Identification of a Potent DNase Activity Associated with RNase T of Escherichia coli*

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RNase T was first identified as an enzyme responsible for end turnover of tRNA in Escherichia coli. Its activity, specific for tRNA-C-C-A, catalyzes the release of tRNA-C-C and AMP. RNase T, along with several other RNases, plays a role in maturation of several other RNA species by a similar limited nucleolytic activity. In previous work, we identified the gene for RNase T, rnt, as a high copy suppressor of the UV sensitivity conferred by deficiency in three single-strand DNA-specific exonucleases, RecJ, exonuclease I, and exonuclease VII. This suggested that RNase T may process DNA substrates as well. In this work, we show that purified RNase T possesses a potent 3’ to 5’ single-strand DNA-specific exonucleolytic activity. Its $K_m$ for single-strand DNA substrates is many orders of magnitude lower than that for tRNA, suggesting that single-strand DNA may be a natural biological substrate for RNase T. We suggest that the DNA activity of RNase T may play a role in end trimming reactions during DNA recombination and/or DNA repair.

RNase T of Escherichia coli was identified as a 3’ exoribonuclease due to its ability to remove the terminal adenine residue from uncharged tRNA-C-C-A molecules (1). In addition to this end turnover reaction, RNase T is one of several enzymes that can remove 3’ residues in the maturation of tRNA (2). RNase T is also responsible for the removal of the two 3’ terminal residues in the processing of SS RNA. In rnt (RNase T ) mutants, no mature 5S RNA is found, but the incompletely processed 5S RNA is present in ribosomes and apparently functions normally in translation (3).

We isolated rnt multiple times in a high copy suppression screen as a gene that could restore UV irradiation resistance to a mutant deficient in the three known single-strand DNA (ssDNA)1 exonucleases of E. coli.2 The three single-strand DNA exonucleases of E. coli, RecJ exonuclease, exonuclease I, and exonuclease VII, play somewhat redundant roles in promoting UV survival. A mutant deficient in all three shows a profound sensitivity, whereas any single mutant has only a minor defect (4). RecJ exonuclease is specific for 5’ ends of single-strand DNA (5), exonuclease I is specific for 3’ ends (6), and exonuclease VII has dual polarity (7). We have proposed that these exonuclease activities are required to process daughter-strand gaps in DNA to allow recombinational repair of replication forks blocked by photodimer lesions (4). The identification of rnt as a suppressor of this repair defect immediately suggested that RNase T may also be able to process DNA substrates. Consistent with this notion RNase T shares motifs with other DNases including exonuclease I and the 3’ to 5’ proofreading exonucleases of the three E. coli DNA polymerases (8, 9).

We report here that overexpression of RNase T concomitantly induces high levels of a DNase activity on ssDNA in crude extracts. We have purified the RNase T protein to homogeneity, both in the native form and as a His6-tagged fusion protein. The DNA activity of RNase T is specific for ssDNA, acts in a 3’ to 5’ polarity, and is distributive in nature. In contrast to its limited degradation of RNA substrates, RNase T is capable of degrading 40-kilobase bacteriophage T7 ssDNA molecules to 70% completion. The kinetics of DNA degradation are comparable with other DNases of E. coli and suggest that RNase T may be a much more potent DNase than RNase A. This suggests that DNA may be a natural substrate for RNase T and that RNase T may play a role in DNA metabolism in vivo.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—The STL2350 strain (xanA2 recJ284:Tn10 Δ(xseA-guaB) sff:3139:Thn10kan) was used for RNase T protein expression and purification due to its attenuated background DNase activity. To induce expression from the T7 $\alpha$10 promoter, the plasmid pTJ300 (10) carrying the gene for the T7 RNA polymerase under control of the $\lambda P_{r}$ promoter and the $\lambda cI857$ repressor gene was introduced into STL2350 by transformation (11) and selection for chloramphenicol resistance. pAL1, conferring resistance to ampicillin, contains a 1.1-kilobase pair Sau3A fragment of E. coli genomic DNA (from strain STL2701 (xanA300:cat recJ2052:Tn10 Δ(xseA-guaB) sff:3139:Thn10kan) inserted into the unique BamHI site of vector pBSKK (Stratagene, Inc.) with the rnt gene under T7 promoter control. PAL1 was isolated in a high copy suppression screen.3 Plasmid pAL5, a derivative of pAL1 with a frameshift mutation 22 base pairs downstream from the initiation codon of rnt, was constructed by cleavage of PAL1 DNA with restriction endonuclease AgeI, “fill-in” synthesis with Klenow fragment (DNA polymerase I) and blunt end DNA ligation. For protein expression experiments, plasmids pAL1, pAL5 or vector pBSSK were transformed into STL2350(pTH30) cells, selecting ampicillin resistance (strain STL4020 is the pTH30/pAL1 transformant of STL2350 used for protein purification).

