JOURNAL OF BACTERIOLOGY, Jan. 1992, p. 56–62
0021-9193/92/010056-07$02.00/0

Vol. 174, No. 1

Broad-Specificity Endoribonucleases and mRNA Degradation in *Escherichia coli*

SUNIL KUMAR SRIVASTAVA, VINCENT J. CANNISTRARO, AND DAVID KENNELL*

Department of Molecular Microbiology, Box 8230, Washington University School of Medicine, St. Louis, Missouri 63110

Received 15 April 1991/Accepted 31 October 1991

Crude extracts from *Escherichia coli* were screened for any broad-specificity endoribonuclease after the cell proteins were fractionated by size. In a mutant lacking the gene for RNase I (molecular mass, 27,156 Da), the only such activities were also in the size range of 23 to 28 kDa. Fractionation by chromatography on a strong cation-exchange resin revealed only two activities. One of them eluted at a salt concentration expected for RNase M and had the specificity of RNase M. It preferred pyrimidine-adenosine bonds, could not degrade purine homopolymers, and had a molecular mass of ~27 kDa (V. J. Cannistraro and D. Kennell, Eur. J. Biochem. 181:363–370, 1989). A second fraction, eluting at a higher salt concentration, was active against any phosphodiester bond but was about 100 times less active than are RNase I and RNase I* (a form of RNase I) in the wild-type cell. On the basis of sizing-gel chromatography, this enzyme had a molecular mass of ~24 kDa. We call it RNase R (for residual). RNase R is not an abnormal product of the mutant *rna* gene; a cell carrying many copies of that gene on a plasmid did not synthesize more RNase R. Our search for broad-specificity endoribonucleases was prompted by the expectation that the primary activities for mRNA degradation are expressed by a relatively small number of broad-specificity RNases. If correct, the results suggest that the endoribonucleases for this major metabolic activity reside in the 24- to 28-kDa size range. Endoribonucleases with much greater specificity must have as primary functions the processing of specific RNA molecules at a very limited number of sites as steps in their biosynthesis. In exceptional cases, these endoribonucleases inactivate a specific message that has such a site, and they can also affect total mRNA metabolism indirectly by a global disturbance of the cell physiology. It is suggested that a distinction be made between these processing and degradative activities.

mRNA was first identified 30 years ago (6, 20) and provided the sought-after link between DNA and protein. Its most unusual property was its extreme instability. Furthermore, each message (i.e., the mRNA for a specific protein) has a unique rate of decay. In *Escherichia coli* this rate can vary from a half-life of 30 s to >8 min at 37°C (5) and to wider ranges at lower growth temperatures (25, 41). This diversity of decay rates could result from the presence of many different RNase activities, each of which is specific for a specific sequence or structure on a certain class of messages. Alternatively, all messages could be degraded by a relatively small number of RNases, and differences in rates could reflect the modulating effects of other parameters of mRNA metabolism, such as the translation frequency (24, 45).

In the work described in this paper, we have screened for all broad-specificity endoribonucleases (endoRNases) in *E. coli* and have only detected such activities in proteins in the size range of 23 to 30 kDa. Besides RNases M (8) and I and I* (9), we observed another endoRNase we call RNase R (for residual, since it has much lower activity than that of the others). We shall argue that the underlying mechanism for degradation of mRNA must be driven by RNases with such broad specificity.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* DK523 was derived by transformation of strain DH5α (BRL) with plasmid pJM589 (pUC19Ω2[430 bp:K-12*rna* 5kb]), which contains a 5-kb *Xho*I DNA fragment with the RNase I (EC 3.1.27.6) gene (*rna*) plus flanking sequences, into the *Sal*I cloning site of pUC19 (33). Plasmid pSS490 (pJM589Ω3[*rna* 322 bp::*kan*]) was constructed by blunt-end ligation of a *kan* gene cartridge (50) into the single *Eag*I site near the center of the *rna* gene (322 bp from the start of the 735-bp coding region of the mature protein) (Fig. 1). The *kan* gene was derived from plasmid pUC4K by excision with *Eco*RI. The 1,282-bp fragment was purified by electrophoresis through 1% agarose. Both the fragment and the *Eag*I-cut plasmid were made blunt-ended by filling in the ends in the Klenow reaction and then ligated in the DNA ligase reaction (31).

Plasmid pSS490 was cut at the two *Pvu*II sites of pUC19, which flank the original *Sal*I cloning site to give a fragment with the entire 5 kb of *E. coli* DNA insert, including the *rna*::*kan* gene, plus about 300 bp of pUC19 DNA. This fragment was ligated into plasmid pIE [Rep (Ts)] (53) after the plasmid was opened at its single *Eco*RI site and the ends were made blunt, to yield plasmid pSS590 (pIEΩ3[*rna*::322 bp::*kan*]).

