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Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K-12

(*rnb*/*pnp*/temperature-sensitive mutant/*in vitro* mutagenesis)

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ABSTRACT **The isolation of a temperature-sensitive allele of RNase II (*rnb*) by *in vitro* mutagenesis has permitted the demonstration that RNase II and polynucleotide phosphorylase (PNPase) are required for cell viability and mRNA turnover in *Escherichia coli*. Double-mutant strains carrying the *pnp-7* and *rnb-500* alleles (PNPase deficient and RNase II thermolabile) ceased growing in Luria broth within 30 min after shift to the nonpermissive temperature. Cessation of growth was accompanied by an accumulation of mRNA fragments 100–1500 nucleotides long. In contrast, single-mutant and wild-type control strains grew normally at the nonpermissive temperature and did not accumulate mRNA. No significant changes in rRNA patterns were observed in any of the strains.**

In vitro, both polynucleotide phosphorylase (PNPase) and ribonuclease II (RNase II) degrade single-stranded RNA exonucleolytically and processively in the 3′ and 5′ direction (1, 2). PNPase catalyzes the reversible phosphorolytic reaction RNA + nPi ⇄ n(nucleotide-5′-diphosphate), whereas RNase II hydrolyzes RNA to nucleotide-5′-monophosphates. Conflicting results have been reported about the *in vitro* roles of these two enzymes. Various researchers have reported that the *in vivo* rate of mRNA degradation was either increased (3), decreased (4), or unchanged (5) in PNPase-deficient mutant strains compared to PNPase$^+$ controls. The *in vivo* role of RNase II is also unclear. Kivity-Vogel and Elson (6) and Lennette *et al.* (7) reported that the *in vivo* rate of mRNA degradation was proportional to the level of RNase II present in the cell. On the other hand, Donovan and Kushner (8) found no difference in the *in vivo* rate of mRNA degradation in either the absence of RNase II activity or in the presence of a 10-fold increase over wild-type levels.

In this communication, it is shown that cells lacking both PNPase and RNase II are inviable. By using the newly isolated *rnb-500* allele, which encodes thermolabile RNase II activity, it has been possible to demonstrate that the loss of cell viability that occurs in a *pnp-7 rnb-500* strain at nonpermissive temperatures is related to an *in vivo* accumulation of RNA species 100–1500 nucleotides long and to an increase in the chemical half-life of total mRNA. In addition, it has been shown that the inviability of such double mutants, as well as the *in vivo* thermolability of RNase II, is partially dependent on the growth medium.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The bacterial strains deficient either in PNPase (*pnp-7*) (9, 10) or RNase II (*rnb-296*) (11) have been described. Strain SK4869 [*pnp-7 rnb-296*/pDK24 (Ap^R *rnb*$^+$)] (Ap^R, ampicillin resistant) was constructed by P1 transduction. Plasmid pDK24 (Ap^R *rnb*$^+$) contains the *rnb* gene as part of a 7.1-kilobase (kb) *Hin*dIII/*Xho* I fragment of *Escherichia coli* DNA cloned into the *Hin*dIII/*Sal* I sites of pBR322 (8). Plasmid pDK04 (*trpE*$^+$ *pyrF*$^+$ *rnb*$^+$) contains the *rnb* gene as part of a 15-kb *Bam*HI fragment of *E. coli* DNA cloned into the *Bam*HI site of the single-copy vector pDF41 (8). Plasmid pKAK7 (Ap^R *pnp*$^+$) contains the *pnp* gene as part of a 6.2-kb *Hin*dIII/*Eco*RI fragment of *E. coli* DNA cloned into the *Hin*dIII/*Eco*RI sites of pBR322 (unpublished results). Plasmid pCDK3 (*argA*$^+$ *recB*$^+$ *recC*$^+$ *thyA*$^+$) contains a 19-kb *Bam*HI fragment of *E. coli* DNA inserted into the *Bam*HI site of pBR325 (12). Plasmid pKK3535 (*rrnB*) contains a 7.5-kb *Bam*HI fragment of *E. coli* DNA inserted into the *Bam*HI site of pBR322 (13).

***In Vitro* Mutagenesis.** Plasmid DNA was mutagenized *in vitro* with hydroxylamine as described by Davis *et al.* (14).