STL3239 (ADE3 adeB15 recJ284::Tn10 endA $\Delta(xth-pncA)$) carries an isoprropyl-1-thio-β-D-galactopyranoside-inducible T7 RNA polymerase gene (12). This strain was used to express and purify a $\beta'_2 H_{5}_{14}$-tagged RNase T fusion protein expressed from plasmid pKD1. The rnt gene was amplified using Pfu polymerase (Stratagene, Inc.) from plasmid pAL1 using primers 5’-GGGGATCCATGGTGCATATGC-3’ and 5’-GGGGTCCAGCTTCTCTCTCTGCGCCGGC-3’ and buffer conditions recommended by the manufacturer. The polymerase chain reaction product was subsequently digested with restriction endonucleases BamHI and SalI and ligated into the pET29a(+) vector (Novagen), producing pKD1, pKD1 was introduced into STL3239 by transformation to kanamycin resistance. Sequence analysis verified the construct was error-free.

All restriction endonucleases, T4 polynucleotide kinase, DNA ligase, and Klenow fragment (DNA polymerase I) were obtained from New
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England Biolabs Inc. Shrimp alkaline phosphatase was obtained from Amersham Pharmacia Biotech. Lysozyme was obtained from United States Biochemical Corp.

**DNA Substrates and DNase Assays—**Uniformly labeled bacteriophage T7-3H DNA with a specific activity of 2.2 × 10^6 cpm/nmol of nucleotide was prepared as described previously (5) using [3H]thymidine (NEN Life Science Products). 3' end-labeled substrate was prepared by changing the Tris pH. When required, protein samples were diluted in a buffer containing 10 mM Tris-HCl (pH 8.5), 10 mM MgCl₂, 1 mM dithiothreitol, and 1 mg/ml bovine serum albumin. Sample incubation, trichloroacetic acid precipitation, and soluble count determination were performed as described previously (4). One unit of DNase activity corresponds to the release of 1 nmol of acid-soluble product in a 20-min reaction at 37 °C.

For agarose gel electrophoresis assays, 1 µg (1 pmol) of 3' or 0.8 µg (0.8 pmol) of 5' end-labeled ssDNA substrate was incubated with either 0.2 ng (7.4 fmol protein monomer) or 1.1 ng (41 pmol protein monomer) of His₆-RNase T monomer. Standard assay conditions were used, and reactions were quenched on ice by addition of EDTA to 5.0 mM. Samples were boiled for 5 min and cooled on ice before loading onto a 1.0% agarose gel (Agarose NA, Amersham Pharmacia Biotech) gel. Gels were run at 80 V for 45 min in TAE buffer (13), dried at 80 °C for 1 h, and exposed to film.

**Protein Expression and Analysis—**Native RNase T protein expression was induced from the T7 promoter of pAL1 by a two-plasmid expression system in which the T7 RNA polymerase gene on plasmid pTJH30 was induced by temperature shift to 42 °C. T7-promoter-mediated expression of the His₆-RNase fusion protein from plasmid pKD1 was induced by isopropyl-1-thio-β-D-galactopyranoside addition (1 mM) to a DE3 lysogen strain STRLA48 containing the T7 RNA polymerase gene resident in the lysogenic phage. Culture growth, [³²⁵]methionine protein labeling, protein expression determinations, and crude extract preparations for both proteins were performed as described previously (10). Protein concentrations were measured by the method of Bradford (14) with standard reagent (Bio-Rad) and bovine serum albumin standard.

All proteins were resolved by 15% SDS-polyacrylamide gel electrophoresis (13) and visualized by Coomassie staining and/or autoradiography.