E. coli DK533 contained *rna*::*kan* in the chromosome in place of the *rna* gene. It was constructed by cointegrate exchange of the *rna* gene in *E. coli* AT2508 (used by us in previous mRNA studies) using pSS590, by the procedures described by Hamilton et al. (21). Strain DK533 had no detectable RNase I in the periplasm and no detectable RNase I* in the spheroplast fraction.

Enzyme purifications. Unless specified otherwise, as in the initial screening assays (see Results), the purification of preparative amounts of an endoRNase essentially followed procedures described in more detail in earlier papers (8, 32). Briefly, the pellet of bacteria was ground with alumina and resuspended in 20 mM Tris-HCl, pH 7.2, containing 1 mM phenylmethylsulfonyl fluoride (Sigma) to inhibit proteases. Ribosomes were removed by ultracentrifugation, and most

* Corresponding author.

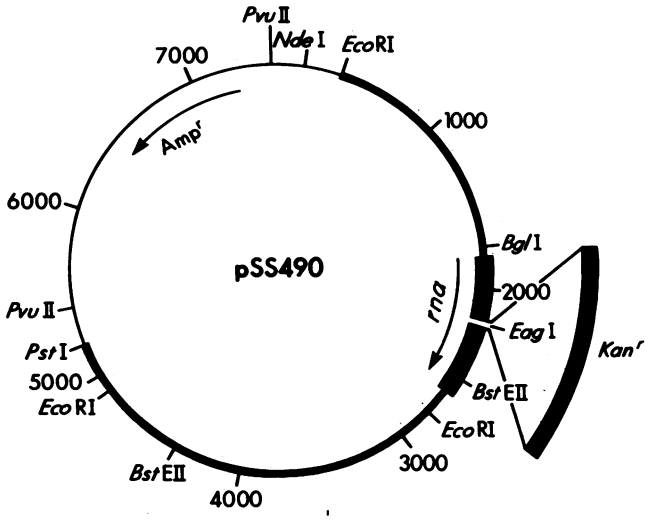

FIG. 1. Plasmid pSS490. The *rna* gene, on a 5-kb *Xho*I fragment from *E. coli* W3110, was cloned into plasmid pUC19 (33). The fragment containing the Kan^r gene was blunt-end ligated into the single *Eag*I site of the *rna* DNA (as shown) to generate pSS490. The *Pvu*II fragment with the *E. coli* DNA from pSS490 was ligated into the replication temperature-sensitive plasmid pIE to give plasmid pSS590, which was used to construct *E. coli* DK533 by gene replacement (Materials and Methods). The thin bar represents pUC19 DNA and the thick bars represent *E. coli* DNA.

of the soluble nucleic acids were removed by protamine sulfate precipitation. The extracts were then batch eluted at a low salt concentration from DEAE weak anion-exchange resin (Whatman) and then from S-Sepharose (Pharmacia) strong cation resin, at a high salt concentration (0.6 M). The resulting active fraction was usually fractionated in a salt gradient either on an S-Sepharose column or on a mono-S column (Pharmacia) by fast protein liquid chromatography (Pharmacia).

Enzyme assays. We have continued to use the simple screening assay for broad-specificity endoRNase activity when monitoring many fractions from a column fractionation (8). An aliquot of the fraction was added to 0.2 μg of an RNA substrate in 20 μl of 10 mM Tris-HCl, pH 7.4, and incubated at 23 or 37°C for an appropriate time before electrophoresis through 1% agarose. Loss of the full-length RNA band signifies activity. Note that this assay will not detect high-specificity processing enzymes that recognize a limited number of unique sites on a limited number of RNA species, nor does it monitor activity by the known 3′-exoribonucleases of *E. coli*, since these processive enzymes are blocked by the many secondary structures present on RNA polymers (27, 47).

In the work described in this paper, we used heterogeneous *E. coli* mRNA as a substrate. The supernatant from ultracentrifugation of a cell extract (150,000 × *g*) was phenol extracted to remove protein and fractionated by electrophoresis through a 20% polyacrylamide gel. A band of the gel that contained the highest concentration of mRNA (species of ~300 nt length) and which was clearly separated from the faster-migrating tRNA population was excised. The mix of mRNAs was eluted from the gel and purified as described elsewhere (10, 11).

Since an RNase might have a specific ion requirement or other requirement for activity, two other assay mixes were used. They were chosen because they have been used with activities that have been implicated in mRNA degradation. The first mix was reported for the assay of RNase E (which is probably also the *ams* activity) (43), and was 10 mM Tris, pH 8.0–100 mM ammonium chloride–1 mM $MnCl_2$–0.1 mM EDTA, pH 7.0–0.1 mM dithiothreitol. The second reaction mix was recommended for assay of RNase K (29) and was 20 mM Tris-HCl, pH 7.9–10 mM $MgCl_2$–0.1 mM EDTA–1 mM dithiothreitol–3% glycerol.