Genetic Procedures. *E. coli* cells were transformed according to the procedure of Kushner (15). P1 transductions were performed as described by Willetts *et al.* (16). Luria broth (L broth) and K medium were described by Dykstra *et al.* (12).

Isolation of an *rnb* Allele Encoding Thermolabile RNase II. To isolate an *rnb* allele that produced thermolabile RNase II, it was first necessary to determine whether plasmid pDK24 (Ap^R Cm^S *rnb*$^+$) (Cm^S, chloramphenicol sensitive) could be displaced from the *pnp-7 rnb-296* strain by another *rnb*$^+$ plasmid that had been mutagenized *in vitro*. Accordingly, an Ap^S Cm^R *rnb*$^+$ plasmid (pDK33) was constructed by subcloning the *rnb* gene from the single-copy plasmid pDK04 (8) into a derivative of pBR325 (pDK33) that had been made Ap^S by deletion of a 126-base-pair *Pvu* I/*Pst* I fragment from the region encoding β-lactamase. When a *pnp-7 rnb-296* double-mutant strain (SK4869) harboring pDK24 (Ap^R Cm^S *rnb*$^+$) was transformed with pDK33 (Ap^S Cm^R *rnb*$^+$), ≈90% of the resulting Cm^R transformants became Ap^S in the absence of Ap selection. In contrast, when a Cm^R Ap^S *rnb*$^-$ plasmid (pDK32) was used to transform the same strain, 100% of the resulting Cm^R transformants remained Ap^R, even in the absence of Ap selection.

Accordingly, pDK33 (Ap^S Cm^R *rnb*$^+$) was mutagenized *in vitro* as described above and subsequently used to transform SK4869 [*pnp-7 rnb296*/pDK24 (Ap^R Cm^S *rnb*$^+$)]. Transformed cells were plated on rich medium containing Cm and incubated at 30°C. After 1 day's growth, Cm^R colonies were printed to duplicate Luria agar plates containing Cm and incubated at either 30°C or 44°C. Colonies that failed to grow at 44°C were purified and retested. Plasmid DNA was isolated from purified temperature-sensitive colonies and used to back-transform SK4869 [*pnp-7 rnb-296*/pDK24 (Ap^R Cm^S *rnb*$^+$)] selecting for Cm^R colonies at 30°C. The resulting Cm^R transformants were tested for their ability to grow at 44°C. Out of ≈16,000 Cm^R Ap^S transformants initially screen-

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Abbreviations: PNPase, polynucleotide phosphorylase; Ap, ampicillin; Cm, chloramphenicol; R, resistant; S, sensitive; kb, kilobase(s).

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ed, two strains were isolated that each contained a plasmid that caused temperature-sensitive growth of SK4869 [*pnp-7 rnb-296*/pDK24 (Ap^R Cm^S *rnb*+)] when pDK24 was displaced by transformation with the mutagenized plasmid. Lysates of the two strains harboring mutagenized plasmid were assayed for RNase II activity. Preliminary results showed that both these strains harbored plasmids (pDK39 and pDK43) containing mutant *rnb* alleles (designated *rnb-500* and *rnb-501*) that encoded thermolabile RNase II activity.

Integration of the *rnb-500* Allele into the Chromosome. Plasmids derived from ColE1, such as pDK39, require DNA polymerase I (*polA*) for autonomous replication (17). CH931 (*polA1*) was transformed with pDK39 (Cm^R *rnb-500*) and lysates of five Cm^R transformants were assayed for RNase II activity at 30°C and 44°C. Preliminary tests showed that one of the five Cm^R transformants contained only thermolabile RNase II activity and this strain, designated SK4785, was studied further. Although the precise DNA recombination event that resulted in the Cm^R strain SK4785 is not known, the important consideration is that this strain contained only thermolabile RNase II activity apparently identical with that encoded by the *rnb-500* allele carried on pDK39 (data not shown).

Enzyme Assays. RNase II activity was assayed quantitatively as described by Ono *et al.* (18). One unit of activity is defined as 1 μmol of adenosine solubilized per hr. The potassium requirement of RNase II permitted its specific determination in crude lysates (18).

PNPase was assayed qualitatively in cell lysates by the $^{32}P \rightleftarrows$ ADP exchange reaction described by Grunberg-Manago (1) and modified by Reiner (9).