**RNase T Purification—**All steps were performed at 4 °C, and ssDNase activity on denatured T7 DNA was determined for each fraction as described above. The standard buffer (Buffer A) throughout the purification contained 20 mM Tris-HCl (pH 7.5), 10% (w/v) glycerol, 0.1 mM EDTA, and 1 mM dithiothreitol; various concentrations of NaCl and sodium phosphate were added as indicated. A 4-liter culture of STL4020 was grown, harvested, and stored frozen as described previously (4). Protein expression was induced at 42 °C for 1.5 h and then continued at 37 °C for 8 h before harvesting cells. Crude extract was prepared by lysing cells in buffer containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM dithiothreitol, 0.1 mM EDTA, and 1.2 mg/ml lysozyme for 1 h on ice. Three cycles of freeze/thawing (2 min 37 °C, 2 min 0 °C) were performed before a supernatant was obtained by high speed centrifugation at 130,000 × g for 20 min (Fraction 1, 91 ml). The crude extract was applied to a 12-ml reactive blue-4 dye column (Sigma) equilibrated with Buffer A + 200 mM NaCl, and the ssDNase activity was eluted from a step gradient of Buffer A + 50 mM NaCl. The active fractions were dialyzed against Buffer A + 50 mM sodium phosphate (Buffer B) and 50 mM NaCl (Fraction 2, 60 ml). Fraction 2 was applied to a 5-mL HTP-hydroxyapatite cartridge column (Bio-Rad) equilibrated in Buffer B containing 50 mM NaCl. A 40-ml linear gradient from 50 to 1000 mM NaCl in Buffer B was applied with the ssDNase activity eluting at 750 mM NaCl (Fraction 3, 20 ml). Fraction 3 was dialyzed against Buffer A + 50 mM NaCl and applied to a 2-ml Affi-Gel Blue (Bio-Rad) column equilibrated in Buffer A containing 50 mM NaCl. The column was eluted with three-step salt gradients of Buffer A + 300 mM, Buffer A + 600 mM, and Buffer A + 2.5 M NaCl.

**Overexpressing RNase T increases Mg²⁺-dependent single-strand DNase activity**

Nuclease assays were performed using crude extracts prepared from cells carrying the indicated plasmids. Cells were induced for protein expression 20 min prior to assay. Assays were performed in the presence or absence of MgCl₂ using ss or ds-3H T7 DNA substrates. Results are the averages of two determinations.

**Table I**

| Plasmid | Substrate | Mg²⁺ | Specific activity |
|---------|-----------|------|-------------------|
| pAL1 | ss | + | 560 |
| pAL5 (ntt frameshift) | ss | + | 36.0 |
| pBSSK | + | + | 32.0 |
| pAL1 | + | + | 3.4 |
| pAL5 | + | + | 2.8 |
| pBSSK | + | + | 3.7 |

**Table II**

Purification of E. coli RNase T single-strand DNase activity and His₆-tagged RNase T

| Fraction | Total activity | Total protein | Specific activity | Recovery | Purification |
|----------|---------------|---------------|------------------|----------|-------------|
| Native RNase T |                   |               |                  |          |             |
| 1. Crude Extract | 59,900 | 1,020 | 58.7 | 100 | 1.0 |
| 2. Sigma Blue 4 | 28,400 | 24.0 | 626 | 47 | 10.6 |
| 3. Hydrolypitate | 12,100 | 5.16 | 2,340 | 20 | 40.0 |
| 4. Affi-Gel Blue | 8,230 | 1.09 | 7,550 | 14 | 129 |
| 5. DEAE Sephadex | 8,230 | 0.53 | 9,920 | 14 | 169 |
| 6. MonoQ | 6,070 | 0.51 | 11,900 | 10 | 203 |

| His₆-tagged RNase T |                   |               |                  |          |             |
| 1. Crude Extract | 23,265 | 47.0 | 495 | 100 | 1.0 |
| 2. Nickel Agarose | 14,539 | 1.1 | 13,710 | 62 | 27.9 |
800 mM, and 1.5 mM NaCl. The majority of the ssDNase activity eluted in 1.5 x NaCl wash (Fraction 4, 22.5 ml) and was dialyzed against Buffer A + 50 mM NaCl. Fraction 4 was applied to a 1-ml DEAE-Sephadex (Amersham Pharmacia Biotech) column prepared according to manufacturer’s instructions in Buffer A (without glycerol). An 18-mL linear gradient from 50 mM NaCl to 1 x NaCl in Buffer A was applied with the ssDNase activity eluting at 800 mM NaCl (Fraction 5, 25 ml). The active fractions were pooled and dialyzed against Buffer A + 50 mM NaCl. The dialysate, containing 0.83 mg of protein was applied to a 1-ml Econopac MonoQ cartridge (Bio-Rad) equilibrated in 20 mM Tris-HCl (pH 8.0) and 0.5 M NaCl (Buffer D) containing 5 mM imidazole. The column was washed with 50 ml of Buffer D containing 60 mM imidazole, and the remaining protein was eluted with Buffer D containing 1 x imidazole. The eluted protein was dialyzed against Buffer C and stored at -20 °C (Fraction 2, 2.0 ml).

