNA probe, pDHEco$^-$ DNA was linearized with *Bam*HI and then transcribed in vitro with T7 RNA polymerase. A typical 20-μl reaction mixture included 20 mM sodium phosphate (pH 7.7), 10 mM dithiothreitol, 8 mM $MgCl_2$, 4 mM spermidine hydrochloride, 1 mM ATP, 1 mM CTP, 1 mM UTP, 0.2 mM GTP, 50 μCi of [α-^{32}P]GTP (3,000 Ci/mmol; Amersham), 2 μg of linearized template, and a saturating amount of T7 RNA polymerase. Incubation was at 37°C for 3 h. The reaction mixture was then passed through a column containing Sephadex G-50 (medium) equilibrated in 10 mM Tris hydrochloride (pH 8)–1 mM EDTA–0.1% sodium dodecyl sulfate to remove unincorporated nucleotides. The *B. subtilis* RNase P RNA probe was prepared in the same way except that the template was pDW10 DNA linearized with *Hin*dIII. Both probes were subjected to partial alkaline hydrolysis (100 mM sodium bicarbonate, 95°C for 5 to 20 min) before use.

RESULTS

Construction of *E. coli* strains in which the only functional RNase P RNA gene resides on a temperature-sensitive plasmid replicon. The method described by Jasin and Schimmel (14) was used to delete the *rnpB* (RNase P RNA) gene from the *E. coli* chromosome. A *recBC* mutant strain of *E. coli* (JC7623) was transformed with a linear fragment of DNA that includes a Cam^r gene bounded by sequences that flank the *rnpB* gene in the chromosome (Fig. 1). By selecting for a double-crossover event within the homologous sequences (Cam^r phenotype), it was possible to replace the chromosomal *rnpB* gene with the Cam^r gene. Since the *rnpB* gene is essential for viability, JC7623 cells that contained pDW160 were used for the transformations. Plasmid pDW160 (Fig. 2), a derivative of pSC101 (8), contains a thermosensitive replicon and carries a wild-type *E. coli rnpB* gene. The plasmid maintains cell viability in the absence of the chromosomal *rnpB* gene, but the resulting cells have a temperature-sensitive phenotype.

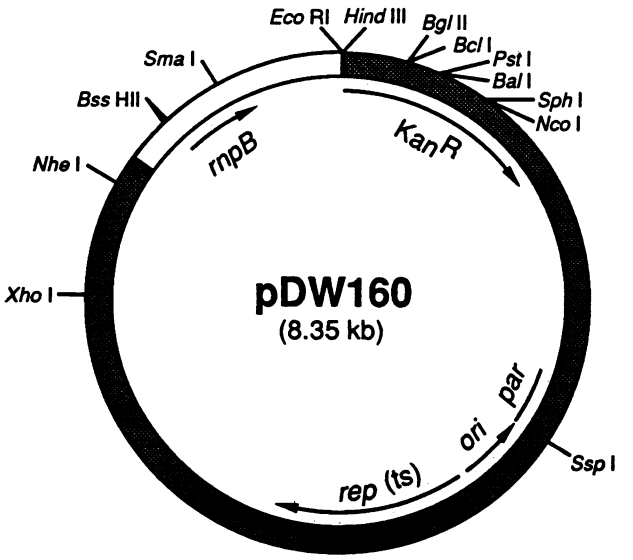

FIG. 2. Schematic diagram of pDW160, a thermosensitive derivative of pSC101 that carries a wild-type *E. coli rnpB* gene. The unshaded portion of the map represents *E. coli* DNA. Some of the unique restriction sites are indicated (there are two *Bss*HII sites separated by only 15 nucleotides).

As detailed in Materials and Methods, an *Sph*I-*Nhe*I double digest of pDW161 (Fig. 1) was used to transform JC7623 cells containing pDW160 to chloramphenicol resistance. Most of the Cam^r colonies obtained on LB agar (19) containing chloramphenicol (30 μg/ml) at 30°C were sensitive to ampicillin; however, some were resistant to that drug. The Amp^r isolates presumably contained recircularized pDW161 and were discarded. Eight of the Amp^s isolates were analyzed further. All proved to be resistant to kanamycin (50 μg/ml), indicating that they contain pDW160, and four were temperature sensitive compared with JC7623 cells containing pDW160. The absence of the *rnpB* gene and the presence of the Cam^r gene at the appropriate locations in the chromosome were verified for two of the thermosensitive isolates by Southern blotting (data not shown). One of these isolates was designated DW1.