RESULTS

Searching for broad-specificity endoRNases. Recently, we identified an endoRNase in the cytoplasm of *E. coli* that degrades natural polymer RNAs very slowly but is very active against small RNA oligomers or homoribopolymers (9). This enzyme (RNase I*) appears to be a form of the periplasmic enzyme RNase I, which readily degrades natural polymer RNA. All evidence points to both enzymes being coded by the same *rna* gene. Together, they account for the bulk of the broad-specificity endoRNase activity in *E. coli*. Both activities are completely absent in strain DK533, which has a *kan* gene inserted in the chromosomal *rna* gene. In order to detect any broad-specificity endoRNases whose activity might be obscured in the presence of RNase I and RNase I*, we screened extracts from strain DK533.

In order to detect activities that might be present in any cell component, we assayed fractions from very crude extracts. Cell proteins were fractionated by size without the usual batch column fractionations (Materials and Methods) routinely used to purify RNase M and RNase I or I* (8, 32). However, it was still essential to remove the bulk of all cell RNA before any screening of fractions, since such RNA would also be a substrate and could obscure an activity against the added substrate. The ribosomes were removed by ultracentrifugation in the presence of a high salt concentration (0.3 M NaCl), and the supernatant nucleic acids were precipitated with excess protamine sulfate. Normally, the excess protamine sulfate has been eliminated by a batch fractionation on S-Sepharose (8), but with this step omitted there was some protamine sulfate which interfered with the assay of fractions in the lower-molecular-mass range at which these small protamine proteins elute (<20 kDa). However, we have never detected endoRNase activity in those smaller size ranges in previous experiments. The total extract from 3 g of *E. coli* DK533 cells, minus ribosomes and most of the soluble nucleic acid fraction, was loaded onto a Superose 12 sizing-gel column. Proteins larger than 300 kDa are excluded from this gel, and fractions were assayed, starting at the void volume, by using the three different assay conditions described in Materials and Methods. Heterogeneous *E. coli* mRNA was used as a substrate in a simple screening assay we have used routinely to detect broad-specificity endoRNase activities when assaying many fractions (Materials and Methods). The results agreed with those seen in many previous size separations which had been preceded by preliminary batch fractionations; the only broad endoRNase activity with any of the three assay conditions was from molecules fractionating in the 24- to 30-kDa size range (Fig. 2). The total activity was much lower than would be present in this size range in an extract from wild-type cells that contained the genes for RNases I and I* (whose molecular mass is 27,156 Da) (33). RNase M also has a mass of ~27 kDa (8), and the migration of the degradation products in Fig. 2 is characteristic of those seen from RNase M action. The limited number of cleavages generate RNA molecules that migrate more slowly than the uncut substrate. That they