**RESULTS**

**Overexpression and Purification of a DNase Activity Associated with RNase T**—The plasmid pAL1 containing the *E. coli* *rnt* gene encoding RNase T was isolated in a screen for high copy suppressors which confer UV resistance to an *E. coli* strain deficient in the three known single-strand DNA exonucleases (4). Induction of *rnt* expression from the T7 promoter of pAL1 increases the production of a 24-kDa protein, consistent with the reported size of RNase T, which is absent from extracts of cells expressing a frameshift mutation within the promoter of pAL1 (strain STL4148) (12). The 1-liter culture of STL4148 (pKD1) for expression of the His<sub>6</sub>-RNase T fusion protein from plasmid pKD1. 0.5 µg of His<sub>6</sub>-RNase T protein (Fraction 2) purified by nickel agarose-chromatography is shown in lane 3.

| Alteration to the complete reaction mixture | Relative activity % |
|-------------------------------------------|---------------------|
| None                                      | 100                 |
| - Enzyme                                  | 1                   |
| - Dithiothreitol                          | 99                  |
| - Bovine serum albumin                    | 90                  |
| + ATP (100 mM)                            | 100                 |
| + Mn<sup>2+</sup> (10 mM)<sup>a</sup>      | 18                  |
| - Mg<sup>2+</sup>                          | 1                   |
| - Mg<sup>2+</sup> + ATP (100 mM)           | 1                   |
| - Mg<sup>2+</sup> + either Co<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, or Zn<sup>2+</sup> (1 or 10 mM) | 1 |

<sup>a</sup> Reaction also contains Mg<sup>2+</sup>.

**Fig. 2.** *E. coli* RNase T and His<sub>6</sub>-RNase T expression and purification. In panel A each fraction from the purification of native RNase T described in Table I was separated by electrophoresis through a 15% SDS-polyacrylamide gel electrophoresis gel and stained with Coomassie Blue. In panel B SDS-polyacrylamide gel electrophoresis was performed on extracts from cells uninduced (lane 1) and induced (lane 2) for expression of the 25-kDa His<sub>6</sub>-RNase T fusion protein from plasmid pKD1. 0.5 µg of His<sub>6</sub>-RNase T protein (Fraction 2) purified by nickel agarose-chromatography is shown in lane 3.

**Fig. 3.** Time course of degradation of denatured T7 DNA. The amounts of purified RNase T protein (Fraction 6) present in each reaction are as indicated.

**TABLE III**

Reaction requirements

| Alteration to the complete reaction mixture | Relative activity % |
|-------------------------------------------|---------------------|
| None                                      | 100                 |
| - Enzyme                                  | 1                   |
| - Dithiothreitol                          | 99                  |
| - Bovine serum albumin                    | 90                  |
| + ATP (100 mM)                            | 100                 |
| + Mn<sup>2+</sup> (10 mM)<sup>a</sup>      | 18                  |
| - Mg<sup>2+</sup>                          | 1                   |
| - Mg<sup>2+</sup> + ATP (100 mM)           | 1                   |
| - Mg<sup>2+</sup> + either Co<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, or Zn<sup>2+</sup> (1 or 10 mM) | 1 |

<sup>a</sup> Reaction also contains Mg<sup>2+</sup>.
and overexpressed from the T7 promoter on plasmid pKD1 in STL2329 (Fig. 1). The protein had an apparent molecular mass of 27 kDa commensurate with the addition of six histidines and a T7 epitope tag. The His$_6$-RNase T protein was purified to homogeneity using nickel agarose chromatography (Fig. 2B and Table II). The specific ssDNase activity of purified His$_6$-tagged RNase T was virtually identical to that for purified native RNase T (Table II) even though the two proteins were purified by different procedures.

**Properties of the DNase Activity of RNase T**—In reactions with denatured bacteriophage T7 DNA (40 kilobases in length), 0.1 μg (4.3 pmol) of purified RNase T (Fraction 6) could degrade 25% of the total DNA (0.5 μg, 1.5 nmol) in 20 min. Addition of larger amounts of protein resulted in increased digestion with up to 70% of the substrate DNA being degraded with 1.0 μg of RNase T (Fig. 3). The extensive degradation of T7 DNA confirms that RNase T has little or no specificity for DNA sequence or structure. No endonuclease activity on ssDNA was observed in reactions with M13 virion circular ssDNA (data not shown), suggesting that the DNase activity of RNase T is exonucleolytic and not endonucleolytic in nature.

