A Multiple Mutant of Escherichia coli Lacking the Exoribonucleases RNase II, RNase D, and RNase BN*

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A multiple mutant strain of Escherichia coli containing mutations affecting the exoribonucleases, RNase II, RNase D, and RNase BN, and also the endonuclease, RNase I, was constructed by P1-mediated transduction. Extracts of the mutant strain were lacking the aforementioned RNase activities. The multiple mutant displayed normal growth in both rich and minimal media at a variety of temperatures, recovered from starvation essentially as the wild-type parent, and could support the growth of a variety of bacteriophages. In addition, RNA synthesis was normal and no precursor RNA accumulation was observed. The properties of the mutant strain indicate that the three exoribonucleases are not essential for the viability of E. coli. The implications of these findings to our understanding of RNA processing and degradation are discussed.

In recent years considerable progress has been made in our understanding of the pathways of RNA processing, although much still remains to be learned about the nucleases actually involved in the various processing steps (1, 2). For many of the endonucleolytic cleavages known to occur, specific nucleases have been identified and their role in processing confirmed by the isolation of appropriate mutant strains (1, 2). The situation with regard to exoribonucleases, which are thought to be involved in terminal trimming reactions, is much less clear.

It is now apparent that cells contain a variety of exoribonucleases, some of which presumably are processing enzymes, and some of which participate in the degradative reactions of RNA metabolism (2). In Escherichia coli, for example, six distinct exoribonucleases have now been identified. These include: RNase II and polynucleotide phosphorylase, which act as nonspecific degradative enzymes (3) and which may be involved in mRNA breakdown (4); RNases D and BN, which have been suggested to play a role in tRNA processing (5-7); RNase R, which is active against tRNA and homopolymers (7, 8); and the recently identified RNAse T, which also acts on tRNA-type substrates in vitro (9). Although mutant strains deficient in most of these enzymes have been isolated, in no case has the absence of a single RNase been correlated with a specific defect in vivo (2). In one instance, that of RNase BN, the absence of this particular enzyme does lead to a defect in the 3′ processing of tRNA precursors, but only those synthesized after bacteriophage infection (6, 10).

In order to further clarify the cellular function of these enzymes, we have been constructing multiple-mutant strains deficient in more than one RNase. This was done since the overlapping specificities of some of these nucleases suggested that the continued presence of one enzyme might compensate for the absence of another. In this paper we describe the characterization of a triple mutant strain lacking RNase II, RNase D, and RNase BN. Surprisingly, our studies of this mutant indicate that all three exoribonucleases can be dispensed with under normal growth conditions.

EXPERIMENTAL PROCEDURES

Bacterial and Bacteriophage Strains—All strains used in this study are E. coli K12 derivatives. The series of strains, CA265, CAN (RNase BN–), CAN/20 (RNase BN–, II–), CAN/20/12 (RNase BN–, II–, D–), and CAN/20/12-E (RNase BN–, II–, D–, T–) were recently described (9). Briefly, the rnb (RNase II–), rrd (RNase D–), and rna (RNase T–) markers were introduced into strain CAN (10, 11) by P1-mediated transduction using trp or Tnl0 as the selectable markers and assaying transductants for the loss of a particular RNAse. The rrd mutation used here is a deletion (12); the nature of the other mutations is not known.

Wild-type bacteriophages T2, T4, T7, and T5 were from our laboratory stock. The amber-suppressor mutant T4 strain plyu1653 was obtained from Dr. William McClain, University of Wisconsin.

Media and Chemicals—Strains were grown in either YT or M9 media (13) supplemented with 0.4% glucose for a rich or minimal medium, respectively. Tetrazacycline-resistant strains were grown and selected on plates or liquid media containing tetracycline at 10 μg/ml.

E. coli tRNA and rRNA were isolated by phenol extraction and isopropanol fractionation as described (14). They were further purified by chromatography on DEAE-cellulose (tRNA) or 1 M NaCl precipitation and chromatography on Sephadex G-75 (rRNA). tRNA-C-C-[3H]C2-3 for RNase D, tRNA-C-[14C]U for RNase I, and tRNA-C-C-A-[3H]C2-3 for RNase T were synthesized as reported (12, 15). [3H]poly(A) and nonradioactive poly(A) were purchased from Miles Laboratories.

