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Analysis of mRNA Decay and rRNA Processing in *Escherichia coli* Multiple Mutants Carrying a Deletion in RNase III

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RNase III is an endonuclease involved in processing both rRNA and certain mRNAs. To help determine whether RNase III (*rnc*) is required for general mRNA turnover in *Escherichia coli*, we have created a deletion-insertion mutation (*Δrnc-38*) in the structural gene. In addition, a series of multiple mutant strains containing deficiencies in RNase II (*rnb-500*), polynucleotide phosphorylase (*pnp-7* or *pnp-200*), RNase E (*rne-1* or *rne-3071*), and RNase III (*Δrnc-38*) were constructed. The *Δrnc-38* single mutant was viable and led to the accumulation of 30S rRNA precursors, as has been previously observed with the *rnc-105* allele (P. Gegenheimer, N. Watson, and D. Apirion, J. Biol. Chem. 252:3064–3073, 1977). In the multiple mutant strains, the presence of the *Δrnc-38* allele resulted in the more rapid decay of pulse-labeled RNA but did not suppress conditional lethality, suggesting that the lethality associated with altered mRNA turnover may be due to the stabilization of specific mRNAs. In addition, these results indicate that RNase III is probably not required for general mRNA decay. Of particular interest was the observation that the *Δrnc-38 rne-1* double mutant did not accumulate 30S rRNA precursors at 30°C, while the *Δrnc-38 rne-3071* double mutant did. Possible explanations of these results are discussed.

mRNA decay in *Escherichia coli* is hypothesized to proceed through the action of both exonucleases and endonucleases (8). The involvement of two exonucleases, polynucleotide phosphorylase (PNPase) (38) and RNase II (42), in mRNA turnover has been well documented (4, 12). These enzymes degrade single-stranded RNA processively in the 3′→5′ direction (23, 42) and have been implicated in both the initial (18) and the terminal (12) steps of mRNA decay. Strains deficient in both of these exonucleases are nonviable and accumulate mRNA fragments 100 to 1,500 nucleotides in length after a shift to the nonpermissive temperature (12). Stem-loop structures such as rho-independent terminators (26) and the so-called REP sequences (32) at the 3′ termini of mRNAs can act as barriers to exonucleolytic decay by these enzymes (27). In contrast, the existence of endonucleolytic activities that degrade mRNAs has only recently been demonstrated. In particular, two enzymes previously identified for their roles in rRNA processing, RNase III and RNase E, appear to be involved in mRNA decay.

RNase E was first characterized as the enzyme responsible for the initial processing of the 5S rRNA from the primary 30S transcript (1). Subsequently, it was shown that RNase E is involved in the specific cleavage of several T4 transcripts, which results in the stabilization or destabilization of adjacent sequences (29). RNase E is also involved in the specific cleavage of *E. coli* messages, which leads to the rapid decay of the *rpsO* transcript (37) and the stabilization of DicF RNA, an inhibitor of cell division (13). One other known substrate for RNase E is RNA I of ColE1 (50). The link between RNase E and mRNA turnover has been established by the analysis of *ams* mutations. Specifically, Arraiano et al. (4) showed that in a mutant strain deficient in the Ams protein, PNPase, and RNase II, the chemical half-life of bulk mRNA increased threefold and was coupled with a dramatic stabilization of discrete mRNA decay intermediates. Recently, Babitzke and Kushner (5) and others (28, 30, 48) provided conclusive evidence that the Ams protein is encoded by the same structural gene as RNase E.

RNase III appears to be specific for double-stranded RNA (39). Its role in the processing of the 16S and 23S rRNAs from the primary 30S transcript has been well documented (15). In RNase III-deficient strains (*rnc-105*) (22), unprocessed 30S precursors accumulate in the cell (16). RNase III is also involved in the processing and/or decay of several bacterial and phage messages. The RNase III cleavage sites in bacteriophages T7, T3, and lambda mRNAs have been summarized (11) and consist of a stem-loop structure with limited sequence homology. It has also been demonstrated that there are RNase III cleavage sites in the *rplL-rpoB* (7), *rpsO-pnp* (34, 45), *dicA-dicF-dicB* (13), and *metY-nusA* (36) intercistronic regions. Cleavage at these sites leads to either the stabilization or the destabilization of the upstream or downstream sequences. RNase III also cleaves its own message, a process that results in the regulation of its own expression (6).

No change in the chemical or functional stability of mRNA was observed in a strain deficient in RNase III (2, 3). In contrast, Talkad et al. (47) observed that in an RNase III-deficient strain, the expression of the *lac*, *gal*, and *trp* operons was altered, and suggested that the expression of many operons may be affected by RNase III activity. In addition, evidence of altered levels of several proteins in a strain deficient in both RNase III and RNase E has been presented (17). Furthermore, Studier showed that *rnc-105* mutants grew more slowly than wild-type controls (44), suggesting that RNase III may be essential for cell viability.

To help determine whether RNase III is essential for cell viability and to determine whether it plays a role in general mRNA turnover, we constructed a deletion in the structural gene. Using this deletion, we characterized a series of

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TABLE 1. *E. coli* strains used

Strain	Genotype	Source or reference
AB1325	*proA2 purB15 his-4 thi-1 lacY1 galK2 xyl-5 mtl-1 rpsL35*	B. Bachmann
DC556	W3110 Tn*10* (Tc^r) near *rnc*	D. Court
MC1061	*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK rpsL*	R. L. Gourse
MG1693	*thyA715*	4
N3431	*rne-3071*	B. Bachmann
N6496	*glyA rnc-105* Km^r	D. Court
SK5665	*thyA715 rne-1*[a]	4
SK6294	*glyA rnc-105* Km^r Tc^r	This study
SK6501	*araD139* Δ*lacU169 rpsL recA56*	F. Moreno
SK6632	*thyA715 pnp-200 rnb-500* Cm^r	57
SK6640	*thyA715 pnp-200 rnb-500 rne-1* Cm^r	57
SK6867	MC1061 *rnc-105* Tc^r	This study
SK7611	MC1061 Δ*rnc-38* Km^r/pMAK705 (*rnc*$^+$ Cm^r)	This study
SK7615	MC1061 Δ*rnc-38* Km^r/pLG339 (Tc^r Km^r)	This study
SK7616	MC1061 Δ*rnc-38* Km^r/pBK2 (*rnc*$^+$ Tc^r Km^r)	This study
SK7621	MC1061 Δ*rnc-38* Km^r	This study
SK7622	*thyA715* Δ*rnc-38* Km^r	This study
SK7623	*thyA715 rnb-500* Δ*rnc-38* Km^r	This study
SK7624	*thyA715 pnp-200 rnb-500* Δ*rnc-38* Cm^r Km^r	This study
SK7625	*thyA715 pnp-200* Δ*rnc-38* Cm^r Km^r	This study
SK7630	*thyA715 pnp-200 rnb-500* Δ*rnc-38 rne-1* Cm^r Km^r	This study
SK7631	*thyA715* Δ*rnc-38 rne-1* Km^r	This study
SK7632	*thyA715 pnp-7* Δ*rnc-38* Km^r	This study
SK7633	*thyA715 pnp-7 rnb-500* Δ*rnc-38* Km^r	This study
SK7634	*thyA715 rnb-500* Δ*rnc-38 rne-1* Km^r	This study
SK7635	*thyA715 pnp-7* Δ*rnc-38 rne-1* Km^r	This study
SK7636	*thyA715 pnp-7 rnb-500* Δ*rnc-38 rne-1* Km^r	This study
SK7639	AB1325 *rne-3071*	This study
SK7687	*thyA715* Δ*rnc-38 rne-3071* Km^r	This study

[a] Formerly *ams-1* (5).