Purified RNase T (Fraction 6) was used to determine optimal conditions for ssDNase activity. Similar to other nucleases, the ssDNase activity of RNase T was dependent upon the presence of a divalent cation; no detectable degradation was seen in its absence (Table III). Both Mg$^{2+}$ and Mn$^{2+}$ divalent cations serve as effective cofactors for RNase T. Whereas optimal activity was achieved with 10 mM Mg$^{2+}$ under our standard assay conditions (Fig. 4A), 95% relative activity was seen with 1 mM Mn$^{2+}$ (Fig. 4B). At higher concentrations, Mn$^{2+}$ inhibited the enzyme in the presence of Mg$^{2+}$ (Table III). Although other divalent cations such as Co$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Ba$^{2+}$, Ca$^{2+}$, and Zn$^{2+}$ were unable to substitute for Mg$^{2+}$ (Table III), they inhibited ssDNase activity in a manner similar to Mn$^{2+}$ (data not shown). RNase T showed optimal activity at pH 8.5 in the presence of Mg$^{2+}$ (Fig. 4C). The ssDNase activity of RNase T was reduced with increasing salt concentrations; a 50% decrease in activity was noted in the presence of 50 mM NaCl (Fig. 4D). Bovine serum albumin enhanced the ssDNase activity of RNase T (Table III). The presence of ATP and the sulfhydryl reducing agent, dithiothreitol, did not alter ssDNase activity (Table III).

![Figure 4](http://www.jbc.org/)  
**Fig. 4. Optimization of RNase T activity.** T7 nuclease assays were performed under standard assay conditions altering either magnesium concentration (panel A) or Tris buffer pH (panel C). Conditions were also altered replacing magnesium with manganese (panel B) or by the addition of NaCl (panel D). All reactions were performed as described in Table III. The 100% relative activity corresponds to 0.5 nmol of acid soluble nucleotides.

The DNase activities of both RNase T protein preparations were examined further on various end-labeled ssDNA substrates (Table IV). Both purified RNase T and the His$_6$-RNase T showed efficient release of the terminal nucleotide from 3’ ssDNA ends. For RNase T (Fraction 6) this 3’ activity was much more efficient than release of terminal nucleotide from 5’ ssDNA ends. Release from 3’ dsDNA ends was apparent only at high concentrations of either enzyme preparation (4,000–6,000-fold more enzyme than that required to detect degradation of 3’ ssDNA ends). Virtually no activity was seen with 5’ labeled dsDNA. This implies that RNase T acts as a 3’ to 5’ exonuclease on ssDNA, the same polarity as its reported activity on RNA.

A 3’ to 5’ polarity of digestion was confirmed by gel electrophoresis of RNase T reactions with end-labeled ssDNA. A 1:135 molar ratio of His$_6$-RNase T protein to 3’-labeled ssDNA (protein monomers: 3’ DNA ends) produced a loss of the terminal 3’ label from nearly all the substrate DNA molecules by 9 min, without detectable shortening of the labeled DNA (Fig. 5A). In contrast, incubation of His$_6$-RNase T with 5’ end-labeled ssDNA at a molar ratio of 51:1 (protein monomers: 5’ DNA ends) resulted in progressive shortening of the labeled DNA with little loss of signal intensity at the earlier time points (Fig. 5B). By 5 min the ssDNA molecules are either degraded completely or have become heterogeneous in size due to asynchronous digestion. These results are consistent with a 3’ to 5’ polarity of DNA degradation by RNase T. Furthermore, the degradation by RNase T must be via a distributive mechanism...
because the substrate molecules appear to be degraded uniformly from the 3′ end. This means that RNase T must dissociate and rebind to its ssDNA substrate during cycles of degradation and contrasts to a processive mechanism of DNA degradation exhibited by ssDNA exonucleases such as exonuclease I (15) and RecJ.4 Electrophoretic analysis of similar time course reactions with purified native RNase T (Fraction 6) produced similar results (data not shown).

Using the 3′ end-labeled ssDNA substrate, the extent of ssDNA degradation was determined for a substrate concentration range of 0.2–40 nM with 1.76 ng/ml (7.5 pM monomer) of ssDNA degradation was determined for a substrate concentration of 14 nM reported for the E. coli RNase H and 5′ end-labeled ssDNA was incubated with 1.1 μg (41 pmol) of His6-RNase T. For both assays 100 ng of DNA was removed for each time point and resolved by agarose gel electrophoresis as described under “Experimental Procedures.”