Two additional strains with the temperature-sensitive properties of DW1 were constructed. One potential use of strain DW1 is for cloning *rnpB* genes from diverse organisms by heterologous complementation (below). To minimize degradation of potentially transforming foreign DNA by the recipient *E. coli* mutant, we moved the *rnpB* deletion into a background that lacks known restriction systems (5). A bacteriophage P1 *vir* lysate of DW1 was used to transduce (19) ER1794 (*mcr mrr*) containing pDW160 to Cam^r. The resultant strain was designated DW2. For genetic studies (23), a *recA* derivative of DW2 was constructed by transduction to Tet^r, using a bacteriophage P1 *vir* lysate of JC10240 (9). The resulting strain was designated DW3. Differences

TABLE 1. Some properties of strains lacking chromosomal *rnpB*

Strain	Genetic background	*recBC*	*recA*	*hsdRSM*	*mcrAB*	*mrr*
DW1	JC7623	−	+	+	+	+
DW2	ER1794	+	+	−	−	−
DW3	ER1794	+	−	−	−	−

between the three strains containing the deletable *rnpB* gene are summarized in Table 1.

Complementation of the *rnpB* gene deletion by RNase P RNA genes from distantly related eubacteria. We investigated whether the *rnpB* genes from a number of distantly related eubacteria are capable of functioning in *E. coli* by transforming strain DW2 with Amp^r plasmids that carry the *rnpB* genes from those organisms (Materials and Methods) and plating the cells on LB agar containing ampicillin (100 μg/ml) at 42°C (Table 2). As expected, when a plasmid that does not contain an *rnpB* gene (Bluescript KS$^+$) was used, Amp^r colonies were obtained at 30°C but not at 42°C. On the other hand, when the cells were transformed with a plasmid that carries the wild-type *E. coli rnpB* (pLN2), the same numbers of Amp^r transformants were obtained at 30 and 42°C. Thus, in this genetic background, the complementation occurs and seems complete as assayed by viability. A plasmid (pDW27) that contains an *rnpB* gene but lacks the normal promoter, containing instead a bacteriophage T7 promoter, weakly complements the deletion. Of the other eubacteria tested in this transformation assay, only the genes from *A. eutrophus*, *B. subtilis*, and *C. vinosum* were capable of producing viable transformants at 42°C: essentially the same numbers of Amp^r transformants were obtained at both temperatures. Amp^r colonies from plates incubated at 42°C were kanamycin sensitive (50 μg/ml). Since pDW160, which carries the *E. coli rnpB* gene, also includes the Kan^r gene, this result indicates that the *E. coli rnpB* gene was eliminated by selection at 42°C and therefore that the cells depend on a foreign RNase P RNA.

The successful complementations of the *rnpB* gene deletion indeed were a consequence of replacing the *E. coli* RNase P RNA with that from a different organism. As shown in Fig. 3 for the transformant with the *B. subtilis* RNase P RNA gene (pDW3), growth at 42°C resulted in the elimination of the temperature-sensitive, *rnpB*-containing plasmid

TABLE 2. Complementation of chromosomal *rnpB* deletion by other *rnpB* genes

Source of *rnpB* gene	Growth at:	
	30°C	42°C
None (pBluescript KS$^+$)	+	−
Escherichia coli(pLN2)	+	+
E. coli(pDW27)	+	+[a]
Agrobacterium tumefaciens(pATU1)	+	−
Pseudomonas fluorescens(pPFL1)	+	−
Chromatium vinosum(pCVI1)	+	+[b]
Alcaligenes eutrophus(pAEU1)	+	+
Bacillus subtilis(pDW3)	+[b]	+
B. brevis(pBBR1)	+	−
B. megaterium(pBME1)	+	−
B. stearothermophilus(pBST1)	+	−[c]

[a] Small, slowly growing colonies.
[b] Mucoid colonies.
[c] Cells lyse at 42°C; no *Bst* RNase P RNA is produced at 30 or 37°C.