Extraction of RNA. At various time intervals, aliquots were removed from growing cell cultures and rapidly chilled on ice. The bacteria were pelleted by centrifugation and resuspended in 50 mM NaCl/4 mM EDTA/0.5% $NaDodSO_4$. Total cellular RNA was recovered by extraction with hot phenol (19).

Assay for RNA Degradation. Cells ($3–4 \times 10^8$ cell per ml) were pulse-labeled for 2 min with 2 μCi (60 nmol) of [^{3}H]uridine per ml (1 Ci = 37 GBq). The labeling was stopped by the addition of rifampicin (500 mg/ml) and nalidixic acid (20 mg/ml) (20). Aliquots (0.5 ml) of cell culture were removed at 1-min intervals and added to 3 ml of ice-cold 20% trichloroacetic acid. The precipitates were collected on Whatman GG/C glass filters, washed three times with 1 ml of ice-cold 95% EtOH/0.1 M HCl. The percentage of [^{3}H]RNA remaining was calculated from the radioactivity (cpm) at a given time minus that for the plateau level of stable RNA divided by the maximum radioactivity in the unstable RNA fraction. mRNA half-life was estimated from a plot of percentage [^{3}H]RNA remaining versus time after addition of rifampicin. Only curves having least-squares measure of fit ≥0.9 were used in mRNA half-life estimations.

RNA·DNA Hybridization. Purified RNA was treated with formaldehyde, electrophoresed through a 1.0% agarose formaldehyde gel, and transferred to nitrocellulose paper as described by Lehrach *et al.* (21) and modified by Barinaga *et al.* (22). Filters were hybridized with DNA that had been radioactively labeled by nick-translation (23).

RESULTS

***In Vitro* Thermolability of RNase II Encoded by *rnb-500*.** Plasmid pDK39 (Cm^R *rnb-500*) was isolated by *in vitro* mutagenesis as described in *Materials and Methods*. SK5035 [*rnb-296*/pDK39 (Cm^R *rnb-500*)] grown at 30°C contained significantly less RNase II activity than lysates of SK5034 [*rnb-296*/pDK33 (Cm^R *rnb*$^+$)] (Table 1). The *in vitro* thermolability of RNase II was measured by preincubating lysates of RNase II-deficient (*rnb-296*) strains that harbored either pDK33 (SK5034) or pDK39 (SK5034) at 44°C and then measuring RNase II activity at 30°C. As shown in Fig. 1, after 30 min at 44°C <5% of the original activity remained in lysates prepared from SK5035 [*rnb-296*/pDK39 (*rnb-500*)], while under identical conditions lysates of SK5034 [*rnb-296*/pDK33 (*rnb*$^+$)] retained 70% of their original RNase II activity.

Table 1. RNase II activity in extracts of various strains

Strain	Genotype	Plasmid	RNase II specific activity
S296	*rnb-296*	None	<0.1
SK5034	*rnb-296*	pDK33:Cm^R *rnb*$^+$	347
SK5035	*rnb-296*	pDK39:Cm^R *rnb-500*	47
CH931	*polA1* Cm^S *rnb*$^+$	None	11.5
SK4785	*polA1* Cm^R *rnb-500*	None	1.7

Strains were grown at 30°C in either L broth (CH931, SK4785, S296) or in L broth with Cm (SK5034, SK5035) to ≈5 × 10^8 cells per ml. Cells were harvested and cell lysates were prepared and assayed at 30°C for RNase II activity as described in *Materials and Methods*.

Integration of the *rnb-500* allele into the chromosome of CH931 (*polA1* Cm^S *rnb*$^+$) resulted in a strain [SK4785 (*polA1* Cm^R *rnb-500*); see *Materials and Methods*] that contained significantly less RNase II activity than lysates of CH931 (*polA1 rnb*$^+$) at 30°C (Table 1). After incubation at 44°C, all detectable RNase activity in lysates of SK4785 was lost in 30 min. In contrast, <20% of the wild-type enzyme activity was inactivated (data not shown).