TABLE 2. Plasmid constructions

Plasmid	Description
pLG339	Low-copy-number vector (Km^r Tc^r) (40)
pLC7-47	14.3-kbp *Eco*RI *rnc*$^+$ chromosomal fragment in *Eco*RI site of ColE1 (48)
pBK2	4.3-kbp *Eco*RI *rnc*$^+$ fragment from pLC7-47 in *Eco*RI site of pLG339 (Km^r Tc^r)
pBK12	4.3-kbp *Eco*RI *rnc*$^+$ fragment from pLC7-47 in *Eco*RI site of pBR325 (Ap^r Tc^r)
pBK13	5.8-kbp *Bam*HI *rnc*$^+$ fragment derived from *Bam*HI digestion of pBK12 (Ap^r)
pUC4K	Km^r plasmid (Pharmacia LKB Biotechnology)
pBK32	1.2-kbp *Hin*cII Km^r fragment from pUC4K in *Eco*RI site of pBR322 made blunt ended with the Klenow fragment of DNA polymerase I (Ap^r Tc^r Km^r)
pBK33	pBK13 with a deletion of the 40-bp *Bss*HII fragment; *Bss*HII ends were made blunt with S1 nuclease and the Klenow fragment of DNA polymerase I (Δ*rnc-33*) (Ap^r) prior to religation
pBK35	1.3-kbp *Eco*RV-*Sca*I fragment from pBK32 inserted into pBK13 with a deletion of the 40-bp *Bss*HII fragment in *rnc*; *Bss*HII ends were made blunt with S1 nuclease and the Klenow fragment of DNA polymerase I (Δ*rnc-38*) (Ap^r Km^r) prior to ligation
pMAK705	Low-copy-number vector with a temperature-sensitive origin of replication (Cm^r) (18)
pBK38	4.6-kbp *Bam*HI-*Eco*RI Δ*rnc-38* Km^r fragment from pBK35 in pMAK705 *Bam*HI-*Eco*RI polylinker sites

isogenic strains deficient in various combinations of RNase III, RNase E, PNPase, and RNase II. The results demonstrate that RNase III is not absolutely required for cell viability, general mRNA degradation, or rRNA processing. In fact, RNase III-deficient strains in general had shorter bulk mRNA half-lives than isogenic RNase III$^+$ controls. In addition, the accumulation of 30S rRNA precursors associated with the RNase III deficiency was suppressed by a mutation in RNase E.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used are described in Table 1. *E. coli* strains deficient in PNPase (*pnp-7* or *pnp-200*) (38, 57), RNase II (*rnb-500*) (12), RNase E (*rne-1* or *rne-3071*) (1, 5, 33), or RNase III (*rnc-105*) (22) have been described. The *pnp-7* allele is a null mutation (21), whereas the *pnp-200*, *rnb-500*, *rne-1*, and *rne-3071* alleles encode thermolabile enzymes. Strains deficient in RNase E (1, 5, 33) or both PNPase and RNase II (12, 57) are nonviable at 44°C. Bacterial strains SK6294, SK6807, and SK7639 were constructed by P1 transductions. Strain SK6294 (*rnc-105* Tc^r) was generated by using DC556 (Tn*10* Tc^r) as the donor strain and N6496 (*rnc-105*) as the recipient strain. Transductants were selected on L agar plates (4) containing tetracycline and subsequently screened for *rnc-105* by visualization of 30S rRNA precursors on an ethidium bromide-stained agarose gel. Strain SK6867 (*rnc-105* Tc^r) was constructed by using SK6294 (*rnc-105* Tc^r) as the donor strain and MC1061 as the recipient strain. Transductants were selected and screened as described above. The construction of strain SK7639 (*rne-3071*) was accomplished by using N3431 (*rne-3071*) as the donor strain and AB1325 (*purB15*) as the recipient strain. Purine prototrophs were screened for the temperature-sensitive growth phenotype associated with *rne-3071*. The construction of strain SK5665 (*rne-1*) (4) as well as of strains SK6632 (*pnp-200 rnb-500*) and SK6640 (*pnp-200 rnb-500 rne-1*) has been documented (57).

The plasmids used in this work are described in Table 2. Plasmid pBK2 was constructed by subcloning the 4.3-kbp *Eco*RI fragment containing *rnc* from pLC7-47 (54) into the *Eco*RI site of pLG339 (43). Plasmid pBK12 was made by subcloning the 4.3-kbp *Eco*RI fragment containing *rnc* from pLC7-47 into the *Eco*RI site of pBR325 (35). Plasmid pBK13 was constructed by digestion of pBK12 with *Bam*HI and religation, which facilitated the removal of all *Bam*HI fragments except for the 5.8-kbp fragment containing *rnc* and vector sequences. Plasmid pBK32 was constructed by subcloning the 1.2-kbp *Hin*cII fragment that contained the aminoglycoside 3′-phosphotransferase (Km^r) gene from pUC4K (51) into pBR322 (10) had been digested with *Eco*RI and made blunt ended with the Klenow fragment of DNA polymerase I. Plasmid pBK35 was constructed by subcloning the 1.3-kbp *Eco*RV-*Sca*I fragment from pBK32 into pBK13 that had been digested with *Bss*HII to delete a 40-bp fragment internal to *rnc*. The large, 5.8-kbp *Bss*HII fragment from pBK13 was gel purified and treated with S1 nuclease and the Klenow fragment of DNA polymerase I to make the fragment blunt ended prior to ligation. Plasmid pBK33 was

constructed by self-ligation of the 5.8-kbp *Bss*HII blunt-ended fragment from pBK13. Plasmid pBK38 was constructed by subcloning the *rnc* deletion-insertion (Δ*rnc-38*) *Bam*HI-*Eco*RI fragment from pBK35 into the *Bam*HI-*Eco*RI sites in the pMAK705 polylinker (19). Plasmids pBHK10 carrying the *trxA* gene (52) and pBK61 carrying the 5S rRNA gene (5) were as previously published.

Genetic procedures, growth curves, and cell viabilities. *E. coli* cells were transformed by the method of Kushner (24). P1 transductions were performed by the technique of Willetts and Mount (55). L broth, K medium, and M56/2 buffer have been described (4). The growth medium for strains containing the *thyA* allele was supplemented with thymine (50 μg/ml). Appropriate antibiotics were added as needed to the following final concentrations: kanamycin (50 μg/ml), ampicillin (200 μg/ml), chloramphenicol (20 μg/ml), and tetracycline (20 μg/ml).

For growth curves, cells were cultured in L broth at 30°C to 40 Klett units (green filter no. 54), at which time they were shifted to 44°C. Klett readings were taken every 30 min. When cultures reached a Klett value of between 70 and 100, they were diluted two- to fivefold with fresh prewarmed medium. Generation times were determined at 30°C for all strains and at 44°C for strains that were not temperature sensitive. For determination of the viability of the strains, aliquots were removed every 30 min during the growth experiments, diluted in M56/2 buffer, plated on L agar plates, and incubated at 30°C for 24 to 48 h.

Chemicals and enzymes. Restriction endonucleases, calf intestinal alkaline phosphatase, S1 nuclease, the Klenow fragment of DNA polymerase I, T4 DNA ligase, and RNase-free DNase I were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and were used as specified by the manufacturer. T7 RNA polymerase was purchased from United States Biochemical Corp. (Cleveland, Ohio) and was used in accordance with the manufacturer's specifications. Amberlite MB-1 was purchased from Sigma Chemical Co. (St. Louis, Mo.). $^{32}P_i$ [α-^{32}P]dATP, [γ-^{32}P]ATP, [^{3}H]uridine, and [^{35}S]methionine were purchased from Dupont, NEN Research Products (Boston, Mass.). [^{3}H]poly(A) was purchased from Amersham Corp. (Arlington Heights, Ill.).

DNA isolation. Plasmid DNA was isolated by the alkaline lysis procedure of Birnboim and Doly (9) for CsCl-purified preparations and by the modified Ish-Horowicz–Burke (20) method for miniscreens. Chromosomal DNA was prepared by a modification of the Sarkosyl technique described by Washburn and Kushner (53).

Enzyme assays. PNPase was assayed in cell lysates by the reversible exchange reaction of $^{32}P_i$ into ADP that was previously described by Reiner (38), except that an incubation temperature of 47°C was used instead of 43°C to allow inactivation of the thermolabile enzyme encoded by *pnp-200*. RNase II activity was assayed as described by Donovan and Kushner (12). Lysates were preincubated for 10 min at 44°C to inactivate thermolabile RNase II and then assayed at 30°C. Enzyme activity was monitored as the release of radioactivity from [^{3}H]poly(A). RNase E activity was determined as described by Babitzke and Kushner (5). The substrate for the RNase E assay was generated as a runoff transcript with T7 RNA polymerase in the presence of [α-^{32}P]CTP (2 Ci/mmol). Plasmid pBK61 digested with *Cla*I was used as the template in the reaction.

The presence of the Δ*rnc-38* allele was confirmed by replica plating colonies to agar plates containing kanamycin. RNase III activity was determined qualitatively by growing cell cultures at 30°C in L broth to 60 Klett units (green filter no. 54), at which time 7-ml aliquots were removed and total cellular RNA was extracted as described by Williams and Rogers (56) and subsequently treated with RNase-free DNase I. RNA samples were fractionated in a 1.5% agarose gel, and the presence (*rnc*) or the absence (*rnc*$^+$) of the 30S rRNA precursor (16) was determined by visual inspection of ethidium bromide-stained gels.