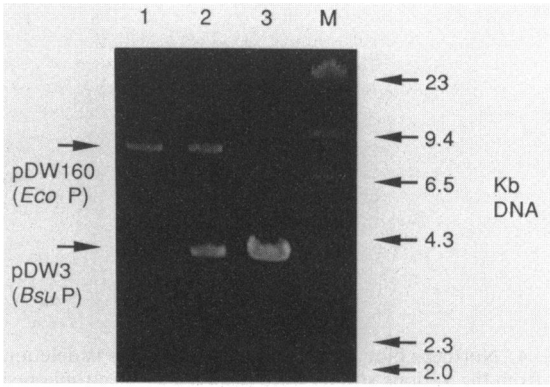

FIG. 3. Plasmid contents of a deletion strain containing a *B. subtilis rnpB* gene at low and high temperatures. DNAs from the various strains were cleaved with *Ssp*I to linearize, resolved by agarose (1%) gel electrophoresis, and detected by ethidium bromide staining. Lanes: 1, strain DW2(pDW160) grown at 30°C; 2, strain DW2(pDW160, pDW3) grown at 30°C; 3, strain DW2(pDW3) grown at 42°C; M, *Hin*dIII-digested phage λ DNA markers. Arrows at the left identify the plasmids visualized; numbers on the right identify the sizes of the indicated marker fragments.

(pDW160), with the retention of pDW3. Figure 4 shows a Northern blot analysis of total RNA from the same strains, using probes specific for the *E. coli* or *B. subtilis* RNA or for a mixture thereof. The *E. coli* RNase P RNA was not detected at 42°C, as expected if the eliminated plasmid contained the only *E. coli rnpB* gene in that strain. The *B. subtilis* RNase P RNA was produced at both 30 and 42°C; at 42°C, growth must depend on the *B. subtilis* RNA. The *B. subtilis*-specific probe identified RNAs of two sizes in DW2(pDW3) (Fig. 4C, lanes 3 and 4). The smaller of these was about (but not exactly) the size of mature RNase P RNA normally produced in *B. subtilis* (lane 5) and presumably derived from processing of the larger form.

We investigated whether the lack of complementation of the *rnpB* gene deletion by RNase P RNA genes from some organisms might be due to lack of expression or instability of the foreign RNAs in *E. coli*. Northern blot analysis was performed on total RNA extracted from *E. coli* cells containing the normal, chromosomal RNase P RNA gene and also plasmids that carry the *rnpB* genes from the other organisms listed in Table 2 (data not shown). In every case except one, failure to complement the *rnpB* gene deletion (Table 2) can be explained by the absence of stable, heterologous RNase P RNA at the restrictive temperature. The *rnpB* gene from *B. megaterium* is an exception; although it did not complement the deletion, it did produce stable RNase P RNA in *E. coli*. Further work will be required to determine why this RNA is unable to function in *E. coli*.

DISCUSSION

The conditional mutants described here will be valuable tools for genetic studies of RNase P RNA structure and function. Mutations constructed in vitro can now be studied in vivo. The strains can be used to screen for mutations that abolish activity in vivo. Together with oligonucleotide cassette mutagenesis, the *rnpB* deletion also can be used to select mutations that do not abolish RNase P RNA activity in vivo (permissible changes). This type of mutational analysis has been used to determine the informational content of amino acid side chains in proteins (26) and should be applicable to RNase P RNA as well. The deletion strains