Viability of PNPase RNase II Double Mutants. To prove that strains deficient in both PNPase and RNase II were inviable, a series of isogenic single (*pnp-7 rnb*$^+$, *pnp*$^+$ *rnb-500*) and double (*pnp-7 rnb-500*) mutant strains was constructed by P1 cotransduction. These transductants were designated SK5003 (Cm^R *pnp-7 rnb-500*), SK5004 (Cm^R *pnp-7 rnb*$^+$), SK5005 (Cm^R *pnp*$^+$ *rnb-500*), and SK5006 (Cm^R *pnp*$^+$ *rnb*$^+$). When grown in L broth, SK5003 (*pnp-7 rnb-500*) ceased growing within one cell doubling (30 min) after shift from 30°C

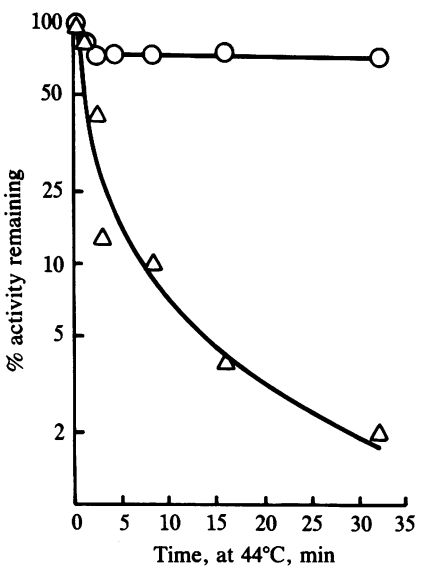

FIG. 1. *In vitro* heat inactivation of RNase II in lysates of SK5034 (*rnb-296*/pDK33:*rnb*$^+$) and SK5035 (*rnb-296*/pDK39:*rnb-500*). Cells were grown in L broth containing Cm at 30°C to a Klett reading of 50. Cells were harvested and cell lysates were prepared. Lysates were incubated at 44°C and aliquots were removed at various times and assayed for RNase II activity at 30°C. ○, SK5034; △, SK5035. When K^+, which is required for RNase II activity, was omitted from the reaction mixtures, the activity fell to <5% of the original values.

to 44°C (Fig. 2A and *Inset*) accompanied by a rapid 10-fold drop in cell viability. Each of the single-mutant strains, SK5004 (*pnp-7 rnb*$^+$), SK5005 (*pnp*$^+$ *rnb-500*), and the wild-type control SK5006 (*pnp*$^+$ *rnb*$^+$) grew well in L broth at 44°C. In contrast, SK5003 (*pnp-7 rnb-500*) continued growing in K medium for 5–6 cell doublings after the temperature shift, during which time the growth rate and viability gradually decreased (Fig. 2B). SK5004 (*pnp-7 rnb*$^+$) had a slightly reduced growth rate at 44°C in K medium compared to either SK5005 (*pnp*$^+$ *rnb-500*) or SK5006 (*pnp*$^+$ *rnb-500*) or SK5006 (*pnp*$^+$ *rnb*$^+$), although all three of these strains grew much better than SK5003 (*pnp-7 rnb-500*) under these conditions (Fig. 2B).

***In Vivo* Accumulation of RNA in the Absence of PNPase and RNase II.** SK5003 (*pnp-7 rnb-500*) and SK5006 (*pnp*$^+$ *rnb*$^+$) were grown in either L broth or K medium at 30°C to a density of ≈1 × 10^8 cells per ml, and the temperature was then shifted to 44°C. At various times, aliquots of the cell cultures were removed and total RNA was extracted. The extracted RNA was electrophoresed through a 1.5% agarose gel and visualized with either ethidium bromide or acridine orange. When grown in L broth, SK5003 (*pnp-7 rnb-500*) progressively accumulated RNA, after a shift to 44°C, that migrated between 16S and 5S rRNA in comparison to constant amounts of 23S and 16S rRNA (Fig. 3A). Within 60 min after SK5003 was shifted to 44°C, the amount of RNA that electrophoretically migrated between 16S and 5S rRNA was >3-fold greater than the amount seen at 30°C, as indicated by densitometric analysis of stained gels containing constant amounts of 23S rRNA (Fig. 3A). SK5006 (*pnp*$^+$ *rnb*$^+$) did not show a similar accumulation of RNA during growth in L broth at 44°C (Fig. 3A).

