NOTES

An Essential Function for the Phosphate-Dependent Exoribonucleases RNase PH and Polynucleotide Phosphorylase

ZHIHUA ZHOU AND MURRAY P. DEUTSCHER*

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06030, and
Department of Biochemistry and Molecular Biology, University of Miami School of Medicine,
Miami, Florida 33101-6129

Received 28 February 1997/Accepted 18 April 1997

Escherichia coli cells lacking both polynucleotide phosphorylase (PNPase) and RNase PH, the only known P_i -dependent exoribonucleases, were previously shown to grow slowly at 37°C and to display a dramatically reduced level of tRNA^{Tyr}su₃⁺ suppressor activity. Here we show that the RNase PH-negative, PNP-negative double-mutant strain actually displays a reversible cold-sensitive phenotype and that tRNA biosynthesis is normal. In contrast, ribosome structure and function are severely affected, particularly at lower temperatures. At 31°C, the amount of 50S subunit is dramatically reduced and 23S rRNA is degraded. Moreover, cells that had been incubated at 42°C immediately cease growing and synthesizing protein upon a shift to 31°C, suggesting that the ribosomes synthesized at the higher temperature are defective and unable to function at the lower temperature. These data indicate that RNase PH and PNPase play an essential role that affects ribosome metabolism and that this function cannot be taken over by any of the hydrolytic exoribonucleases present in the cell.

Of the eight distinct 3'-to-5' exoribonucleases that have been identified and isolated from *Escherichia coli*, only two, polynucleotide phosphorylase (PNPase) and RNase PH, catalyze a P_i -dependent degradation of RNA leading to the release of nucleoside diphosphates rather than monophosphates (6, 7). Although these enzymes also catalyze the synthesis of RNA from nucleoside diphosphates, at the in vivo concentration of phosphate, both enzymes would be expected to participate primarily in RNA-degradative reactions (6, 14, 20). At present, PNPase and RNase PH are the only P_i -requiring nucleases known. The primary role of PNPase is thought to be in mRNA degradation (8), whereas that of RNase PH is in tRNA metabolism (5, 15, 17, 19). What has not been clear are the specific functions served by these enzymes that require a phosphorytic rather than a hydrolytic mode of degradation.

As an approach to answering this question, mutant cells lacking both enzymes were constructed (15, 24). Such cells grew poorly at 37°C, whereas cells lacking only one of the two RNases were essentially unaffected. Moreover, the absence of both P_i -dependent exoribonucleases dramatically decreased the level of tRNA^{Tyr}su₃⁺ suppressor activity to only ~10% of that in wild-type or single-mutant cells at 31°C (15, 24). From these initial studies, it appeared that PNPase and RNase PH have overlapping specificities in vivo and that they might play an important role in tRNA metabolism.

In this paper, the properties of an RNase PH-negative, PNPase-negative double-mutant strain are examined in more detail. The data from this study indicate that cells devoid of both RNase PH and PNPase not only grow poorly at 37°C

but are extremely cold sensitive in their growth. Surprisingly, despite the decreased suppressor activity, tRNA synthesis and processing in the double-mutant strain are apparently normal. In contrast, ribosome structure and function are severely affected, particularly at lower temperatures. These findings indicate that in addition to their roles in tRNA and mRNA metabolism, the P_i -dependent exoribonucleases also serve an essential function that affects ribosomes.

Cold-sensitive phenotype of RNase PH-negative, PNPase-negative cells. On YT plates, the cells of the double mutant CA244 Rph⁻ Pnp⁻ (*lacZ trp relA spoT rph::cam pnp::Tn5*) (15, 24) displayed a slow-growth phenotype that became more pronounced as the incubation temperature was decreased. Thus, at 31°C, 2 to 3 days of incubation were needed for the production of visible colonies, and no growth was observed even after 1 week when cells were incubated at 21°C. Single mutants lacking RNase PH produced colonies similar in size to those of the wild type at all the temperatures examined, while PNPase single-mutant colonies were slightly smaller at all temperatures tested. Interestingly, much less of a growth defect was observed on minimal-medium (M9-glucose) plates. At 37°C, colonies of double-mutant cells were similar in size to those of the single mutants and the wild type. At 31°C, the double-mutant colonies were smaller but the difference in growth compared to the single mutants and wild type was much less pronounced than on rich medium.