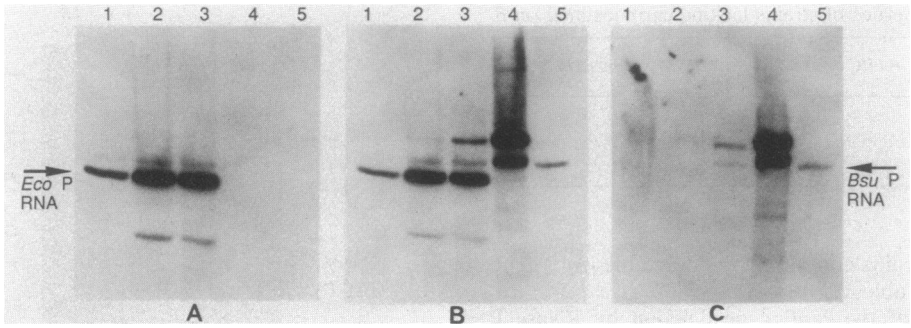

FIG. 4. Northern blot analysis of RNase P RNAs in deletion strains containing a *B. subtilis rnpB* gene at low and high temperatures. Total RNAs from the various strains were resolved by electrophoresis in a 5% polyacrylamide gel and electrophoretically transferred to a charged nylon membrane. Hybridization with the different blots was carried out with probes specific for the *E. coli* (A), *E. coli* plus *B. subtilis* (B), and *B. subtilis* (C) RNase P RNAs (see Materials and Methods). Lanes: 1, *E. coli* MRE600 grown at 37°C; 2, strain DW3(pDW160) grown at 30°C; 3, strain DW2(pDW160, pDW3) grown at 30°C; 4, strain DW2(pDW3) grown at 42°C; 5, *B. subtilis* 168 grown at 37°C. Arrows indicate positions of the native RNase P RNAs. The hybridizing bands smaller than the RNase P RNAs presumably derived from a low level of fragmentation of the native RNAs during growth or isolation.

may, however, be most useful as tools for reversion analysis. Intragenic reversion of selected mutations can be used to obtain information about the structure of the RNase P RNA; extragenic revertants could provide information about the interaction of the RNase P RNA with the RNase P protein or with other macromolecules in vivo.

The strains with the deletable *rnpB* gene may also be useful for cloning *rnpB* genes from diverse organisms. The observation that at least some plasmid-borne, heterologous *rnpB* genes are capable of complementing the deletion indicates that the approach is feasible; its potential value is evidenced by the fact that complementation is successful even though the heterologous genes (e.g., that of *B. subtilis*) may be so different from the *E. coli rnpB* gene that they