When SK5003 was grown in K medium at 44°C, a similar accumulation of RNA was not seen (Fig. 3B). RNA did not accumulate at 44°C in either SK5004 (*pnp-7 rnb*$^+$) or SK5005 (*pnp*$^+$ *rnb-500*) when these strains were grown in either L broth or K medium (data not shown). After the samples of extracted RNA were treated with RNase A prior to electrophoresis, all of the stained regions disappeared except for the DNA band (top band in Fig. 3 A and B). Treatment with pancreatic DNase I did not eliminate either the rRNA bands or the material that migrated in the region between the 16S and 5S species (data not shown).

Chemical Half-Life of Pulse-Labeled RNA. The chemical half-life of pulse-labeled RNA (mRNA) was measured in SK5003 (*pnp-7 rnb-500*), SK5004 (*pnp-7 rnb*$^+$), SK5005 (*pnp*$^+$ *rnb-500*), and SK5006 (*pnp*$^+$ *rnb*$^+$). When strains were grown in L broth at 30°C, the half-life of pulse-labeled RNA was

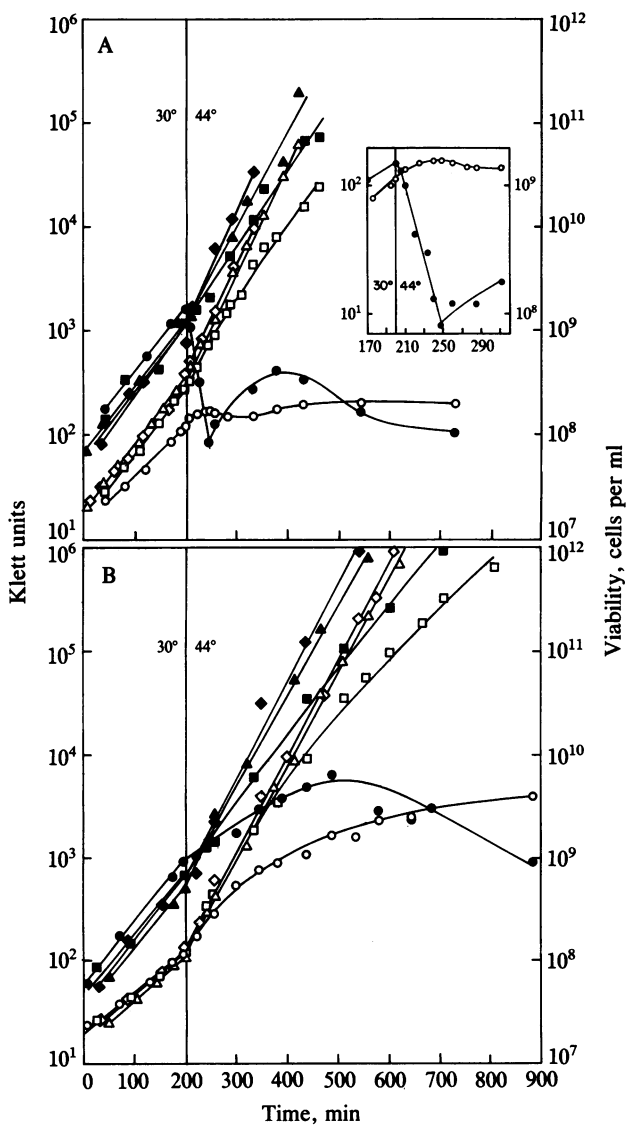

FIG. 2. Growth rate and cell viability of single- and double-mutant strains at 30°C and 44°C. Cells were grown in either L broth (A) or in K medium (B) at 30°C until reaching logarithmic growth, and then they were shifted to 44°C for further growth. When cell cultures reached a Klett reading of ≈100, they were diluted 1:10 into fresh prewarmed medium. Data were plotted after multiplying Klett units and cells per ml by the appropriate dilution factor. Solid symbols, cell viability; open symbols, Klett readings. ○, SK5003 (*pnp-7 rnb-500*); □, SK5004 (*pnp-7, rnb*$^+$); △, SK5005 (*pnp*$^+$ *rnb-500*); ◇, SK5006 (*pnp*$^+$ *rnb*). (*Inset*) SK5003 (*pnp-7 rnb-500*).