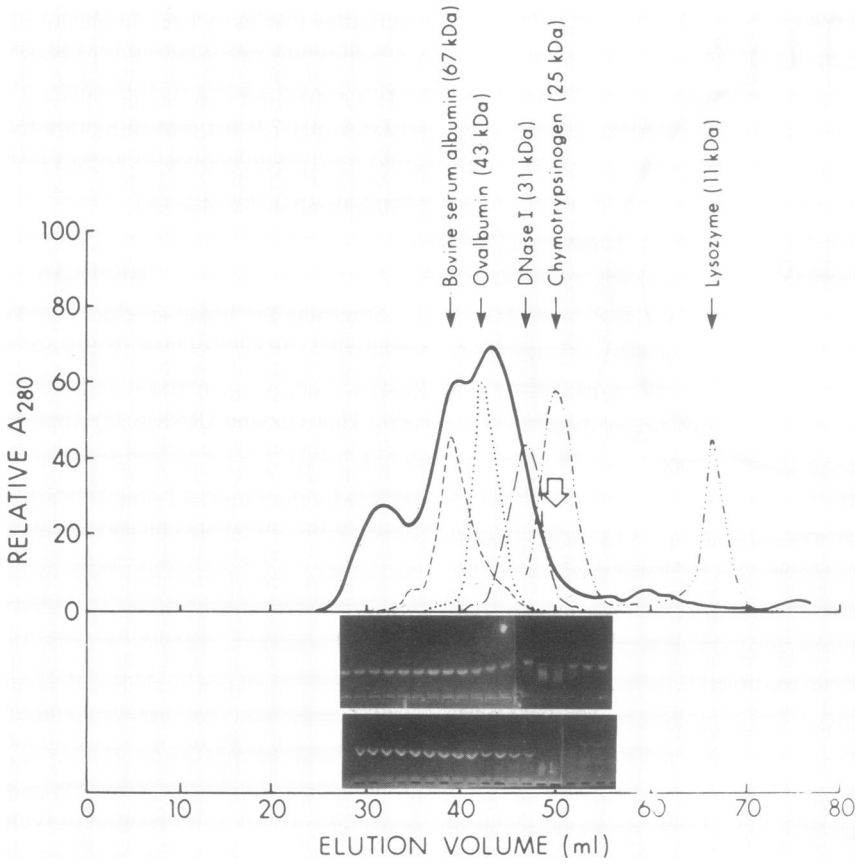

FIG. 2. The presence of broad-specificity endoRNase activity in a crude extract of *E. coli* DK533 fractionated by size on a Superose 12 column. To minimize loss of any possible RNase activity, the extract was applied without any prior column fractionation (see Results). Fractions from the column were assayed for activity by using heterogeneous *E. coli* mRNA as a substrate. The total protein profile and molecular weight standards, which were run separately, are shown at the top, and the endoRNase activity in the 23- to 29-kDa size range are shown at the bottom. When undegraded, the S-RNA appears as a distinct band visible by ethidium bromide staining. The two rows of lanes are positioned to correspond to the fractions immediately above them. The top row of lanes shows a short-time assay. The active fractions lose the full-length RNA molecules, which are replaced by slower-migrating forms. This is the pattern seen by a limited RNase M digestion. The lower row shows the absence of any activity in fractions containing proteins with masses of >30 kDa even with a very long (overnight) reaction with substrate. The same patterns were seen with each of the assay mixes described in Materials and Methods.

were cut molecules was verified by their migration in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). Apparently, the large polymer nondenatured substrates form compact molecules that migrate faster in agarose than do the large oligonucleotide degradation products. With longer reaction times most of the fluorescent bands were lost in those fractions, partly because of the overlapping presence of another enzyme activity described below.

Purification of a residual endoRNase. The RNase activity observed in the size fractionation described above could have been accounted for by the presence of RNase M alone. However, other RNases might be present in that size fraction. All three of the known broad-specificity endoRNases, RNases, I, I*, and M, are bound tightly by strong cationic exchangers, and we have used such resins to separate RNase M from RNases I and I*. Since the total activity recovered from the *rna* mutant was much lower than that recovered from the parent strain, larger starting cell masses had to be used than could be processed in an initial gel-sizing step. A total of 150 g of mutant cells was treated as described in Materials and Methods, including batch elutions from DE52 and S-Sepharose preparative columns, before the active fraction was applied to an S-Sepharose column for gradient elution. As seen earlier (8), a large mass of proteins was not bound, but two peaks of activity could be resolved by the salt gradient (Fig. 3). The first one appeared at 0.36 M NaCl on the gradient, which agrees with the concentration at which RNase M elutes (8). Assays for specificity showed that it indeed was RNase M, i.e., it degraded S-RNA and pyrimidine homopolymers but not purine homopolymers (8, 9, 32) (Fig. 4).

The second activity eluted at 0.48 M NaCl, which is close to the concentration at which RNase I (and RNase I*) elute from such a column. It catalyzed the degradation of both purine and pyrimidine homopolymers and heterogeneous RNA (Fig. 4). Using poly(C) as a substrate, we estimated that this activity is at least 100 times lower in the cell than is RNase I* in the parent strain. We call this enzyme RNase R (for residual).

RNase R is not an abnormal product of the mutant *rna* gene. Expression of the *rna*::Kan^r gene from the normal *rna* promoter would generate a polypeptide of 134 amino acids (~15 kDa), with the first 107 amino acids of RNase I plus 27 amino acids from the linkers and G tail region of pUC4K and terminating at a TGA codon. There is an AUG in frame near the beginning of the distal segment of *rna* that would code for

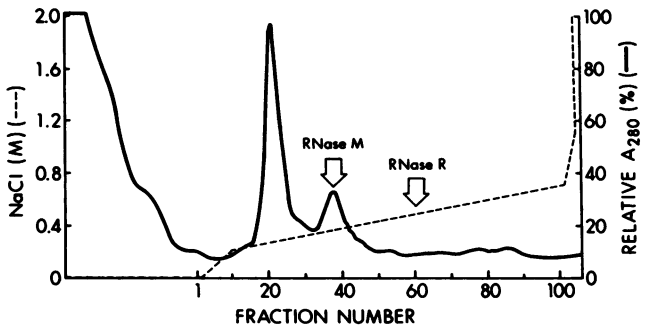

FIG. 3. Endoribonuclease activity from *E. coli* DK533 in fractions eluting from an S-Sepharose strong cation exchanger. The extract applied to the column had been prepared by the procedures described in Materials and Methods. The activity eluting at 0.36 M NaCl was RNase M, as expected from previous work (8). A second low-level activity eluted at 0.48 M NaCl.

a polypeptide of ~14 kDa. If both the distal and proximal polypeptides were synthesized, they could conceivably associate and complement each other to give an enzyme in the size range and with the low activity of RNase R. The following observations argued against this possibility. (i) RNase R was very resistant to treatment at 100°C for 4 min at pH 4. A hybrid dimer would not be expected to be so stable. (ii) We transformed DK533 with pSS490, the original plasmid containing the *rna*::Kan^r insert, which was used to exchange the *rna*::Kan^r for the normal *rna* gene to give DK533. If RNase R were a product of *rna*::Kan^r gene, then it should be synthesized much faster in the resultant strain DK549 than in DK533, since the former has at least 100 copies of the gene. RNase R was partially purified from each strain, and its activity per mass was the same. These results demonstrated that RNase R was not a product of the *rna*::Kan^r gene; therefore, RNase R must be an endoRNase encoded by a different gene.