Preparation of Extracts—Cells were grown to about A500 of 1.5 OD, collected by centrifugation, and suspended in one-fifth the volume of 10 mM Tris-Cl, pH 7.5, 0.1 mM dithiothreitol, 0.1 mM EDTA. The cell suspensions were subjected to sonication for about 15 s to rupture the cells and were centrifuged to remove cell debris. Aliquots of the supernatant fractions were assayed for RNase activity against various substrates.

RNase Assays—Activity of the various RNases was assayed under optimal conditions by determination of acid-soluble radioactivity released from the appropriate substrate as follows: [3H]poly(A) for RNase II, tRNA-C-C-A-[3H]C2-3 for RNase D, tRNA-C-[14C]U for RNase BN, [3H]tRNA in the presence of EDTA for RNase I, and tRNA-C-C-[3H]C2-3 for RNase T. Details of the various reaction mixtures have been described (9), and the specific assay conditions are described in the legend to Table I.

RESULTS

Enzymatic Characterization of Multiple Mutant Strains—Strain CAN/20-12, lacking the exoribonucleases, RNase BN, RNase II, and RNase D, was constructed by introduction of mutant rnb and rrd loci into strain CAN. In the additional derivative, strain CAN/20-12E, the contaminating endonucle-
ase, RNase I, was also eliminated. The enzymatic defects present in the individual mutant strains have been described previously (11, 12, 16). However, to ensure the accuracy of the genetic manipulations and to eliminate the possibility of unexpected compensatory changes, the loss of various RNase activities was followed in the series of strains leading to the multiple mutants (Table I). In strain CAN, the absence of RNase BN led to a 20% decrease in hydrolysis of tRNA-C-[^14]C[U, and an additional 10% of the activity was lost upon removal of RNase II in strain CAN/20. Since RNase D is essentially inactive under RNase BN assay conditions, removal of this enzyme had no further effect. The apparently small decrease in tRNA-C-[^14]C[U hydrolysis obtained upon RNase BN removal is due to the large amount of RNase I present in extracts prepared by sonication. This was demonstrated by the introduction of an rna allele which eliminated all the remaining activity against tRNA-C-[^14]C[U, and essentially all activity against tRNA when assayed in the presence of EDTA.

The data in Table I also show that introduction of the rnb mutation in strain CAN/20 eliminates RNase II activity as measured by poly(A) hydrolysis, and the presence of the rnb mutation in strain CAN/20-12 removes 85% of the activity against tRNA-C-C-A-[^14]C[2,3]. In contrast, there is relatively little decrease in hydrolysis of tRNA-C-[^14]C[A upon removal of the three exoribonucleases. This latter observation has led to the recent identification of a new exoribonuclease, termed RNase T (9). This enzyme also could account for the residual activity against tRNA-C-C-A-[^14]C[2,3] seen in the triple mutant strain since this artificial tRNA precursor is a substrate for purified RNase T (9).

In view of the relatively smaller decrease in tRNA-C-[^14]C[U hydrolysis in strain CAN and its derivatives, the absence of RNase BN was confirmed by measuring the plating efficiency of wild-type bacteriophage T4 and the amber-suppressor mutant psu",-BU33 (Table II). Previous work has shown that strains lacking RNase BN cannot support the growth of phage strains dependent on the synthesis of psu",tRNA^ser (6, 10, 17). The data in Table II confirm the absence of RNase BN since none of the CAN derivatives are able to support the growth of the mutant phage, whereas wild-type T4 plates normally on all the RNase mutants. These findings also indicate that the absence of three exoribonucleases has no significant effect on infection by bacteriophage T4.