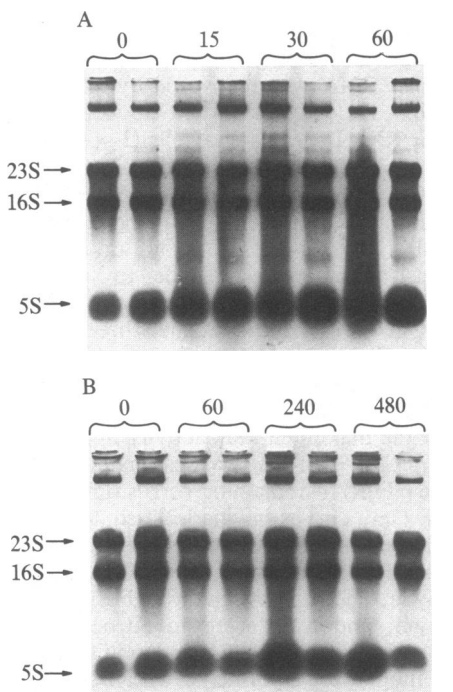

FIG. 3. Relative RNA content in SK5003 and SK5006. Strains were grown at 30°C in either L broth (A) or in K medium (B) and then shifted to 44°C for further growth. At various times before and after the temperature shift, aliquots of the cell cultures were removed, and RNA was extracted and electrophoresed through a 1.5% agarose gel using TBE (89 mM Tris/8.9 mM borate/2 mM EDTA, pH 8.3) buffer. Numbers above each pair of lanes indicate the time (min) after the temperature shift when RNA was extracted. For each pair, the first lane shows RNA extracted from SK5003 (*pnp-7 rnb-500*) and the second lane shows RNA extracted from SK5006 (*pnp*$^+$ *rnb*$^+$). An attempt was made to load approximately the same amount of rRNA in each lane.

Table 2. Half-life of pulse-labeled RNA at 30°C and 44°C in strains carrying various combinations of *rnb* and *pnp* alleles

Strain	Growth medium	Genotype	Half-life, min 30°C	44°C
SK5003	L broth	*pnp-7 rnb-500*	4.2	4.0
SK5003	K medium	*pnp-7 rnb-500*	4.1	2.4
SK5004	L broth	*pnp-7 rnb*$^+$	4.3	1.8
SK5005	L broth	*pnp*$^+$ *rnb-500*	4.4	1.8
SK5006	L broth	*pnp*$^+$ *rnb*$^+$	4.2	1.7
SK5006	K medium	*pnp*$^+$ *rnb*$^+$	4.0	1.7

Duplicate cell cultures were grown either in broth or K medium at 30°C and then one set was shifted to 44°C for further growth. The half-life of pulse-labeled RNA was measured either at 30°C or 60 min after the shift to 44°C.

≈4.3 min for all four strains (Table 2). When measured 60 min after the strains were shifted to 44°C, the half-lives of pulse-labeled RNA in SK5004, SK5005, and SK5006 all decreased to ≈1.8 min, whereas the RNA half-life in SK5003 remained approximately the same (4.0 min) as at 30°C (Table 2). In contrast, when SK5003 was grown in K medium, the mRNA half-life decreased significantly after a shift from 30°C to 44°C (Table 2), although the decrease was not as great as that seen with the wild-type control (SK5006, Table 2).

***In Vivo* Thermolability of RNase II Encoded by the rnb-500 Allele.** To test the apparent *in vivo* thermolability of RNase II, SK5003 was grown at 30°C in either L broth or K medium and then shifted to 44°C. At various times before and after the shift, aliquots of each culture were removed and assayed for RNase II activity at 30°C. When SK5003 was grown in L broth, the relative specific activity of RNase II dropped >75% within 6 min after a shift to 44°C (Fig. 4). Upon further growth at 44°C, the activity temporarily increased and then steadily decreased to an undetectable level. In contrast, when SK5003 was grown in K medium, the initial drop in RNase II specific activity was identical to that observed in L broth after the shift to 44°C, but then it steadily increased with time of incubation at 44°C until the *in vivo* levels of RNase II activity at 44°C were ≈60% of the levels seen prior to the temperature shift (Fig. 4). The RNase II activity in *rnb*$^+$ strains decreased initially after shift to 44°C but then steadily increased (Fig. 4).