A more quantitative measure of the growth rates of the various strains was obtained from determination of doubling times in liquid culture (Table 1). At 42°C, the doubling time of the RNase PH-negative, PNPase-negative double mutant in YT-glucose medium was twice that of the wild type; at 37°C, this difference increased to 5-fold, and at 31°C, the double-mutant cells grew more than 10 times more slowly. In contrast, RNase PH-negative or PNPase-negative single-mutant cells

* Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, P.O. Box 016129, Miami, FL 33101-6129. Phone: (305) 243-3150. Fax: (305) 243-3955. E-mail: mdeutsch@mednet.med.miami.edu.

TABLE 1. Growth rates of RNase PH-negative and PNP-negative mutant strains^a

Relevant phenotype (plasmid)	Doubling time (min) at temperature ^b :		
	31°C	37°C	42°C
Wild type	50	27	24
Rph ⁻	49	27	24
Pnp ⁻	55	33	30
Rph ⁻ Pnp ⁻	480 ^c	130	50
Rph ⁻ Pnp ⁻ (pBRPH)	55	34	31
Rph ⁻ Pnp ⁻ (pKAK7)	44	29	26

^a Cells were grown directly from colonies in YT medium supplemented with 0.2% glucose at the temperatures indicated.

^b Doubling times were calculated from the A_{550} values determined at 30-min intervals.

^c This strain tends to revert to a faster-growing form at 31°C. The doubling time shown is the maximum value observed.

had doubling times similar to those of the wild type at all three temperatures. These data demonstrate that cells lacking both RNase PH and PNPase display a severe cold-sensitive growth phenotype and that some metabolic process is strongly dependent on the presence of a P_i -requiring RNase.

To ensure that the observed phenotype is, in fact, due to the absence of RNase PH and PNPase, strain CA244 Rph⁻ Pnp⁻ (15, 24) was transformed with plasmid pBRPH (derived from plasmid pPP1, which was obtained from K. F. Jensen) and, separately, with plasmid pKAK7 (obtained from S. Kushner), which carry only the wild-type *rph* and *pnp* genes, respectively. Introduction of either plasmid completely reversed the cold-sensitive growth phenotype of the double mutant (Table 1). These findings indicate that the loss of RNase PH and PNPase completely accounts for the observed cold sensitivity.

The reversibility of the double-mutant strain's cold sensitivity was examined in a temperature shift experiment (Fig. 1). Wild-type and mutant cell cultures growing at 42°C were diluted and shifted to 31°C for the periods of time indicated in Fig. 1; for a portion of the culture, this was followed by a shift back to 42°C. Upon decreasing the temperature to 31°C, growth of the wild-type cells continued (Fig. 1A), although with a slower doubling time than at 42°C. The RNase PH-negative, PNPase-negative cells (Fig. 1B), in contrast, rapidly ceased growing upon temperature downshift and remained in this condition for the remainder of the experiment (over 6 h). When the temperature was returned to 42°C, both wild-type and double-mutant cells resumed growth at their 42°C rate after an identical lag period of about 1 h. These data suggest that the affected cellular component responsible for the cold sensitivity of the RNase PH-negative, PNPase-negative mutant is able to function at 42°C but rapidly loses activity at 31°C. However, this component can be reactivated as rapidly as its wild-type counterpart upon elevation of the temperature to 42°C.

Effect of RNase PH and PNP mutations on tRNA biosynthesis. Earlier studies (15) had shown that su3 suppressor activity expressed from a single-copy plasmid is greatly reduced in an RNase PH-negative, PNPase-negative strain. To determine whether this decreased activity reflected a reduced level of tRNA^{Tyr}su₃⁺, Northern blot analysis (18) was performed to examine whether the amount or state of tRNA^{Tyr}su₃⁺ was affected by the absence of RNase PH and PNPase. Surprisingly, as shown in Fig. 2, RNase PH-negative, PNPase-negative cells (lane 4) showed no reduction in the amount of tRNA^{Tyr}su₃⁺ compared to the wild type (lane 1) or to the single mutants (lanes 2 and 3), nor was there any accumulation of