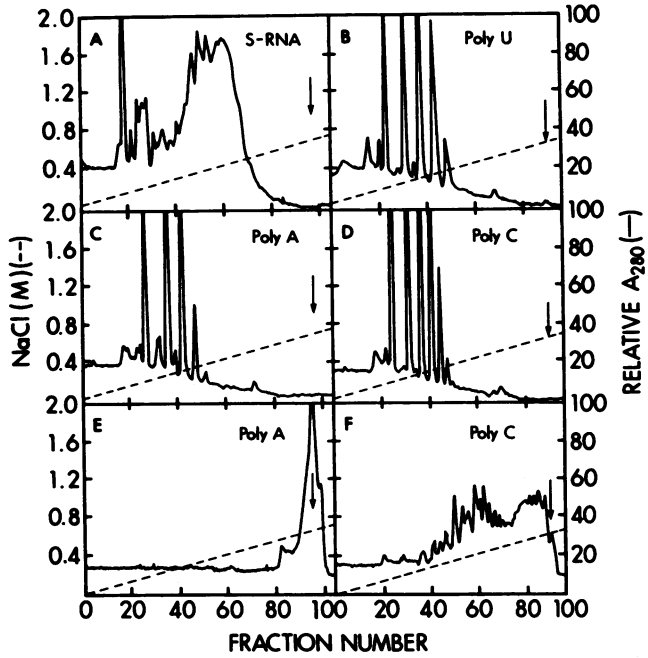

FIG. 4. The degradation of RNA homopolymers and *E. coli* S-RNA by RNase R (panels A through D) and RNase M (panels E and F). The homopolymers (Sigma) were ~300 nt in length each. [Poly(G) is a poor substrate for all of the *E. coli* RNases.] S-RNA is RNA in the supernatant of a cell extract ultracentrifuged at 150,000 × *g*. The RNA substrate indicated in each panel was 10 μg in a 250-μl reaction mixture with 30 mM Tris-HCl, pH 7.5, and 20 μl from a 2-ml fraction from the S-Sepharose fractionation described in the legend to Fig. 3. The reaction was performed at 23°C for 15 h. The arrow in each panel shows the position of the full-length substrate (9).

DISCUSSION

Enzymological perspectives on degradation. The primary sequences of the messenger RNAs of a cell have a complexity as diverse as that of the primary sequences of all the proteins that they code. The ordered degradation of the hundreds of messages (mRNAs for specific proteins) is a major metabolic activity equivalent in magnitude to approximately half of the total cell RNA being synthesized at a given time. Each message has a unique half-life that remains remarkably constant in a given growth condition. This latter characteristic leads to one of two possible contrasting perspectives on how this major activity is managed. It ascribes the diversity of decay rates to a diversity of targets, with a unique enzyme activity for each target. While it is absurd to suppose that there are about 1,000 unique enzymes for the approximately 1,000 different messages synthesized by *E. coli* every several generations (23), the initial targets of inactivation could be grouped into message classes of say, 20 or 30 unique sites. However, even this kind of consolidation is hard to imagine. If it were a part of the coding sequence, it would demand a constraint on the nucleotide sequence and thus the amino acid sequence of the protein product for all proteins in a particular class. A more likely common sequence would be one in the nontranslated leader region that precedes the message or the 3′ tail that follows it. For a number of reasons, degradation usually starts near the 5′ end (24), and in the only case shown rigorously, does so for *lac* mRNA (7). However, a search for such a sequence or structure in 27 randomly chosen *E. coli* leaders failed to find any more homologies for any internal segments of nucleotides than that seen with a collection of "fake" leaders of the same lengths but with random sequences (24).

The other perspective is that there are specific targets, but that they are of such broad specificity that they are common to all messages, and only one enzyme is involved in the initial inactivating event. If decay generally starts near the 5′ end, and if there is no 5′-exoribonuclease, such an enzyme must be a endoRNase. The diverse decay rates would result from secondary parameters that influence the rate, such as the ribosome density. Most of the in vivo cleavages of mRNA from the *lac* operon occur between Py-A residues, and the remainder occur between Py-G or Py-Py residues (9), and an endoRNase (RNase M) with that specificity has been purified (8). This one enzyme could account for the initial inactivating cleavage and the subsequent clipping of the polymer RNA into oligonucleotides that are further degraded to mononucleotides by the processive 3′-exonucleases RNase II and polynucleotide phosphorylase (16, 26, 46) to a resistant small oligonucleotide core. This core could be further degraded to mononucleotides by RNase I* (9) or by another RNase that may also be specific for ribooligonucleotides (15). In theory, these four or five RNases could account for all mRNA decay in *E. coli*. But what of the other RNases?