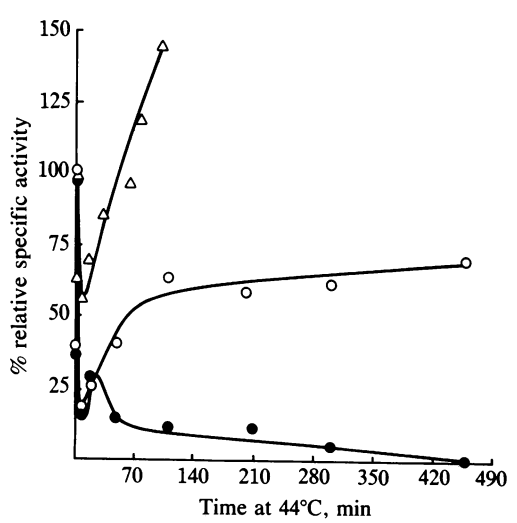

FIG. 4. *In vivo* heat inactivation of RNase II. Cells were grown at 30°C in either L broth or K medium to a Klett reading of 30 and then shifted to 44°C for further growth. At various times before and after the shift, aliquots of the cell cultures were removed, cell lysates were prepared, and RNase II activity was measured at 30°C. ○, SK5003 (*pnp-7 rnb-500*) grown in K medium; ●, SK5003 grown in L broth; △, wild-type *rnb*$^+$ strain grown in K medium. When K$^+$ was omitted from the reaction mixtures, activity fell to <5% of the original values.

Analysis of Accumulated RNA. After a shift to the nonpermissive temperature, SK5003 (*pnp-7 rnb-500*) accumulated RNA fragments of 1.5 kb (16S rRNA) to 0.1 kb (5S rRNA) in size (Fig. 3*A*). To determine whether this accumulated RNA was either mRNA, rRNA, or both, portions of the RNA samples shown in Fig. 3*A* were treated with DNase I, electrophoresed through a 1% agarose/formaldehyde gel (21, 24), and transferred to a nitrocellulose filter paper. The filter was probed with radioactivity labeled plasmid DNA (either pDK07 or pCDK3).

pDK07 and pCDK3 encode seven and eight structural genes, respectively, but do not contain either tRNA or rRNA sequences (25). Fig. 5*A* demonstrates that labeled pDK07 plasmid DNA hybridized to the accumulated RNA described in Fig. 3*A*. Similar results were obtained with the pCDK3 plasmid DNA probe (Fig. 5*B*). The RNA species migrating between 16S and 5S rRNA (Fig. 3*A*) did not, however, appreciably hybridize to a labeled *rrnB* ribosomal DNA probe (Fig. 5*C*).

DISCUSSION

In vivo mutagenesis of the cloned structural gene for RNase II has led to the isolation of a mutant allele (*rnb-500*) encoding RNase II activity that is thermolabile at 44°C *in vitro* and *in vivo*, especially when cells are grown in L broth (Fig. 4). This contrasts to a previously isolated allele (*rnb-464*) that produced an RNase II having thermolabile activity *in vitro* at 45°C–47°C but not *in vivo* (26). Since strains carrying this *rnb-500* allele are temperature sensitive for growth when they are also deficient in PNPase (Fig. 2*A*), this result indicates that RNase II and PNPase are required for some essential cellular functions. Two findings indicate that one of these functions is the degradation of mRNA.

At the nonpermissive temperature (44°C), SK5003 (*pnp-7 rnb-500*) degraded pulse-labeled RNA (mRNA) at a significantly slower rate than strains that contained wild-type levels of either RNase II or PNPase (Table 2). Hence, either RNase II or PNPase can degrade mRNA in the absence of the other enzyme, and in either case mRNA is degraded equally well. This finding is consistent with the results of Kinscherf and Apirion (27), who reported the apparent ability of PNPase to degrade mRNA in the absence of RNase II. However, in that study mRNA degradation was measured in cells incubated at 49°C. Thus, the possibility existed that at such a nonphysiological temperature other RNA metabolizing enzymes may have been inactivated.