Assay for mRNA degradation. The chemical decay of pulse-labeled RNA was assayed as described previously (5, 12). Cells grown to 40 Klett units (green filter no. 54) at 30°C in either L broth or K medium were pulse labeled for 1 min with 3 μCi (90 nmol) of [5,6-^{3}H]uridine per ml. The labeling was terminated by the addition of 500 μg of rifampin, 20 μg of nalidixic acid, and 200 μg of uridine per ml. Cells were shifted to 44°C, and duplicate 0.5-ml aliquots were removed at 1- or 2-min intervals and added to 3 ml of 20% ice-cold trichloroacetic acid. The precipitates were collected on Whatman GF/C filters and washed three times with ice-cold 10% trichloroacetic acid and three times with ice-cold 95% ethanol–0.1 M HCl. The percentage of [^{3}H]RNA remaining was calculated from the radioactivity at a given time minus that in the stable RNA fraction present 60 min after rifampin addition, divided by the maximum radioactivity in the unstable fraction. The half-lives were determined by linear regression analysis. Only curves having a least-squares fit of ≥0.95 were used.

Analysis of plasmid-encoded proteins. Plasmid-encoded proteins were identified in the maxicell strain SK6501 (*recA56*) by the method of Sancar et al. (41). Proteins were labeled with [^{35}S]methionine and analyzed on a 12.5% polyacrylamide-sodium dodecyl sulfate gel as described by Laemmli (25). The gel was dried, and autoradiography was performed at room temperature.

Southern blot analysis. Chromosomal DNA from MC1061 (*rnc*$^+$) and SK7621 (Δ*rnc-38*) was purified, digested with *Eco*RI, fractionated on an 0.8% agarose gel, and transferred to a Biotrans nylon membrane (ICN Biomedicals Inc., Irvine, Calif.) as previously described (40). The DNA was fixed to the membrane by baking at 80°C for 1 h, and hybridizations were carried out at 42°C with 50% formamide as described by the manufacturer of the membrane. Autoradiography was performed at −70°C. The DNA probe for *rnc* was prepared by digesting pBK13 with *Bam*HI and *Eco*RI. The 1.3-kbp fragment containing the *rnc* gene was purified on a low-melting-point agarose gel and radiolabeled by the random primer method with [α-^{32}P]dATP (3,000 Ci/mmol) and the Klenow fragment of DNA polymerase I (14). The DNA probe for the aminoglycoside 3′-phosphotransferase (Km^r) gene was prepared by digesting pBK32 with *Pst*I. The 1.2-kbp Km^r fragment was purified on a low-melting-point agarose gel and radiolabeled as described above. The DNA fragment used to probe for the deleted region of Δ*rnc-38* was prepared by digesting pBK13 with *Bss*HII. The 40-bp fragment was gel purified on a 12% polyacrylamide gel containing 10% glycerol. Oyster glycogen (20 μg/ml) was used to improve the recovery during ethanol precipitation. The purified DNA fragment was dephosphorylated by treatment with calf intestinal alkaline phosphatase, purified as described above, and subsequently 5′ end labeled with [γ-^{32}P]ATP (3,000 Ci/mmol) and T4 polynucleotide kinase (40).

Northern (RNA) blot analysis. RNA used in the Northern blot analysis was isolated by harvesting cells as described by Arraiano et al. (4), and total cellular RNA was extracted as described above. Seven-microgram RNA samples were frac-

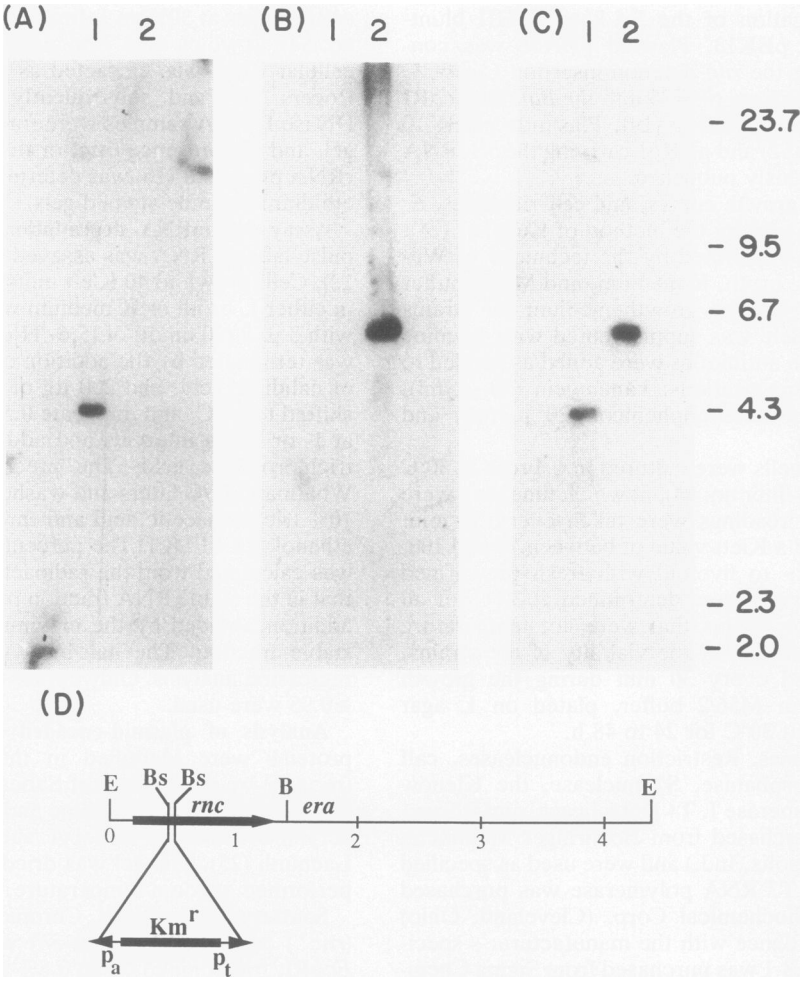

FIG. 1. Southern blot of a Δ*rnc-38* strain. (A) 40-bp probe homologous to the region deleted from Δ*rnc-38*. Lanes: 1, MC1061 (wild type); 2, SK7621 (Δ*rnc-38*). (B) Km^r probe. Lanes: 1, MC1061; 2, SK7621. (C) *rnc* probe. Lanes: 1, MC1061; 2, SK7621. Numbers on the right mark the positions of *Hin*dIII-digested λ molecular size standards (in kilobase pairs). (D) Restriction map (in kilobase pairs) of the chromosomal location of Δ*rnc-38*. Restriction enzymes are *Eco*RI (E), *Bam*HI (B), and *Bss*HII (Bs). p_a and p_t denote Ap^r and Tc^r promoters derived from pBK38, respectively. The large arrow denotes the direction of *rnc* transcription. Small arrows denote the directions of Ap^r and Tc^r transcription. The Tc^r promoter is necessary for expression of the essential *era* gene located immediately downstream of *rnc*.

tionated in 6% polyacrylamide gels containing 7 M urea (5) and electroblotted to Biotrans nylon membranes as described previously (4). The RNA was fixed to the membranes by irradiation with UV light for 90 s at 1 J/m^2/s. Hybridizations were performed at 42°C with 50% formamide as outlined by Thomas (49), except that 0.5% casein replaced bovine serum albumin, tRNA (50 μg/ml) replaced calf thymus DNA, and the sodium dodecyl sulfate concentration was increased to 0.75%. Autoradiography was carried out at −70°C. The DNA probe for the *trxA* (thioredoxin) message was prepared by digesting pBHK10 with *Hin*cII, and the 472-bp fragment containing *trxA* was gel purified on NA45 paper (Schleicher & Schuell, Keene, N.H.) and radiolabeled by the random primer method as described above.

RESULTS

Isolation of the *rnc* deletion strain. To construct a deletion mutation in *rnc*, we removed a 40-bp *Bss*HII restriction fragment from the structural gene in vitro and replaced it with a 1.3-kbp DNA fragment encoding the structural gene for aminoglycoside 3′-phosphotransferase (Km^r); this mutation was designated Δ*rnc-38*. The mutant retained the coding capacity for the first 71 of the 227 amino acid residues found in RNase III (31). The construct was made in such a way that the promoter for the tetracycline resistance gene, derived from pBR322, would allow transcription to proceed downstream into the essential *era* gene (46) once the construct had been used to replace the wild-type copy of *rnc* in the chromosome (Fig. 1D).