Processive versus degradative activities. Other RNase activities have been reported to affect the decay rates of one or more messages. RNase III, with double-strand as well as sequence specificity, was shown to cleave T7 early mRNA between its messages (17) and at a specific site on 45S precursor rRNA (17, 38). Two independent groups showed that cleavage at an RNase III site (Sib) about 250 nt downstream from the end of the phage λ*int* mRNA caused that message to decay faster (19, 44). This unusual observation led to reports of the presence of stem-loop structures at the 3′ ends of messages which led to faster decay of the messages when removed (37, 52) and, alternatively, of attack by RNase III at a 5′ site (42). However, there have been relatively few such cases. In fact, it was shown years ago that while loss of RNase III gave a variety of pleiotropic effects, many of which may result from faulty translation (48), there is no evidence for any general effect on mRNA degradation (1, 2, 48). It is clear that RNase III has a very stringent enzyme specificity and makes cuts at a very limited number of sites on very specific RNA molecules.

Similarly, RNase E must have a very stringent enzyme specificity, since it cleaves (i) 9S RNA (but not many other RNAs) and does so only at two specific sites (43), (ii) the small RNA 1 at one site (51), (iii) specific sites in some phage RNAs (28, 36), and (iv) a specific site on the ribosomal protein S20 message (30). However, most RNA substrates tested were not degraded (34), and the substrate specificity so far appears to include a 9-nt sequence and, possibly, secondary structure (2). (A computer search for the 9 nt in more than 1,000 *E. coli* DNA sequences revealed only a few matches; of interest, three were in *pap* messages for pilus proteins). The *rne* and *ams* genes are probably the same (4, 13, 35, 49). An *ams* mutant shows slower mass decay of mRNA but normal functional decay rates (39, 40), and the normal endonucleolytic cleavages are still observed (3, 36). The primary function of RNase E, and how it affects mRNA mass decay, may be difficult to identify. Chanda et al. (12) cloned and sequenced a 595-bp DNA fragment which suppressed the Ams$^-$ phenotype from the *E. coli* genome. The sequence was almost identical to a region of the *gro*EL gene that codes for a 65-kDa protein monomer that is involved as part of a large multimeric complex in phage maturation and other protein-protein interactions (22). However, Claverie-Martin et al. (14) were unable to complement *ams* with plasmids carrying the *gro*E operon. The issue may need further resolution. Also, the sequence cloned by Chanda et al. shows no homology to any part of the sequence of *rne* (13), but if Chanda et al. were correct, it raises the possibility that RNase E affects complex molecular interactions, one side-effect of which is a slower mass decay of mRNA.

We propose that a distinction be made between processing and degradation. The former term should be restricted to its known role of very specific modifications of a precursor molecule in the process of its maturation to a final form. In contrast, degradation of a molecule is synonymous with its destruction. One is an anabolic activity, and the other is a catabolic activity. The reason it is important to distinguish between the two is that the enzymes expected to be most significant in the degradation of a whole class of macromolecules, such as protein or RNA populations, would have specificity, but such specificity would be expected to be relatively broad based. Such seems to be the case for proteases involved in large-scale protein turnover (18) and for the RNases that are known to participate in large-scale degradation of RNA, i.e., RNase I and polynucleotide phosphorylase as well as RNase I during non-growth phases (32).

We suspect that loss of RNase E or RNase III activity can affect mRNA degradation indirectly via the incomplete maturation of molecules that are involved in mRNA metabolism. The most likely candidates are components of translation. In exceptional cases, a processing enzyme may also have evolved a direct role in the decay of a specific message. It is not clear how to classify RNase K (29) until more is learned of its specificity. RNase M has the necessary broad specificity, i.e., for the vulnerable Pyn-A bonds scattered throughout all mRNA molecules, to liberate appropriately sized oligonucleotides for further attack by the 3′-exonucleases. Enzymologically, the initial cleavages would be necessary, since these exonucleases could not handle large polymer molecules because their activity would be blocked at the many double strands that would form (27, 47).

RNase R—a backup activity? RNase R cleaved any RNA nucleotide bonds. If the perspective presented above is correct, such a broad specificity would suggest a function as a general degradative enzyme. However, its activity in the cell was extremely low and could not have been detected in the wild-type cell containing a functional *rna* gene. Unless it is sequestered from all mRNA, it probably contributes to some low level of endonucleolytic cleavages, but compared to the activity of RNase M toward Pyd-A bonds, the 3′-exonucleases, and the proposed activity of RNase I* against oligonucleotides, its role would be marginal in the normal cell. A more important function could be to provide a backup activity in the absence of one or more of the major degradative enzymes. Also, it could have been a major activity in a progenitor strain which had different RNA degradative requirements. The similarities in size and charge of all the known endoRNases could reflect a common genetic origin, with each species arising by gene duplication followed by mutation and selection to an altered form that provided some advantage to the organism.