FIG. 5. Degradation of end-labeled linear DNA substrates. In panel A, 1 μg (1 pmol) of 3′ end-labeled ssDNA was incubated with 0.2 ng (8.7 fmol) of His6-RNase T. In part B 0.8 μg (0.8 pmol) of 5′ end-labeled ssDNA was incubated with 1.1 μg (41 pmol) of His6-RNase T. For both assays 100 ng of DNA was removed for each time point and resolved by agarose gel electrophoresis as described under “Experimental Procedures.”

DISCUSSION

We have demonstrated that RNase T of E. coli possesses a Mg2+-dependent 3′ to 5′ exonucleolytic activity on ssDNA. This ssDNase activity most likely accounts for the ability of rnt, in high copy, to suppress the UV repair defects of an E. coli mutant deficient in other ssDNA exonucleases, RecJ exonuclease, exonuclease I, and exonuclease VII. Unlike these latter ssDNases (7, 15), RNase T degrades DNA via a distributive mechanism. It may be for this reason that multiple copies of the rnt gene are required to overcome the repair deficiency conferred by mutations in the processive ssDNA exonucleases. Further investigation should reveal what role RNase T plays in aspects of DNA metabolism including DNA repair, recombination, and mutation avoidance. Mutants lacking RNase T grow more slowly than wild-type strains and recover poorly from starvation (16). Although this has been interpreted as a failure of RNA processing, DNA metabolism defects may also account for this phenotype (17).

We have found that optimal DNase activity of RNase T occurs under the same conditions determined optimal for its RNase activity (1). The DNase and RNase activity demonstrate the same spectrum of activity for most of the divergent cations tested. Whereas Co2+ has been shown to provide partial RNase activity (1), it is unable to provide any DNase activity. The K_m of RNase T for ssDNA 3′ end degradation was 5 nM, which contrasts to a K_m of 14 μM reported for the activity of RNase T on tRNA (1), suggesting that DNA rather than RNA may be the preferred substrate for RNase T. The K_m of DNA degradation by RNase T is comparable with other known DNases including exonuclease I (K_m = 12 nM (18)) and RecJ (K_m = 1.5 nM).4 In contrast to the limited degradation of RNA substrates, RNase T can extensively degrade ssDNA substrates (approximately 30 kilobases in length). We do not, however, discount a role of RNase T in RNA metabolism. RNase T clearly affects RNA processing in vivo. In cca mutant strains deficient in tRNA nucleotidyltransferase, functional RNase T promotes the accumulation of tRNA-C-C molecules. Mutations in the rnt gene restore more normal growth rates to cca mutants presumably by blocking this end degradation (17). Likewise, two nucleotide 3′ extensions of 5S rRNA accumulate in rnt mutants (3).

RNase T appears to be conserved in a number of proteobacterial species including E. coli, Hemophilus influenza, Vibrio algino lyticus, and Pasteurella hemolytica but has not been found outside this division. In addition, the protein shares significant homology to the DNA polymerase III proofreading exonuclease domain of Gram-positive bacteria. RNase T shares a motif structure with several other 3′ to 5′ DNases, including E. coli exonuclease I and the proofreading exonuclease domains of the three E. coli DNA polymerases (8, 9). Four of these conserved acidic amino acids can be seen in the crystal structure of E. coli polymerase I (Klenow fragment) to coordinate two divalent cations required for phosphodiester bond cleavage (19). Because both its RNase and DNase activities are 3′ to 5′ in polarity, our expectation is that both DNA and RNA substrates are cleaved via the same active site on the enzyme, but this remains to be experimentally confirmed. Likewise, it is unknown whether dimerization of RNase T, required for its tRNA processing activity (20), is also required for ssDNase activity.

Duality of polynucleotide substrates for exonucleases has been reported previously. Two 5′ exonucleases from Saccharomyces cerevisiae, the products of the XRN1 (alternatively SEP1 or KEM1) and HKE1 genes, degrade both RNA and DNA substrates, although RNA substrates are apparently utilized more efficiently by both enzymes (21). Likewise, the 5′ exonucleases of T4 RNase H and E. coli DNA polymerase I degrade both RNA-DNA and DNA-DNA duplexes (22, 23). Because specificity for RNA or DNA is often not experimentally determined, ambiguity of polynucleotide substrates for enzymes may be a more common occurrence than is appreciated.

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