To demonstrate that RNase III was not produced by the plasmid-encoded Δ*rnc-38* allele, we transformed maxicell strain SK6501 (*recA56*) with plasmids pBK32, pBK13 (*rnc*$^+$), pBK33 (Δ*rnc-33*), and pBK35 (Δ*rnc-38*) (Table 2). The [^{35}S]methionine-labeled proteins encoded by the plasmids were separated as described in Materials and Methods and are shown in Fig. 2. One distinct polypeptide band whose synthesis was directed by the *rnc*$^+$ insert of pBK13 was identified (lane 2). The apparent molecular mass of this polypeptide is 27 kDa, in close agreement with the reported 26-kDa molecular mass that was observed for RNase III (54). The predicted molecular mass of this enzyme is 25 kDa

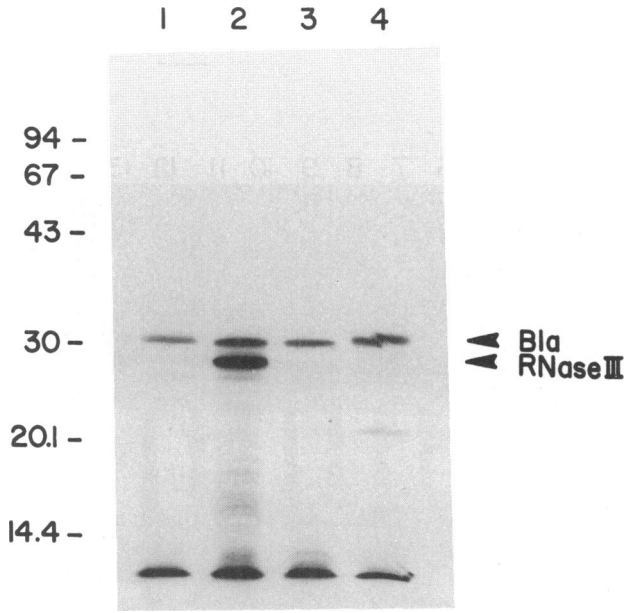

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel of ^{35}S-labeled proteins expressed in maxicells. Lanes: 1, pBK32 (vector); 2, pBK13 (*rnc*$^+$); 3, pBK33 (Δ*rnc-33*); 4, pBK35 (Δ*rnc-38*). Arrowheads mark the positions of β-lactamase (Bla) and RNase III, and numbers mark the positions of protein molecular size standards (in kilodaltons) (Pharmacia Inc., Piscataway, N.J.).

(31). This polypeptide was not produced by vector pBK32 (lane 1), by pBK33, in which *rnc* was disrupted by a 40-bp deletion (lane 3), or by pBK35, in which *rnc* was disrupted by a 40-bp deletion and a 1.3-kbp Km^r insertion (lane 4). In lane 4, a faint band with an apparent molecular mass of approximately 20 kDa was present. The identity of this polypeptide is unknown. It is unlikely that it is a truncated form of RNase III, since it was not present in the lane corresponding to pBK33 (lane 3), in which *rnc* was disrupted at the same position as in pBK35.

The construct containing the Δ*rnc-38* allele was subsequently moved into pMAK705, a vector that is temperature sensitive for DNA replication and that can be used to generate gene replacements in *E. coli* (19). The resulting plasmid, pBK38, was used to transform strain MC1061 (*rnc*$^+$). Cointegrates were selected by plating at the nonpermissive temperature for plasmid replication (44°C) in the presence of chloramphenicol. Three of the cointegrate colonies were pooled together and grown for approximately 20 generations at 30°C (the permissive temperature) in L broth containing chloramphenicol to allow resolution of the cointegrates. The culture was subsequently plated at 30°C. Plasmid miniscreens indicated that 9 of 10 resolved cointegrates contained *rnc*$^+$ on the plasmid, suggesting that Δ*rnc-38* remained in the chromosome. Subsequently, the *rnc*$^+$ plasmid from one of the isolates (SK7611) was displaced by pLG339. P1 grown on the resulting strain (SK7615) was used to generate Km^r Δ*rnc-38* transductants of MC1061. One such isolate was designated SK7621.

To confirm that Δ*rnc-38* was correctly inserted into the chromosome, we digested chromosomal DNA from either the deletion-insertion mutant strain or the parental wild-type strain with *Eco*RI, electrophoresed it through an 0.8% agarose gel, and subsequently transferred it to a nylon membrane. Initially, the 40-bp *Bss*HII DNA fragment that had been deleted from Δ*rnc-38* was used as a probe. One band of approximately 4.3 kbp was detected in the lane corresponding to the wild-type strain (MC1061) (Fig. 1A, lane 1) but not in the lane corresponding to the mutant strain (SK7621) (Fig. 1A, lane 2). After the probe was stripped, the membrane was rehybridized with a restriction fragment that contained the Km^r gene. One band of approximately 5.6 kbp was detected in the lane corresponding to the deletion-insertion strain (Fig. 1B, lane 2) but not in the lane corresponding to the wild-type strain (Fig. 1B, lane 1). Again, the probe was stripped and the membrane was probed with a DNA fragment that contained *rnc*. In the lane corresponding to the wild-type strain, a 4.3-kbp band was detected (Fig. 1C, lane 1), whereas a 5.6-kbp band was detected in the lane corresponding to the mutant strain (Fig. 1C, lane 2). The hybridization patterns matched those expected if Δ*rnc-38* had replaced the wild-type allele in the chromosome (Fig. 1D). These results confirm the report of Takiff et al. (46) that RNase III is not essential for cell viability.

Total cellular RNA was extracted from strains MC1061 (*rnc*$^+$), SK6867 (*rnc-105*), SK7615 (Δ*rnc-38*), SK7616 (Δ*rnc-38*) containing pBK2 (*rnc*$^+$), and SK7621 (Δ*rnc-38*). Equivalent amounts of RNA were fractionated in a 1.5% agarose gel. RNA extracted from RNase III-deficient strains (*rnc-105* or Δ*rnc-38*) contained 30S rRNA precursors (Fig. 3A, lanes 2, 3, and 5). In contrast, no 30S species were seen in the wild-type strain (lane 1) or when Δ*rnc-38* was complemented by pBK2 (*rnc*$^+$) (lane 4).

Construction of multiple mutant strains. A set of isogenic strains were constructed by P1 transduction with SK7621 (Δ*rnc-38* Km^r) as the donor strain and strains that contained various combinations of the *pnp-7*, *pnp-200*, *rnb-500*, *rne-1*, and *rne-3071* alleles as the recipients (4, 5, 57) (Table 1). Transductants were selected on the basis of their ability to grow in the presence of kanamycin and were screened for PNPase, RNase II, RNase III, and RNase E activities as described in Materials and Methods (data not shown).

In vivo accumulation of 30S rRNA precursors. Total cellular RNA was extracted from various strains grown at 30°C and electrophoresed through a 1.5% agarose gel (Fig. 3B). As was seen previously, 30S rRNA precursors accumulated in the Δ*rnc-38* single mutant strain but not in the wild-type strain (lanes 1 and 2). Of particular interest was the fact that at the permissive temperature (30°C), there was a complete absence of 30S rRNA precursors in RNA extracted from strains that contained both the Δ*rnc-38* and the *rne-1* alleles (lanes 8 to 10). This phenomenon was not a function of growth rate, since 30S rRNA precursors were observed when RNA was extracted from SK7622 (Δ*rnc-38*) grown in L broth (generation time, 41 min), K medium (generation time, 86 min), or K medium with glycerol as the sole carbon source (generation time, 116 min) (Table 3 and Fig. 3B, lanes 2 to 4). 30S rRNA precursors were observed, however, in RNA extracted from the Δ*rnc-38 rne-3071*, *rnb-500* Δ*rnc-38*, and *pnp-200* Δ*rnc-38* double mutant strains, as well as the *pnp-200 rnb-500* Δ*rnc-38* triple mutant strain (lanes 5 to 7 and 11), results suggesting that the absence of 30S rRNA precursors was peculiar to strains that contained both Δ*rnc-38* and *rne-1*. No 30S rRNA precursors were observed in either the *rne-1* or the *rne-3071* single mutant strain (lanes 12 and 13).