FIG. 1. Temperature shift experiments comparing wild-type (WT) and RNase PH-negative, PNPase-negative (PH⁻PNP⁻) mutant cells. Cultures were grown at 42°C in YT-0.2% glucose medium to early log phase ($A_{550} \approx 0.3$) and then temperature shifted by dilution into 4 volumes of YT-0.2% glucose medium kept at 31°C. The shifted cultures were incubated at 31°C for up to 6 h. After about 2 h, portions of the cultures that had been incubated at 31°C were then shifted back to 42°C. For the wild type, this was accomplished by a 1:5 dilution in YT-glucose. For the RNase PH-negative, PNPase-negative strain, half of the 31°C culture was shifted back to 42°C without further dilution. Absorbance was monitored every 15 or 20 min for all cultures until late log phase was reached. (Top) Wild type; (Bottom) RNase PH-negative, PNPase-negative mutant. The arrow indicates the time of shift back to 42°C.

tRNA precursors despite the fact that suppressor activity was dramatically reduced in the same cells. Thus, the ~90% reduction in suppressor activity apparently was not due to a reduced amount of suppressor tRNA.

Five other tRNAs, encoded by the chromosome rather than by a plasmid, were also examined by Northern blotting (data not shown). As with the plasmid-borne tRNA gene, there was no defect in the maturation of tRNA^{Tyr}, tRNA^{Ser}, tRNA^{Val}, tRNA^{Cys}, or tRNA^{Arg} in cells lacking RNase PH and PNPase. These data show that it is unlikely that the cold sensitivity of the RNase PH-negative, PNPase-negative double mutant is due to a defect in the processing of tRNA precursors. Rather, as will be shown below, the RNase PH-negative, PNPase-negative double-mutant cells contain altered ribosomes which could affect the ability of the suppressor tRNA to function properly.

Effect of RNase PH and PNP mutations on ribosomes. Cold-sensitive mutants of *E. coli* often are defective in ribosome

FIG. 2. Northern blot analysis of $tRNA^{Tyr^{su3+}}$ in wild-type and mutant cells. Vector pOU61 (16) or plasmid pOUCCA encoding the suppressor tRNA (15) were transformed into wild-type and single- or double-mutant strains. Cells were grown at 31°C and harvested at an A_{550} of ~ 1.0 . Total small RNAs were isolated as described previously (18). Electrophoresis was performed on a 6% polyacrylamide–8.3M urea sequencing gel run at 1,600 V until the xylene cyanol dye had migrated 29 cm. RNA was transferred to a GeneScreen Plus membrane (DuPont) by electroblotting. tRNA was detected with the 14-mer deoxyoligonucleotide AGATTTAGAGTCTG, complementary to the anticodon region. Lanes for cells with plasmid pOUCCA: 1, wild-type cells; 2, PNPase-negative cells; 3, PH-negative cells; 4, PH-negative, PNPase-negative cells. Lane 5, wild-type cells with plasmid pOU61.

metabolism (1, 4, 10, 12, 23, 25). Therefore, we compared the status of ribosomes isolated from differentially labeled wild-type and double-mutant cells during steady-state growth at three different temperatures (Fig. 3). Ribosomes were prepared in low- Mg^{2+} buffer (0.3 mM) so that the 30S and 50S ribosomal subunits could be assessed individually. Shown in Fig. 3C are ribosomal subunits isolated from cells grown at 31°C. Major differences between the ribosome profiles of the wild type and the RNase PH-negative, PNPase-negative mutant were seen at this temperature. The double-mutant cells contained only a small quantity of isolable 50S subunits; in addition, particles sedimenting at a size smaller than 30S accumulated, suggesting either degradation or incomplete assembly of the 50S subunits. At 37°C, more particles of the sizes 30S and 50S accumulated in the double mutant; however, there was still a major deficiency of the 50S particles compared to the wild type (Fig. 3B). At 42°C, the ribosomal subunit profile of the mutant was much closer to that of the wild type, although even at this temperature the ratio of 50S to 30S subunits remained somewhat abnormal (Fig. 3A). Thus, these data reveal a close correlation between the degree to which ribosome profiles are altered and the relative growth rates of the mutant cells at the three temperatures (Table 1), supporting the conclusion that the ribosome alterations are related to the cold sensitivity of the mutant cells.