It is important to identify all the RNases that could function in the degradation of bulk mRNA. RNase R is such an enzyme. It could provide a compensatory function in mutants lacking a major degradative enzyme, such as RNase M, and thus the role of the latter could be obscured. A precedent for such a situation is provided by the two 3′ exoribonucleases, both of which must be missing before the role of either in mRNA mass decay can be detected (26).

ACKNOWLEDGMENTS

Paul Wanda participated in these experiments while on sabattical leave from Southern Illinois University, Edwardsville, Ill., and we benefitted from advice on genetic constructions from Suhas Phadnis, Toni Kazic, and Anil Chauhan.

This work was supported by research grant GM34127 from the NIH.

REFERENCES

1. **Apirion, D., J. Neil, and N. Watson.** 1976. Consequences of losing ribonuclease III on the *Escherichia coli* cell. Mol. Gen. Genet. **144**:185–190.
2. **Apirion, D., and D. R. Gitelman.** 1980. Decay of RNA in RNA processing mutants of *Escherichia coli*. Mol. Gen. Genet. **177**:339–343.
3. **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.
4. **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.
5. **Blundell, M., E. Craig, and D. Kennell.** 1972. Decay rates of different mRNA in E. coli and models of decay. Nature (London) New Biol. **238**:46–49.
6. **Brenner, S., F. Jacob, and M. Meselson.** 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature (London) **190**:576–581.
7. **Cannistraro, V. J., and D. Kennell.** 1985. Evidence that the 5′ end of *lac* mRNA starts to decay as soon as it is synthesized. J. Bacteriol. **161**:820–822.
8. **Cannistraro, V. J., and D. Kennell.** 1989. Purification and characterization of ribonuclease M and mRNA degradation in *Escherichia coli*. Eur. J. Biochem. **181**:363–370.
9. **Cannistraro, V. J., and D. Kennell.** 1991. RNase I*, a form of RNase I, and mRNA degradation in Escherichia coli. J. Bacteriol. **173**:4653–4659.
10. **Cannistraro, V. J., M. N. Subbarao, and D. Kennell.** 1986. Specific endonucleolytic cleavage for decay of *Escherichia coli* mRNA. J. Mol. Biol. **192**:257–274.
11. **Cannistraro, V. J., B. M. Wice, and D. E. Kennell.** 1985. Isolating and sequencing the predominant 5′-ends of a specific mRNA in cells. II. End-labeling and sequencing. J. Biochem. Biophys. Methods **11**:163–175.
12. **Chanda, P. K., M. Ono, M. Kuwano, and H.-F. Kung.** 1985. Cloning, sequence analysis, and expression of alteration of the mRNA stability gene (ams^+) of *Escherichia coli*. J. Bacteriol. **161**:446–449.
13. **Chauhan, A. K., A. Miczak, L. Taraseviciene, and D. Apirion.** 1991. Sequencing and expression of the rne gene of *Escherichia coli*. Nucleic Acids Res. **19**:125–129.
14. **Claverie-Martin, F., M. R. Diaz-Torres, S. D. Yancey, and S. R. Kushner.** 1989. Cloning of the altered mRNA stability (*ams*) gene of *Escherichia coli* K-12. J. Bacteriol. **171**:5479–5486.
15. **Datta, A. K., and S. K. Niyogi.** 1975. A novel oligoribonuclease of Escherichia coli. II. Mechanism of action. J. Biol. Chem. **250**:7313–7319.
16. **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.
17. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs in vivo by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. **70**:3296–3300.
18. **Goldberg, A. L., and S. A. Goff.** 1986. The selective degradation of abnormal proteins in bacteria, p. 287–314. *In* W. S. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworths, Stoneham, Mass.
19. **Gottesman, M., A. B. Oppenheim, and D. Court.** 1982. Retroregulation: control of gene expression from sites distal to the gene. Cell **29**:727–728.
20. **Gros, F., H. Hiatt, W. Gilbert, C. J. Kurland, R. W. Riseborough, and J. D. Watson.** 1961. Unstable ribonucleic acid revealed by pulse labelling of *Escherichia coli*. Nature (London) **190**:581–585.
21. **Hamilton, C. M., 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.
22. **Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis.** 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) **333**:330–334.
23. **Kennell, D.** 1968. Titration of the gene sites on DNA by DNA-RNA hybridization. II. The *Escherichia coli* chromosome. J. Mol. Biol. **34**:85–103.
24. **Kennell, D.** 1986. The instability of messenger RNA in bacteria, p. 101–142. *In* W. S. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworths, Stoneham, Mass.