Growth properties of multiple mutant strains. Growth experiments were carried out in L broth. The quadruple mutant strain SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*) ceased growing within 30 min after the shift to the nonpermissive temperature (Fig. 4F), whereas the triple mutant strain SK7624 (*pnp-200 rnb-500* Δ*rnc-38*) did not stop grow-

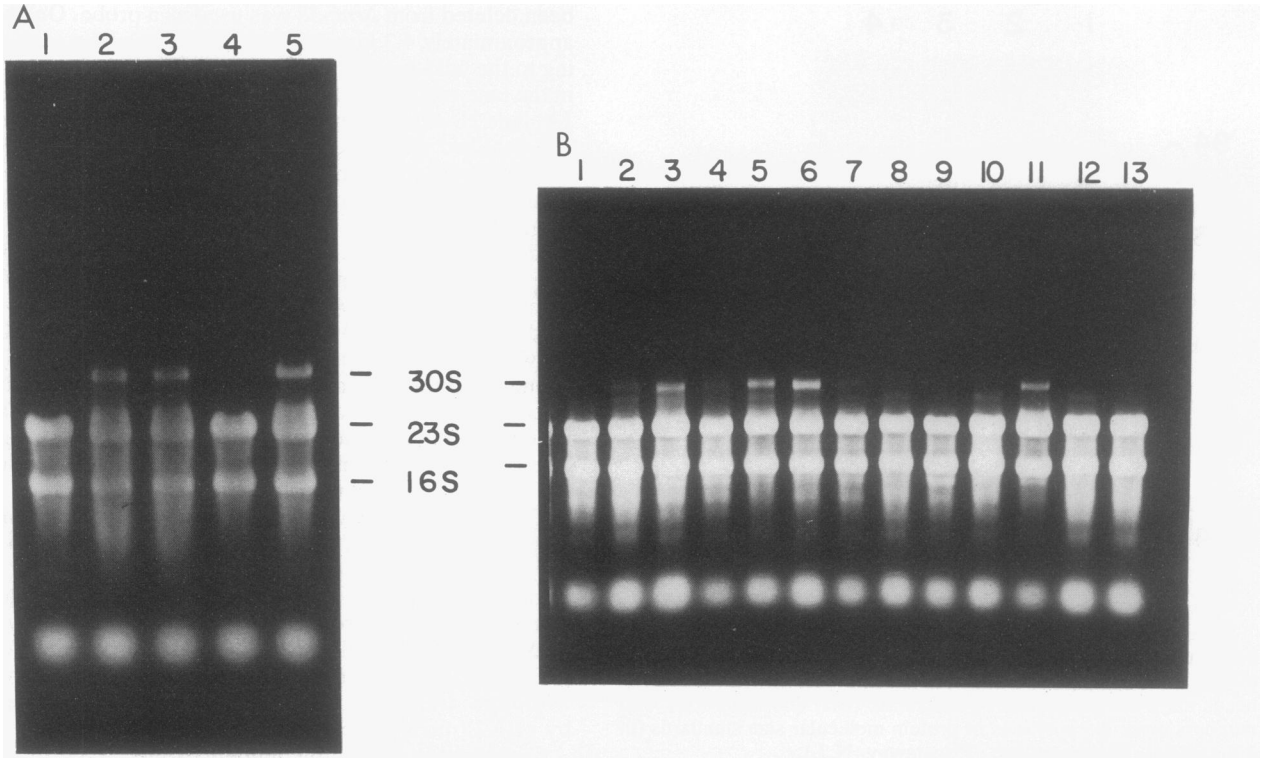

FIG. 3. In vivo accumulation of unprocessed 30S rRNA precursors. Agarose gels (1.5%) were run under nondenaturing conditions. (A) RNA isolated from strains used in the isolation of the *rnc* deletion strain. Cells were grown in L broth at 30°C (see Materials and Methods). Each lane contained 5 μg of RNA. Lanes: 1, MC1061 (wild type); 2, SK6867 (*rnc-105*); 3, SK7615 (Δ*rnc-38*) containing pLG339; 4, SK7616 (Δ*rnc-38*) containing pBK2 (*rnc*$^+$); 5, SK7621 (Δ*rnc-38*). (B) RNA isolated from multiple mutant strains grown at 30°C. Cells were grown in L broth unless otherwise indicated (see Materials and Methods). Each lane contained 7.5 μg of RNA. Lanes: 1, MG1693 (wild type); 2, SK7622 (Δ*rnc-38*); 3, SK7622 (Δ*rnc-38*) (K medium-glucose); 4, SK7622 (Δ*rnc-38*) (K medium-glycerol); 5, SK7623 (*rnb-500* Δ*rnc-38*); 6, SK7625 (*pnp-200* Δ*rnc-38*); 7, SK7624 (*pnp-200 rnb-500* Δ*rnc-38*); 8, SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*); 9, SK7634 (*rnb-500* Δ*rnc-38 rne-1*); 10, SK7631 (Δ*rnc-38 rne-1*); 11, SK7687 (Δ*rnc-38 rne-3071*); 12, SK5665 (*rne-1*); 13, N3431 (*rne-3071*). The positions of the 30S, 23S, and 16S rRNAs are indicated.

ing until 90 min after the shift to 44°C (Fig. 4C). The double mutant strain SK7631 (Δ*rnc-38 rne-1*) (Fig. 4D) and the triple mutant strain SK7634 (*rnb-500* Δ*rnc-38 rne-1*) (Fig. 4E) continued growing for about 120 min after the same temperature shift. In all of the strains tested, no significant drop in viability, measured as CFU per milliliter, was observed at the nonpermissive temperature (Fig. 4).

Generation times were determined in L broth at 30°C for all of the mutant strains and at 44°C for strains that were viable at this temperature (Table 3). Strains deficient in both PNPase and RNase II or RNase E by itself were nonviable at 44°C. Of particular interest was the increase in the generation times at 30°C of strains that contained the Δ*rnc-38* allele relative to their respective isogenic *rnc*$^+$ strains. This effect was most dramatic when SK6640 (*pnp-200 rnb-500 rne-1*) (38 min) was compared with SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*) (73 min). In addition, the generation time of SK7636 (*pnp-7 rnb-500* Δ*rnc-38 rne-1*) (125 min) was twice that of its isogenic *rnc*$^+$ strain that had been previously observed (4).

Chemical decay of pulse-labeled RNA. The chemical half-life of total pulse-labeled RNA was determined at 44°C in K medium for strains carrying the Δ*rnc-38*, *pnp-7*, *pnp-200*, *rnb-500*, and *rne-1* alleles in various combinations (Table 3). It was apparent from these experiments that the complete absence of RNase III did not significantly increase the half-life of bulk mRNA. In fact, the pulse-labeled RNA half-lives observed in SK5665 (*rne-1*), SK6632 (*pnp-200 rnb-500*), and SK6640 (*pnp-200 rnb-500 rne-1*) actually decreased when the Δ*rnc-38* allele was introduced into these strains (Table 3). Donovan and Kushner (12) previously presented evidence that RNase II produced from the *rnb-500* allele was not as thermolabile when cells were grown in K medium rather than L broth. Accordingly, mRNA half-life experiments were also carried out in L broth for MG1693 (wild type), SK6640 (*pnp-200 rnb-500 rne-1*), and SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*) (Table 3). Although the chemical half-lives observed for strains carrying the *rnb-500* allele were longer when the cells were grown in L broth rather than K medium, a decrease in the half-life was still observed for the quadruple mutant strain relative to the triple mutant strain (Table 3).