It should be noted that even though ribosomes isolated from cells grown at 42°C appear nearly normal by sedimentation analysis, they apparently cannot function at lower temperatures. As presented in Fig. 1B, mutant cells downshifted from 42 to 31°C rapidly ceased growing. Radioactive labeling following the downshift revealed that protein synthesis also shut off completely (Fig. 4B); RNA accumulation, on the other hand, proceeded in the mutant, although at a slower rate (Fig. 4A). Based on sedimentation analysis, the preexisting ribosomes synthesized at 42°C were stable for at least 45 min at 31°C (data not shown). Thus, these data suggest that while ribosomes made at 42°C can function at that temperature, they nevertheless must differ in some way from wild-type ribosomes.

Effect of RNase PH and PNP mutations on ribosomal RNA.

To further assess the effect of the RNase PH and PNPase mutations on ribosomes, the integrity of the rRNA was also examined. As shown in Fig. 5, there was a dramatic difference in the rRNA profiles of the wild-type and RNase PH-negative, PNPase-negative cells. At 42°C (Fig. 5A) there was a significant reduction in the ratio of 23S to 16S rRNA in the mutant compared to the wild-type ratio, with a concomitant increase in RNA smaller than 16S. This deficiency in the amount of 23S

FIG. 3. Sedimentation analysis of ribosomal subunits from wild-type and RNase PH-negative, PNPase-negative mutant cells. S30 supernatant extracts were prepared from wild-type (^{32}P -labeled) and RNase PH-negative, PNPase-negative (3H -labeled) cells grown in BT medium supplemented with 0.2% glucose (9) and either $^{32}P_i$ or [3H]uridine. At an A_{550} of ~ 0.1 , cells were ruptured in a French press and then centrifuged at $30,000 \times g$ for 20 min. Approximately 200 μ l of a mixture of 3H - and ^{32}P -labeled S30 fractions was layered on 12-ml 5 to 20% sucrose gradients in 10 mM Tris-HCl (pH 7.8)–30 mM NH_4Cl –0.3 mM $MgCl_2$ –6 mM mercaptoethanol, and the gradients were centrifuged for 15.5 h at 22,000 rpm in a Beckman SW41 rotor. (A) Cells grown at 42°C; (B) cells grown at 37°C; (C) cells grown at 31°C. Open circles, wild type; closed circles, mutant.

rRNA was even more pronounced at 37°C (Fig. 5B). At that temperature, much less RNA was present in the 23S region and there was a large increase in RNA sedimenting between 4S and 16S. A similar experiment, run under slightly different conditions, was also performed with RNA prepared from cells grown at 31°C. At this temperature, a broad peak sedimenting between 4S and 16S was found and almost no 23S RNA was present (data not shown). These data indicate that the altered ribosomal-subunit profiles seen in Fig. 3 result from extensive rRNA degradation, especially of 23S RNA.

The data presented here show that RNase PH and PNPase, the only two P_i -dependent RNases known, play an essential role in ribosome metabolism in *E. coli*. What specific function

FIG. 4. Protein and RNA synthesis after a temperature shift from 42 to 31°C. Cultures (5.5 ml) were grown from single colonies at 42°C in YT-glucose medium containing either [35 S]Protein Labeling Mix (2.5 μ Ci per ml, 1,175 mCi per μ mol) (Dupont/NEN) or [5,6- 3 H]uridine (1.5 to 3.0 μ Ci per ml, 44 mCi per μ mol). At an A_{550} of ~ 0.3 , each culture was diluted into 4 volumes of the same labeled medium and shifted to 31°C. Samples of 1.0 ml were withdrawn at the indicated times and precipitated with 15% trichloroacetic acid, and then radioactivity was determined. Data are expressed relative to the amount of RNA and protein present at the time of the shift. (A) [3 H]uridine incorporation; (B) [35 S] incorporation. Open circles, wild type; closed circles, RNase PH-negative, PNPase-negative mutant.

these nucleases might serve remains to be determined. Both enzymes are 3'-to-5' exoribonucleases (6, 7). Yet, none of the other hydrolytic 3'-to-5' exoribonucleases present in *E. coli* can substitute for RNase PH or PNPase in this process under the usual cellular conditions. We have found that overexpression of one hydrolytic exoribonuclease, RNase II, can partially complement the cold-sensitive phenotype of the RNase PH-negative, PNPase-negative double mutant, but not as well as can be done by even a single chromosomal copy of either the *rph* or *pnp* gene (26). Thus, whatever process is carried out by RNase PH or PNPase, a P_i -dependent exoribonuclease is critical. The specific requirement for a P_i -dependent nuclease raises the interesting possibility that P_i levels might influence ribosome metabolism and, ultimately, protein synthesis in vivo. Under low- P_i conditions, RNase PH and PNPase would be relatively inactive, resulting in a situation similar to that in the double mutant.