25. **Kennell, D., and I. Bicknell.** 1973. Decay of messenger ribonucleic acid from the lactose operon of *Escherichia coli* as a function of growth temperature. J. Mol. Biol. **74**:21–31.
26. **Kinscherf, T. G., and D. Apirion.** 1975. Polynucleotide phosphorylase can participate in decay of mRNA in *Escherichia coli* in the absence of ribonuclease II. Mol. Gen. Genet. **139**:357–362.
27. **Klee, C. B., and M. F. Singer.** 1968. The processive degradation of individual polyribonucleotide chains. I. *Micrococcus lysodeikticus* polynucleotide phosphorylase. J. Biol. Chem. **243**:923–927.
28. **Kokoska, R. J., K. J. Blumer, and D. A. Steege.** 1990. Phage f1 mRNA processing in Escherichia coli: search for the upstream products of endonuclease cleavage, requirement for the product of the altered mRNA stability (ams) locus. Biochimie **72**:803–822.
29. **Lundberg, U., A. von Gabain, and O. Melefors.** 1990. Cleavages in the 5′ region of the ompA and bla mRNA control stability: studies with an *E. coli* mutant altering mRNA stability and a novel endoribonuclease. EMBO J. **9**:2731–2741.
30. **Mackie, G. A.** 1991. Specific endonucleolytic cleavage of the messenger RNA for ribosomal protein S20 of *Escherichia coli* requires the product of the *ams* gene in vivo and in vitro. J. Bacteriol. **173**:2488–2497.
31. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Meador, J., III, B. Cannon, V. J. Cannistraro, and D. Kennell.** 1990. Purification and characterization of Escherichia coli RNase I. Comparisons with RNase M. Eur. J. Biochem. **187**:549–553.
33. **Meador, J., III, and D. Kennell.** 1990. Cloning and sequencing the gene encoding *Escherichia coli* ribonuclease I: exact physical mapping using the genome library. Gene **95**:1–7.
34. **Misra, T. K., and D. Apirion.** 1979. RNase E, an RNA processing enzyme from *Escherichia coli*. J. Biol. Chem. **254**:11154–11159.
35. **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.
36. **Mudd, E. A., P. Prentki, D. Belin, and H. M. Krisch.** 1988. Processing of unstable bacteriophage T4 gene 32 mRNAs into a stable species requires *Escherichia coli* ribonuclease E. EMBO J. **7**:3601–3607.
37. **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.
38. **Nikolaev, N., L. Silengo, and D. Schlessinger.** 1973. Synthesis of a large precursor to ribosomal RNA in a mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **70**:3361–3365.
39. **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.
40. **Ono, M., and M. Kuwano.** 1980. Chromosomal location of a gene for chemical longevity of messenger ribonucleioc acid in a temperature-sensitive mutant of *Escherichia coli*. J. Bacteriol. **142**:325–326.
41. **Pedersen, S., S. Reeh, and J. D. Friesen.** 1978. Functional mRNA half lives in E. coli. Mol. Gen. Genet. **166**:329–336.
42. **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.
43. **Roy, M. K., B. Singh, B. K. Ray, and D. Apirion.** 1983. Maturation of 5-S rRNA: Ribonuclease E cleavages and their dependence on precursor sequences. Eur. J. Biochem. **131**:119–127.
44. **Schindler, D., and H. Echols.** 1981. Retroregulation of the int gene of bacteriophage 1: control of translation completion. Proc. Natl. Acad. Sci. USA **78**:4475–4480.
45. **Schneider, E., M. Blundell, and D. Kennell.** 1978. Translation and mRNA decay. Mol. Gen. Genet. **160**:121–129.
46. **Sekiguchi, M., and S. S. Cohen.** 1963. The selective degradation of phage-induced ribonucleic acid by polynucleotide phosphorylase. J. Biol. Chem. **238**:1319–1330.

47. **Singer, M. F., and G. Tolbert.** 1965. Purification and properties of a potassium-activated phosphodiesterase (RNase II) from *Escherichia coli*. Biochemistry **4**:1319–1330.
48. **Talkad, V., D. Achord, and D. Kennell.** 1978. Altered mRNA metabolism in ribonuclease III-deficient strains of *Escherichia coli*. J. Bacteriol. **135**:528–541.
49. **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.
50. **Taylor, L. A., and R. E. Rose.** 1988. A correction in the nucleotide sequence of the Tn903 kanamycin resistance determinant in pUC4K. Nucleic Acids Res. **16**:358–358.
51. **Tomcsanyi, T., and Apirion, D.** 1985. Processing enzyme ribonuclease E specifically cleaves RNA I. J. Mol. Biol. **185**:713–720.
52. **von Gabain, A., J. G. Belasco, J. L. Schottel, A. C. Y. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.
53. **Yaskowiak, E., M. Cotoras, G. Lee, and P. E. March.** 1988. Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, H32, p. 150.