Northern blot analysis of *trxA* mRNA. Because the Δ*rnc-38* allele seemed to accelerate the degradation of total pulse-labeled mRNA (Table 3), it was of interest to determine whether this same phenomenon occurred with a specific transcript. The *trxA* (thioredoxin) message was chosen because its decay pattern in a *pnp-7 rnb-500 rne-1* triple mutant strain has been established (4). Total RNA isolated at various times after a shift to the nonpermissive temperature was separated under denaturing conditions on 6% polyacrylamide gels and probed with a DNA fragment specific for *trxA*. As shown in Fig. 5A, the wild-type decay pattern indicated that the full-length transcript was being degraded into discrete smaller fragments. By 16 min after the shift to

TABLE 3. Generation times and mRNA half lives

Strain	Genotype[a]				Generation time (min) in:			Half-life (min)[b] in:	
	pnp	*rnb*	*rnc*	*rne*	L broth		K medium, 30°C	L broth	K medium
					30°C	44°C			
MG1693	+	+	+	+	30	23	77	3.5	3.5
SK5665	+	+	+	*1*	35	Nonviable	ND[c]	ND	7.4
SK6632	*200*	*500*	+	+	37	Nonviable	ND	ND	4.6
SK6640	*200*	*500*	+	*1*	38	Nonviable	137	14.3	7.3
SK7622	+	+	Δ*38*	+	41	29	86	ND	4.1
SK7623	+	*500*	Δ*38*	+	44	33	ND	ND	4.0
SK7624	*200*	*500*	Δ*38*	+	63	Nonviable	ND	ND	3.9
SK7625	*200*	+	Δ*38*	+	44	34	ND	ND	4.0
SK7630	*200*	*500*	Δ*38*	*1*	73	Nonviable	125	9.8	5.6
SK7631	+	+	Δ*38*	*1*	63	Nonviable	ND	ND	6.8
SK7632	*7*	+	Δ*38*	+	57	48	ND	ND	ND
SK7633	*7*	*500*	Δ*38*	+	75	Nonviable	ND	ND	ND
SK7634	+	*500*	Δ*38*	*1*	75	Nonviable	ND	ND	8.0
SK7635	*7*	+	Δ*38*	*1*	104	Nonviable	ND	ND	ND
SK7636	*7*	*500*	Δ*38*	*1*	125	Nonviable	ND	ND	6.9

[a] +, wild type for the specified gene; mutant allele numbers are indicated.
[b] The half-life of total pulse-labeled RNA was determined at 44°C as described in Materials and Methods. Values are averages of two experiments.
[c] ND, not determined.

44°C, nearly all of the message had been degraded (Fig. 5A). In contrast, the *trxA* decay pattern in the Δ*rnc-38* strain was very different (Fig. 5A). The *trxA* transcript decayed more rapidly without the appearance of discrete breakdown products.

The decay pattern observed for SK6640 (*pnp-200 rnb-500 rne-1*) resembled that observed for a previously described (4) *pnp-7 rnb-500 rne-1* strain, with smaller oligonucleotides appearing between 8 and 16 min after the shift to the nonpermissive temperature (Fig. 5B). When the *rnc* deletion was introduced into this strain (SK7630), the decay pattern resembled that of the Δ*rnc-38* single mutant strain, except that the overall degradation was somewhat slower. Densitometric scans of the autoradiograms confirmed these observations. The half-lives of the *trxA* mRNAs were 2.3 min (wild type), 1.6 min (Δ*rnc-38*), 5.0 min (*pnp-200 rnb-500 rne-1*), and 4.2 min (*pnp-200 rnb-500* Δ*rnc-38 rne-1*).

DISCUSSION

The results described above demonstrate that cells completely devoid of RNase III remain viable and contain 30S rRNA precursors but do not show a general stabilization of mRNA species. In fact, when the Δ*rnc-38* allele was introduced into strains that were already deficient in combinations of PNPase, RNase II, and RNase E, the half-life of bulk pulse-labeled RNA actually decreased relative to that in the *rnc*$^+$ parental strains (Table 3). This accelerated decay phenomenon observed in the bulk RNA half-life experiments was also seen when the decay of the *trxA* transcript was examined (Fig. 5). A comparison of the *trxA* decay pattern in the deletion strain (SK7622) with that in the wild-type control strain (MG1693) indicated the rapid loss of the full-length transcript without the production of any detectable decay intermediates. Similar results were also seen when the decay pattern in SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*) was compared with that in SK6640 (*pnp-200 rnb-500 rne-1*) (Fig. 5B). Again, the degradation of the *trxA* transcript was more rapid, and no decay intermediates were detected. The origin and significance of the RNA species of higher molecular weights seen in all of the lanes corresponding to the *rnc* deletion strain (SK7622) are not known at this time.

It is known that RNase III is involved in the rate-limiting step in the degradation of the transcript encoding PNPase. For strains carrying the *rnc-105* allele, it was found that PNPase levels were increased 3- to 10-fold (34, 45). This result could explain why the Δ*rnc-38* single mutant strain resulted in a more rapid decay of the *trxA* message. Since PNPase is a processive 3′→5′ exonuclease, elevated levels of this enzyme would prevent the accumulation of decay intermediates. Recently, Yancey and Kushner (57) showed in vitro that the level of *pnp-200*-encoded PNPase is reduced by 70% within 30 min at 50°C compared with wild-type enzyme levels. The fact that PNPase levels are elevated in strains deficient in RNase III, combined with the observation that PNPase encoded by *pnp-200* is not completely inactivated in vitro at elevated temperatures, could account for why the rate of decay of *trxA* mRNA is increased in the quadruple relative to the triple mutant strain. However, these observations cannot explain why the bulk mRNA half-life for the quadruple mutant strain carrying *pnp-7* (SK7636, 6.9 min) (Table 3) is reduced compared with the half-life seen previously for its *rnc*$^+$ parent strain (8.9 min) (4), since no PNPase is produced in strains that carry the *pnp-7* allele (21).

Additionally, Donovan and Kushner (12) reported that *rnb-500*-encoded RNase II was not as thermolabile when strains carrying this mutation were grown in K medium rather than L broth. Even this fact cannot explain why the increased decay rate was observed, because the cells used in the Northern analysis (Fig. 5) were grown in L broth and the bulk RNA chemical half-life for SK7630 (*pnp-200 rnb-500* Δ*rnc-38 rne-1*) was still shorter than that for its *rnc*$^+$ parental strain (Table 3). Since RNase III controls the decay of its own message (6) as well as that of PNPase (34, 45), it is possible that RNase III cleavage regulates additional enzymes involved in RNA metabolism. One possible candidate would be RNase II.

A surprising result was the complete absence of 30S rRNA precursors at the permissive temperature in multiple mutants