**Growth Properties of Multiple Mutant Strains**—All the mutant strains grew as well as the wild-type parent, CA265, on plates with rich medium at 30, 37, 42, and 44 °C. Likewise, no differences were observed in liquid culture among the various strains grown at temperatures between 30 and 43 °C. Essentially identical results were obtained on minimal plates. All the mutant strains were able to grow at temperatures up to 42-43 °C, with only slight differences in colony size between the parental and mutant strains.

As a further test of the growth properties of the mutant strains under more stressful conditions, we determined their ability to recover from extended periods of starvation. It is known that during starvation, cells degrade a considerable amount of their RNA and require a period of resynthesis prior to beginning logarithmic growth (18). If any of the exoribonucleases deficient in the mutant strains were required during RNA synthesis, a delay in recovery or a lack of recovery from the starvation might be expected. However, as shown in Fig. 1A, all the strains recovered equally well after a starvation period of 42 h at 37 °C. After 90 h of starvation (Fig. 1B), some delayed recovery of the mutant strains does become evident, although all the strains do recover. Whatever the cause of the slightly delayed recovery, it is already apparent in strain CAN and is no worse in the other mutants, indicating that at most only RNase BN could be involved. These data indicate that none of the exoribonucleases is essential to the viability of E. coli and that, even in the absence of all three enzymes, growth is almost the same as the wild-type parent.

**Growth of Bacteriophage in Mutant Strains**—Inasmuch as host RNases may play a role during phage infection, we have also examined whether the absence of multiple exoribonucleases has any effect on phage growth in the mutant strains (Table I). For bacteriophages T3, T4, and T5 no significant differences were observed in plating efficiency or plaque size in any of the mutant hosts compared to strain CA265. In contrast, we repeatedly observed that the plating efficiencies of phages T2 and T7 were slightly decreased in all the mutant strains. In addition, the plaque size for T7 grown on CA265 was larger than that of phage grown on the mutant. The explanation for these differences is not known, but as the effect is already evident in strain CAN, it either is a consequence of the absence of RNase BN or of another mutation introduced during isolation of strain CAN. However, since in no case is a major decrease in phage growth observed, the exoribonucleases are also nonessential during infection.

**RNA Synthesis in Mutant Strains**—Although growth of the mutant strains was normal, it was still possible that RNA synthesis or processing might have been affected, but not sufficiently to become rate-limiting for growth. However, labeling of wild-type and mutant cells for 15 min with ^32P, followed by gel electrophoresis (Fig. 2), indicated that the mutants synthesized the same species of RNA as found in the wild-type parent, and no precursors of low-molecular-weight RNAs accumulated. Based on these data we concluded that RNA metabolism has not been affected significantly by the absence of the three exoribonucleases.

**DISCUSSION**

The studies presented here indicate that neither RNase II, RNase D, nor RNase BN, alone or in combination, are

### Table I

**Enzymatic activities of E. coli ribonuclease mutants**

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<td>CA265</td>
<td>100</td>
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<tr>
<td>CAN/20-12(BN^-II-D^-)</td>
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<td>15</td>
<td>127</td>
<td>83</td>
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<tr>
<td>CAN/20-12(BN^-II-D^-I-)</td>
<td>1</td>
<td>&lt;1</td>
<td>18</td>
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TABLE II
Plating efficiency of bacteriophages on RNase mutant E. coli strains

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<th>E. coli strain</th>
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<td></td>
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<tr>
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<tr>
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The plating efficiency of bacteriophages on RNase mutant E. coli strains was measured. Cells were grown to an A500 of about 1, and 0.4 ml were incubated with an appropriate dilution of phage for 10 min at 37 °C. After incubation, the mixture was overlayed onto YT plates with 2.5 ml of soft agar. Plates were incubated at 37 °C overnight.

**REFERENCES**


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*This strain was constructed by P1 transduction of the rnb deletion in strain SK5032, kindly provided by Drs. Sidney Kushner and William Donovan, into strain CAN using an adjacent Tn5 as the selectable marker. All the ampicillin-resistant transductants assayed were RNase II. The rnb deletion was then introduced as described under "Experimental Procedures" for construction of strain CAN/20-12.*