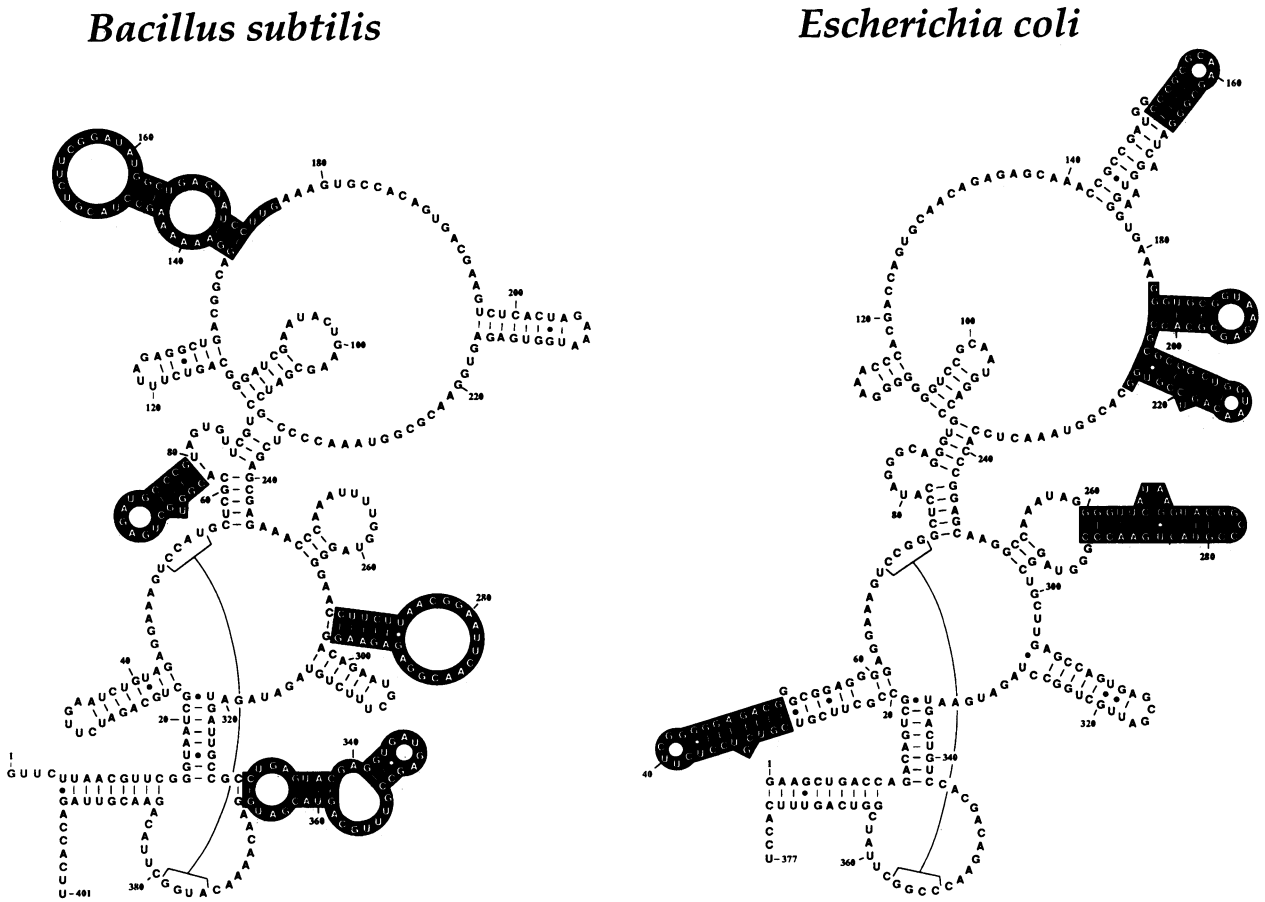

FIG. 5. Secondary-structure models of *B. subtilis* and *E. coli* RNase P RNAs. The models are based on phylogenetic comparison (13). Structural domains in one type of RNA that do not have a homolog in the other RNA are highlighted (see text for discussion).

cannot be identified by heterologous hybridization (11). Thus, the sensitivity of the complementation test may extend beyond that obtainable with heterologous hybridization.

The *rnpA49* mutant already has been used to clone RNase P RNA genes from several close relatives of *E. coli* by heterologous complementation (15). We obtained the same qualitative result when the various *rnpB* genes listed in Table 2 were tested for their ability to complement the *rnpA49* mutation (data not shown). Nevertheless, we believe that complementation of the deletions described here inspires more confidence than does complementation of the *rnpA49* mutation. The strain with the latter mutation contains a temperature-sensitive RNase P protein, and the mechanism of complementation of the *rnpA* mutation by the *rnpB* gene is obscure (3). Moreover, in at least one instance, complementation of the *rnpA* allele has generated a false-positive clone. A fragment of the *Methanothermus fervidus* 16S rRNA gene is capable of rescuing the *rnpA* mutation (12). It fails, however, to complement an *rnpB* gene deletion (DW2).

The RNase P RNAs of different eubacteria vary substantially in sequence. Much of the variation is due to phylum-specific occurrence of short hairpin helices inserted into a core of conserved secondary structure and homologous sequence. The phylum-specific structures in the RNase P RNAs of *B. subtilis* and *E. coli* are indicated in Fig. 5. The fact that the *B. subtilis rnpB* gene is capable of functioning as the sole source of RNase P RNA in *E. coli* indicates that the phylum-specific domains of the *E. coli* RNA are not absolutely essential for growth. It is known that these phylum-specific domains are dispensable for pre-tRNA processing in vitro, since a synthetic RNase P RNA (*Min*1 RNA) consisting only of phylogenetically conserved structural features is nearly as active as the native RNA enzymes (31). The *Min*1 RNA gene will not complement the deletion mutation described here. However, activity of the synthetic RNA in vitro is dependent on such high ionic strength that it would not be expected to function in vivo. It remains conceivable that the phylum-specific structural elements in the RNase P RNAs play some role in vivo and that the observed complementation is due to the presence of a large amount of marginally active RNase P RNA. We think it likely, however, that the phylum-specific structures are analogous to expansion sequences in rRNAs, i.e., evolutionarily volatile sequences that are likely to be functionless but do not impair the RNA and so are tolerated by the cell (6, 10).

It has been known for some time that heterologous holoenzymes formed by combining the RNA and protein subunits of RNase P from *E. coli* and *B. subtilis* are active in vitro (11). Nevertheless, the ability of the *B. subtilis rnpB* gene to function as the only source of RNase P RNA in *E. coli* seems remarkable in view of the extensive dissimilarity between the primary and secondary structures of the two molecules. This finding indicates that despite these apparent differences, the higher-order structures of the functional components of the two RNase P RNAs must indeed be very similar.

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