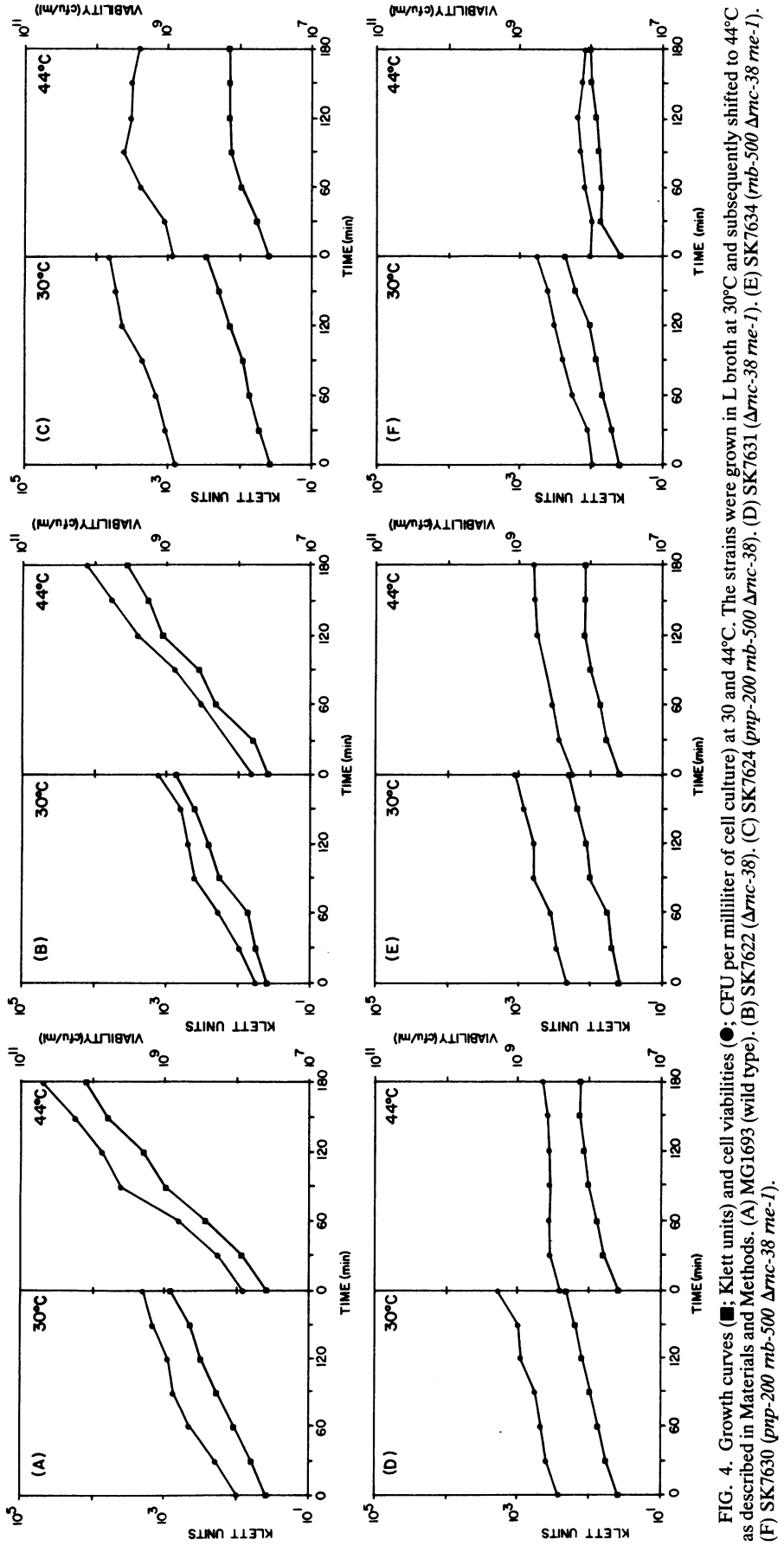

FIG. 4. Growth curves (■; Klett units) and cell viabilities (●; CFU per milliliter of cell culture) at 30 and 44°C. The strains were grown in L broth at 30°C and subsequently shifted to 44°C as described in Materials and Methods. (A) MG1693 (wild type). (B) SK7622 (*Δmc-38*). (C) SK7624 (*pnp-200 rnb-500 Δmc-38*). (D) SK7631 (*Δmc-38 rne-1*). (E) SK7634 (*rnb-500 Δmc-38 rne-1*). (F) SK7630 (*pnp-200 rnb-500 Δmc-38 rne-1*).

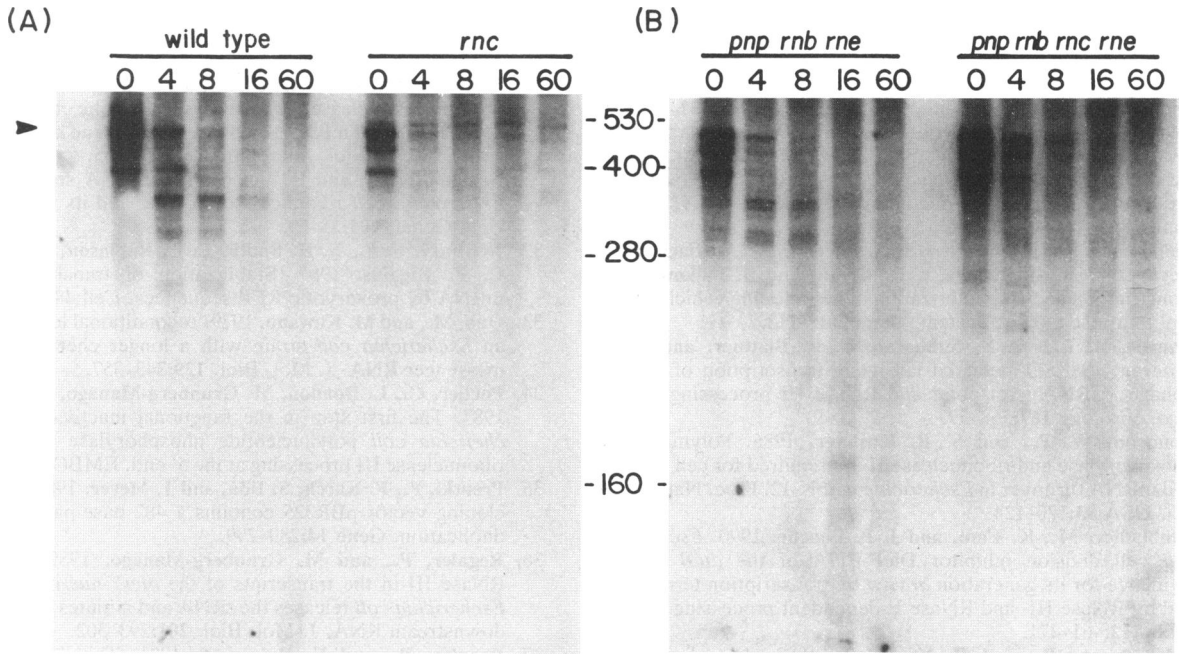

FIG. 5. Northern analysis of *trxA* mRNAs in MG1693 (wild type), SK7622 (*Δrnc-38*), SK6640 (*pnp-200 rnb-500 rne-1*), and SK7630 (*pnp-200 rnb-500 Δrnc-38 rne-1*). Cells were grown in L broth at 30°C and subsequently shifted to 44°C as described in Materials and Methods. (A) MG1693 in first five lanes and SK7622 in last five lanes. (B) SK6640 in first five lanes and SK7630 in last five lanes. Numbers above each lane indicate the time (in minutes) after the temperature shift at which the RNA was extracted. The positions of RNA molecular size standards (in nucleotides) (GIBCO BRL Life Technologies Inc., Gaithersburg, Md.) are indicated. The full-length *trxA* transcript is noted by an arrowhead.

harboring both the *Δrnc-38* and the *rne-1* alleles (Fig. 3B, lanes 8 to 10). Although the generation time of SK7631 (*Δrnc-38 rne-1*) was significantly slower than that of SK7622 (*Δrnc-38*) (63 and 41 min, respectively), 30S rRNA precursors were observed over a wide range of growth rates in SK7622 (41 to 116 min) (Fig. 3B). This result would suggest that the absence of 30S rRNA precursors was not a function of growth rate. Interestingly, 30S rRNA precursors were observed in a *Δrnc-38 rne-3071* double mutant (Fig. 3, lane 11). Even though genetic analysis has placed both the *rne-1* and the *rne-3071* lesions in the amino-terminal region of the RNase E protein (5), this result suggests that the two alleles are significantly different. More important is the fact that a primary defect in rRNA processing can be suppressed by the alteration of a second endonuclease that is also required for rRNA processing. Whether the ability to generate 16S and 23S rRNAs in *Δrnc-38 rne-1* multiple mutants results from a modification of RNase E activity or the intervention of some other RNase will require further analysis of the *rne-1*-encoded RNase E protein.

In conclusion, it is apparent that while rRNA processing can be dramatically affected by the complete absence of RNase III without the loss of cell viability (see above and reference 46), alterations in mRNA turnover (*rne* or *pnp rnb* mutants; 4, 12, 33) have more serious consequences. These observations suggest that rRNA processing can be achieved by a variety of enzymes with compensating activities. In contrast, apparently small perturbations in mRNA turnover (compare the bulk RNA half-lives for MG1693 [wild type] and SK6632 [*pnp-200 rnb-500*]: 3.5 and 4.1 min, respectively) lead to nonviability at 44°C. Furthermore, the more rapid decay of pulse-labeled RNA in strains carrying *Δrnc-38* rather than *rnc*$^+$ does not suppress the nonviability (Table 3). Thus, it would appear that the lethality arising from an alteration in normal mRNA turnover may result not so much from the failure to decay mRNAs rapidly enough but from the consequences of the longer stability of specific mRNAs or their decay products.

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REFERENCES

1. **Apirion, D., and A. B. Lasser.** 1978. A conditional mutant of *Escherichia coli* which affects the processing of ribosomal RNA. J. Biol. Chem. **253:**1738–1742.
2. **Apirion, D., and N. Watson.** 1975. Mapping and characterization of a mutation in *Escherichia coli* that reduces the level of ribonuclease III specific for double-stranded ribonucleic acid. J. Bacteriol. **124:**317–324.
3. **Apirion, D., and N. Watson.** 1975. Unaltered stability of newly synthesized RNA in strains of *Escherichia coli* missing a ribonuclease specific for double-stranded RNA. Mol. Gen. Genet. **136:**317–326.
4. **Arraiano, C. M., S. D. Yancey, and S. R. Kushner.** 1988. Stabilization of discrete mRNA breakdown products in *ams pnp rnb* multiple mutants of *Escherichia coli* K-12. J. Bacteriol. **170:**4625–4633.
5. **Babitzke, P., and S. R. Kushner.** 1991. The Ams (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **88:**1–5.
6. **Bardwell, J. C. A., P. Regnier, S.-M. Chen, Y. Nakamura, M.**