The finding that either PNPase II or RNase II can degrade mRNA in the absence of other enzymes partly explains previous apparently conflicting reports. Studies that found no difference in the rate of mRNA degradation between PNPase$^+$ and PNPase-modified strains used RNase II$^+$ strains at 37°C (5). In contrast, experiments indicating that PNPase did affect the rate of mRNA degradation were done after cells were heat shocked at 47°C (4), a condition that may have inactivated wild-type RNase II (Fig. 4). Other research indicating that the rate of mRNA degradation was proportional to the level of RNase II activity was done with strains that were modified in PNPase activity, a condition that the present study shows to be significant.

The relative decrease in the rate of degradation of pulse-labeled RNA that occurred when SK5003 (*pnp-7 rnb-500*) was shifted to the nonpermissive temperature coincided with a substantial *in vivo* accumulation of RNA fragments ranging in size from 100 to 1500 nucleotides (Fig. 3*A*). That these RNA fragments were mRNA was demonstrated by RNA·DNA

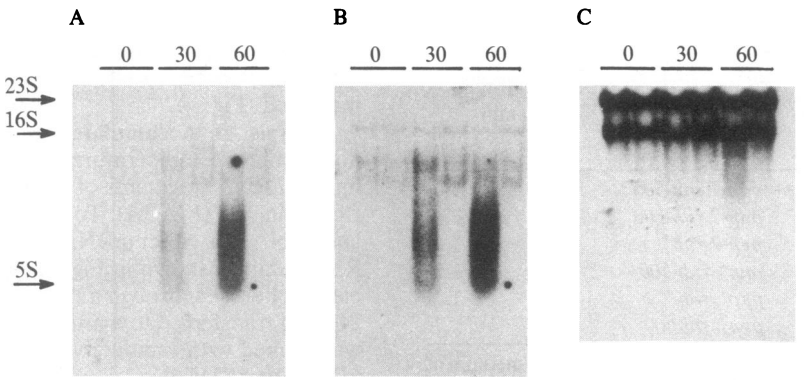

FIG. 5. RNA blot analysis of accumulated RNA. RNA was prepared from SK5003 (*pnp-7 rnb-500*) and SK5006 (*pnp*$^+$ *rnb*$^+$) as described in the legend to Fig. 3. RNA was electrophoresed through a 1.0% agarose formaldehyde gel, transferred to a nitrocellulose filter, and the filter was probed with radioactivity labeled pDK07 plasmid DNA (*A*), pCDK3 plasmid DNA (*B*), or *rrnB* DNA (*C*). Numbers above each pair of lanes indicate the time (min) after the temperature shift when RNA was extracted. For each pair, the first lane shows RNA extracted from SK5003 and the second lane shows RNA extracted from SK5006. Numbers on the left indicate the positions of rRNA. Approximately the same amount of total RNA was loaded in each lane.

hybridization analysis in which a radioactively labeled fragment of DNA, encoding only mRNAs, specifically hybridized to the accumulated RNA (Fig. 5 *A* and *B*). In contrast, hybridization with a rDNA probe showed a minor change in the rRNA pattern after 60 min (Fig. 5*C*). These findings do not rule out the possibility that RNase II and PNPase affect rRNA metabolism in some way. However, if they do, the effect is much less noticeable than that observed with mRNA.

In contrast to its growth characteristics in L broth, SK5003 (*rnb-500 pnp-7*) unexpectedly continued growing for several generations in K medium at the nonpermissive temperature (Fig. 2*B*). Furthermore, SK5003 did not accumulate RNA at the nonpermissive temperature during growth in K medium (Fig. 3*B*). The longer time of growth at the nonpermissive temperature in K medium as well as the absence of accumulated RNA may be explained by the finding that the apparent *in vivo* levels of RNase II activity were significantly greater at the nonpermissive temperature when SK5003 was grown in K medium than when grown in L broth (Fig. 4). It is not clear at this time why RNase II activity in cells carrying the *rnb-500* allele was apparently less thermolabile *in vivo* when cells were grown in K medium than when grown in L broth.

Overall, there is a correlation among cell viability, the *in vivo* accumulation of RNA, the rate of degradation of mRNA, and the *in vivo* levels of RNase II and PNPase. It is possible that the primary cause of growth cessation at the nonpermissive temperature is a failure of the cells to degrade mRNA at a sufficient rate in the absence of both RNase II and PNPase to balance the rate of mRNA synthesis. Preliminary results indicate that RNA synthesis stops rapidly after SK5003 is shifted to the nonpermissive temperature.

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