The phenotype of the RNase PH-negative, PNPase-negative double mutant is reminiscent of that seen in a number of other cold-sensitive mutants that are affected in various aspects of ribosome biogenesis (1, 4, 10, 12, 23, 25). The defect in the

FIG. 5. Sedimentation analysis of RNA from wild-type and PH-negative, PNPase-negative mutant cells. Total RNA was isolated from wild-type (32 P-labeled) and RNase PH-negative, PNPase-negative (3 H-labeled) cells by using sodium dodecyl sulfate (1%), EDTA (10 mM), and phenol. Approximately 150 μ l of a mixture of 3 H- and 32 P-labeled total RNA was analyzed as described in the legend to Fig. 3 except that centrifugation was for 24 h at 25,000 rpm in a Beckman SW41 rotor. (A) RNA isolated from cells grown at 42°C; (B) RNA isolated from cells grown at 37°C. Open circles, wild type; closed circles, RNase PH-negative, PNPase-negative mutant.

RNase PH-negative, PNPase-negative cells is observed at all temperatures, but at 31°C it is so severe that very few intact large subunits are present, which explains why mutant cells are essentially unable to grow at that temperature. The reduction in the number of intact 50S subunits is accompanied by degradation of the 23S rRNA and the formation of products sedimenting between 4S and 16S. These findings are most consistent with an assembly defect at 31°C in which rRNA is synthesized but, because the 50S subunit cannot be assembled properly, its RNA is left unprotected and is ultimately degraded. At 42°C, the assembly defect is overcome and ribosomes are made. However, these ribosomes appear to be abnormal, as shifting to 31°C shuts off protein synthesis immediately. How the absence of RNase PH and PNPase might lead to such effects on ribosome biogenesis remains to be determined. In addition to direct effects on rRNA processing, effects that are more indirect, such as maturation of an mRNA for a ribosomal protein or for an assembly or modification protein (11), also can be envisaged. Distinguishing among these possibilities will be challenging, as much remains to be learned about the details of ribosome biogenesis.

It should be noted that PNPase has been reported to be a cold shock protein (2, 13). It has also been shown that *E. coli* and *Bacillus subtilis* strains lacking PNPase display cold-sensitive growth at temperatures of 23°C and below (21). However, we have not observed cold sensitivity of the PNPase mutant at

31°C (Table 1). A PNPase mutation has also been implicated in increased sensitivity of *E. coli* to antibiotics (22). RNase PH of *B. subtilis* has been found to suppress a number of cold-sensitive mutations of *E. coli* (3). These diverse observations, together with the information presented here, identify a variety of unexplained functions for the P_i-dependent exoribonucleases. Clearly, further studies will be necessary to unravel the complexities of these unusual nucleases.

We thank Sidney Kushner and Kaj Frank Jensen for providing plasmids.

This work was supported by grant GM16317 from the National Institutes of Health.