Grunberg-Manago, and D. L. Court. 1989. Autoregulation of RNase III operon by mRNA processing. EMBO J. **8:**3401–3407.
7. **Barry, G., C. Squires, and C. L. Squires.** 1980. Attenuation and processing of RNA from the *rplJL-rpoBC* transcription unit of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **77:**3331–3335.
8. **Belasco, J. G., and C. F. Higgins.** 1988. Mechanisms of mRNA decay in bacteria: a perspective. Gene **72:**15–23.
9. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
10. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.
11. **Daniels, D. L., M. N. Subbarao, F. R. Blattner, and H. A. Lozeron.** 1988. Q-mediated late gene transcription of bacteriophage λ: RNA start point and RNase III processing sites *in vivo*. Virology **167:**568–577.
12. **Donovan, W. P., and S. R. Kushner.** 1986. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **83:**120–124.
13. **Faubladier, M., K. Cam, and J.-P. Bouche.** 1990. *Escherichia coli* cell division inhibitor DicF-RNA of the *dicB* operon. Evidence for its generation *in vivo* by transcription termination and by RNase III- and RNase E-dependent processing. J. Mol. Biol. **212:**461–471.
14. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
15. **Gegenheimer, P., and D. Apirion.** 1981. Processing of prokaryotic ribonucleic acid. Microbiol. Rev. **45:**502–541.
16. **Gegenheimer, P., N. Watson, and D. Apirion.** 1977. Multiple pathways for primary processing of ribosomal RNA in *Escherichia coli*. J. Biol. Chem. **252:**3064–3073.
17. **Gitelman, D. R., and D. Apirion.** 1980. The synthesis of some proteins is affected in RNA processing mutants of *Escherichia coli*. Biochem. Biophys. Res. Commun. **96:**1063–1070.
18. **Guarneros, G., and C. Portier.** 1990. Different specificities of ribonuclease II and polynucleotide phosphorylase in 3′ mRNA decay. Biochimie **72:**771–777.
19. **Hamilton, C. A., M. Aldea, B. K. Washburn, P. Babitzke, and S. R. Kushner.** 1989. New method for generating deletions and gene replacements in *Escherichia coli*. J. Bacteriol. **171:**4617–4622.
20. **Ish-Horowicz, D., and J. F. Burke.** 1981. Rapid and efficient cosmid cloning. Nucleic Acids Res. **9:**2989–2998.
21. **Ivarie, R., and S. R. Kushner.** Unpublished data.
22. **Kindler, P., T. U. Keil, and P. H. Hofscheider.** 1973. Isolation and characterization of a ribonuclease III deficient mutant of *Escherichia coli*. Mol. Gen. Genet. **126:**53–69.
23. **Klee, C. B., and M. F. Singer.** 1968. The processive degradation of individual polyribonucleotide chains. II. *Micrococcus lysodeikticus* polynucleotide phosphorylase. J. Biol. Chem. **243:**923–927.
24. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with ColE1 derived plasmids, p. 17–23. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Biomedical Press, Amsterdam.
25. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
26. **Mackie, G. A.** 1987. Posttranscriptional regulation of ribosomal protein S20 and stability of the S20 mRNA species. J. Bacteriol. **169:**2697–2701.
27. **McLaren, R. S., S. F. Newbury, G. S. C. Dance, H. C. Causton, and C. F. Higgins.** 1991. mRNA degradation by processive 3′-5′ exoribonucleases *in vitro* and the implications for prokaryotic mRNA decay *in vivo*. J. Mol. Biol. **221:**81–95.
28. **Melefors, O., and A. von Gabain.** 1991. Genetic studies of cleavage-initiated mRNA decay and processing of ribosomal 9S RNA show that the *Escherichia coli ams* and *rne* loci are the same. Mol. Microbiol. **5:**857–864.
29. **Mudd, E. A., A. J. Carpousis, and H. M. Krisch.** 1990. *Escherichia coli* RNase E has a role in the decay of bacteriophage T4 mRNA. Genes Dev. **4:**873–881.
30. **Mudd, E. A., H. M. Krisch, and C. F. Higgins.** 1990. RNase E, an endoribonuclease, has a general role in the chemical decay of *Escherichia coli* mRNA: evidence that *rne* and *ams* are the same genetic locus. Mol. Microbiol. **4:**2127–2135.
31. **Nashimoto, H., and H. Uchida.** 1985. DNA sequencing of the *Escherichia coli* ribonuclease III gene and its mutations. Mol. Gen. Genet. **201:**25–29.
32. **Newbury, S. F., N. H. Smith, E. C. Robinson, I. D. Hiles, and C. F. Higgins.** 1987. Stabilization of translationally active mRNA by prokaryotic REP sequences. Cell **48:**297–310.
33. **Ono, M., and M. Kuwano.** 1979. A conditional lethal mutation in an *Escherichia coli* strain with a longer chemical lifetime of messenger RNA. J. Mol. Biol. **129:**343–357.
34. **Portier, C., L. Dondon, M. Grunberg-Manago, and P. Regnier.** 1987. The first step in the functional inactivation of the *Escherichia coli* polynucleotide phosphorylase messenger is a ribonuclease III processing at the 5′ end. EMBO J. **6:**2165–2170.
35. **Prentki, P., F. Karch, S. Iida, and J. Meyer.** 1981. The plasmid cloning vector pBR325 contains a 482 base-pair-long inverted duplication. Gene **14:**289–299.
36. **Regnier, P., and M. Grunberg-Manago.** 1989. Cleavage by RNase III in the transcripts of the *metY-nusA-infB* operon of *Escherichia coli* releases the tRNA and initiates the decay of the downstream RNA. J. Mol. Biol. **201:**293–302.
37. **Regnier, P., and E. Hajnsdorf.** 1991. The decay of mRNA encoding ribosomal protein S15 of *Escherichia coli* is initiated by a RNase E-dependent endonucleolytic cleavage which removes the 3′ stabilizing stem and loop structure. J. Mol. Biol. **217:**283–292.
38. **Reiner, A. M.** 1969. Characterization of polynucleotide phosphorylase mutants of *Escherichia coli*. J. Bacteriol. **97:**1437–1443.
39. **Robertson, H. D., R. E. Webster, and N. D. Zinder.** 1967. A nuclease specific for double-stranded RNA. Virology **32:**718–719.
40. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137:**692–693.
42. **Spahr, P. E.** 1964. Purification and properties of ribonuclease II from *Escherichia coli*. J. Biol. Chem. **239:**3716–3726.
43. **Stoker, N. G., N. F. Fairweather, and B. G. Spratt.** 1982. Versatile low-copy-number plasmid vectors for cloning in *Escherichia coli*. Gene **18:**335–341.
44. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124:**307–316.
45. **Takata, R., T. Mukai, and K. Hori.** 1987. RNA processing by RNase III is involved in the synthesis of *Escherichia coli* polynucleotide phosphorylase. Mol. Gen. Genet. **209:**28–32.
46. **Takiff, H. E., S.-M. Chen, and D. L. Court.** 1989. Genetic analysis of the *rnc* operon of *Escherichia coli*. J. Bacteriol. **171:**2581–2590.
47. **Talkad, V., D. Achord, and D. Kennell.** 1978. Altered mRNA metabolism in ribonuclease III-deficient strains of *Escherichia coli*. J. Bacteriol. **135:**528–541.
48. **Taraseviciene, L., A. Miczak, and D. Apirion.** 1991. The gene specifying RNase E (*rne*) and a gene affecting mRNA stability (*ams*) are the same gene. Mol. Microbiol. **5:**851–855.
49. **Thomas, P.** 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol. **100:**255–266.
50. **Tomcsanyi, T., and D. Apirion.** 1985. Processing enzyme ribonuclease E specifically cleaves RNA I, an inhibitor of primer formation in plasmid DNA synthesis. J. Mol. Biol. **185:**713–720.
51. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.

52. **Wallace, B. J., and S. R. Kushner.** 1984. Genetic and physical analysis of the thioredoxin (*trxA*) gene of *Escherichia coli* K-12. Gene **32:**399–408.
53. **Washburn, B. K., and S. R. Kushner.** 1991. Construction and analysis of deletions in the structural gene (*uvrD*) for DNA helicase II of *Escherichia coli*. J. Bacteriol. **173:**2569–2575.
54. **Watson, N., and D. Apirion.** 1985. Molecular cloning of the gene for the RNA-processing enzyme RNase III of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**849–853.
55. **Willetts, N. S., and D. W. Mount.** 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducing with *thyA*. J. Bacteriol. **100:** 923–934.
56. **Williams, M. G., and P. Rogers.** 1987. Expression of *arg* genes in *Escherichia coli* during arginine limitation dependent upon the stringent control of translation. J. Bacteriol. **169:**1644–1650.
57. **Yancey, S. D., and S. R. Kushner.** 1990. Isolation and characterization of a new temperature-sensitive polynucleotide phosphorylase mutation in *Escherichia coli* K-12. Biochimie **72:**835–843.