REFERENCES

1. Bryant, R. E., and P. S. Sypherd. 1974. Genetic analysis of cold-sensitive ribosome maturation mutants of *Escherichia coli*. J. Bacteriol. **117**:1082–1092.
2. Clarke, D. J., and B. C. A. Dowds. 1994. The gene coding for polynucleotide phosphorylase in *Photobacterium* sp. strain K122 is induced at low temperatures. J. Bacteriol. **176**:3775–3784.
3. Craven, M. G., D. J. Henner, D. Alessi, A. T. Schauer, K. A. Ost, M. P. Deutscher, and D. I. Friedman. 1992. Identification of the *rph* (RNase PH) gene of *Bacillus subtilis*: evidence for suppression of cold-sensitive mutations in *Escherichia coli*. J. Bacteriol. **174**:4727–4735.
4. Dammel, C. S., and H. F. Noller. 1993. A cold-sensitive mutation in 16S rRNA provides evidence for helical switching in ribosome assembly. Genes Dev. **7**:660–670.
5. Deutscher, M. P., G. T. Marshall, and H. Cudny. 1988. RNase PH: an *Escherichia coli* phosphate-dependent nuclease distinct from polynucleotide phosphorylase. Proc. Natl. Acad. Sci. USA **85**:4710–4714.
6. Deutscher, M. P. 1993. Promiscuous exoribonucleases of *Escherichia coli*. J. Bacteriol. **175**:4577–4583.
7. Deutscher, M. P. 1993. RNA maturation nucleases, p. 377–406. In S. M. Linn, S. R. Lloyd, and R. J. Roberts (ed.), Nucleases, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. Donovan, W. P., and S. R. Kushner. 1986. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **83**:120–124.
9. Feunteun, J., R. Monier, C. Vola, and R. Rosset. 1974. Ribosomal assembly defective mutants of *Escherichia coli*. Nucleic Acids Res. **1**:149–169.
10. Geyl, D., A. Bock, and H. G. Wittmann. 1977. Cold-sensitive growth of a mutant of *Escherichia coli* with an altered ribosomal protein S8: analysis of revertants. Mol. Gen. Genet. **152**:331–336.
11. Green, R., and H. F. Noller. 1996. In vitro complementation analysis localizes 23S rRNA posttranscriptional modifications that are required for *Escherichia coli* 50S ribosomal subunit assembly and function. RNA **2**:1011–1021.
12. Guthrie, C., H. Nashimoto, and M. Nomura. 1969. Studies on the assembly of ribosomes *in vivo*. Cold Spring Harbor Symp. Quant. Biol. **34**:69–75.
13. Jones, P. G., R. A. VanBogelen, and F. C. Neidhardt. 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol. **169**:2092–2095.
14. Kelly, K. O., and M. P. Deutscher. 1992. Characterization of *Escherichia coli* RNase PH. J. Biol. Chem. **267**:17153–17158.
15. Kelly, K. O., N. B. Reuven, Z. Li, and M. P. Deutscher. 1992. RNase PH is essential for tRNA processing and viability in RNase-deficient *Escherichia coli* cells. J. Biol. Chem. **267**:16015–16018.
16. Larsen, J. E. L., K. Gerde, J. Light, and S. Molin. 1984. Low-copy-number plasmid-cloning vectors amplifiable by derepression of an inserted foreign promoter. Gene **28**:45–54.
17. Li, Z., and M. P. Deutscher. 1994. The role of individual exoribonucleases in processing at the 3' end of *Escherichia coli* tRNA precursors. J. Biol. Chem. **269**:6064–6071.
18. Li, Z., and M. P. Deutscher. 1995. The tRNA processing enzyme RNase T is essential for maturation of 5S RNA. Proc. Natl. Acad. Sci. USA **92**:6883–6886.
19. Li, Z., and M. P. Deutscher. 1996. Maturation pathways for *E. coli* tRNA precursors: a random multienzyme process *in vivo*. Cell **86**:503–512.
20. Littauer, U. Z., and H. Soreq. 1982. Polynucleotide phosphorylase, p. 518–553. In P. D. Boyer, (ed.), the enzymes, vol. XV, part B. Academic Press, New York, N.Y.
21. Luttinger, A., J. Hahn, and D. Dubnau. 1996. Polynucleotide phosphorylase is necessary for competence development in *Bacillus subtilis*. Mol. Microbiol. **19**:343–356.
22. McMurtry, L. M., and S. B. Levy. 1987. Tn5 insertion in the polynucleotide phosphorylase (*pnp*) gene in *Escherichia coli* increases susceptibility to antibiotics. J. Bacteriol. **169**:1321–1324.
23. Nashimoto, H., W. Held, E. Kaltschmidt, and M. Nomura. 1971. Structure and function of bacterial ribosomes. XII. Accumulation of 21S particles by some cold-sensitive mutants of *Escherichia coli*. J. Mol. Biol. **62**:121–138.
24. Reuven, N. B., and M. P. Deutscher. 1993. Multiple exoribonucleases are required for the 3' processing of *Escherichia coli* tRNA precursors *in vivo*. FASEB J. **7**:143–148.
25. Tai, P.-C., D. P. Kessler, and J. Ingraham. 1969. Cold-sensitive mutations in *Salmonella typhimurium* which affect ribosome synthesis. J. Bacteriol. **97**:1298–1304.
26. Zhou, Z., and M. P. Deutscher. Unpublished data.