Escherichia coli RNase E has a role in the decay of bacteriophage T4 mRNA

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Bacteriophage T4 mRNAs are markedly stabilized, both chemically and functionally, in an *Escherichia coli* strain deficient in the RNA-processing endonuclease RNase E. The functional stability of total T4 messages increased 6-fold; we were unable to detect a T4 message whose functional stability was not increased. There was a 4-fold increase in the chemical stability of total T4 RNA. The degree of chemical stabilization of six specific T4 mRNAs examined varied from a maximum of 28-fold to a minimum of 1.5-fold. In the RNase E-deficient strain, several minutes delay and a slower rate of progeny production led to a reduction in final phage yield of ~50%. Although the effect of the *rne* temperature-sensitive mutation could be indirect, the simplest interpretation of our results is that RNase E acts directly in the degradation of many T4 mRNAs.

[**Key Words:** Bacteriophage T4; mRNA decay; mRNA processing; mRNA stability; ribonuclease E]

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Escherichia coli RNase E is an endoribonuclease that has been shown to process 9S RNA to a precursor of 5S rRNA (Ghora and Apirion 1978) and also to process RNA1, the inhibitor of ColE1 plasmid replication (Tomcsányi and Apirion 1985) both in vitro and in vivo. RNase E did not appear to have a general role in mRNA decay in *E. coli* (Apirion and Gitelman 1980), although the synthesis of some *E. coli* proteins was affected by the mutation (Gitelman and Apirion 1980). The enzyme is involved in the processing of mRNA from bacteriophage T4 genes 32 and 59 in vivo. Processing of these messages resulted in destabilization of the portion of the mRNA upstream of the cleavage site (Mudd et al. 1988; Carpousis et al. 1989) and thus may have a role in retroregulation (Schmeissner et al. 1984) of upstream gene expression. A comparison of the cleavage sites found in the noncoding RNAs and the T4 mRNAs revealed similarities in sequence at the cleavage sites and in the potential to form RNA secondary structure just downstream of the sites (Tomcsányi and Apirion 1985; Mudd et al. 1988; Carpousis et al. 1989).

As yet, the nucleases that determine the rate of functional decay of total mRNA in *E. coli* or its phage have not been identified. A mutation in the *E. coli* *ams* gene was found to have a five- to sixfold effect on the chemical stability of total *E. coli* RNAs, but it did not affect functional stability significantly (Kuwano et al. 1977; Ono and Kuwano 1979). *E. coli* RNases III (see Portier et al. 1987), E (Mudd et al. 1988; Carpousis et al. 1989), and other as yet unidentified endonucleases (Cannistraro et al. 1986; Båga et al. 1988; Melefors and von Gabain

1988; Uzan et al. 1988) have been implicated in the decay of a few specific bacterial or phage mRNAs. In this paper we present evidence suggesting that *E. coli* RNase E has a major role in the functional and chemical decay of many bacteriophage T4 mRNAs.

Results

E. coli RNase E affects the functional stability of T4 mRNAs

The functional stability of mRNA can be estimated from the ability of the mRNA to direct the synthesis of proteins after transcription initiation has been blocked with rifampicin. Figure 1B shows the effect of the temperature-sensitive *rne* mutation on the functional stability of T4 mRNAs at the nonpermissive temperature. At different times following the addition of rifampicin, infected *rne*⁺ and *rne*⁻ cells were pulse-labeled with ¹⁴C-labeled amino acids and the labeled T4 proteins analyzed by SDS-PAGE. As assayed by the protein synthetic rates, the functional stabilities of all the T4 mRNAs whose gene products are detected in Figure 1B appear to increase in the *rne*⁻ strain. A message whose stability is not affected by this mutation cannot be readily detected. If such mRNAs exist, their protein products must be minor species. The total proteins synthesized at each time point were determined by integration of densitometric scans of each lane in Figure 1B. From this, the estimated functional half-life of the messages in the *rne*⁺ strain is 7 min, whereas it is 42 min in the *rne*⁻ strain. The overall effect of the *rne* mutation is therefore a sixfold functional stabilization of the messages. It should be noted that this measurement of functional half-life of total mRNA is biased in that mRNAs with

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Figure 1. Protein synthesis at (A) 30°C or (B) 43°C in phage-infected *rne*⁺ and *rne*⁻ *E. coli* cells before and after rifampicin treatment. In B, the cells were infected with T4, and after 6 min, rifampicin was added to a final concentration of 150 µg/ml. Before the addition of rifampicin (time 0), or at the times shown (minutes) after the rifampicin treatment, samples of cells were removed, pulse-labeled for 3 min with ¹⁴C-labeled amino acids, and chased with excess cold amino acids for 3 min. The times represent the mid-point of each pulse; for example, 8 min represents a pulse begun at 6.5 min after the addition of rifampicin and terminated by the chase at 9.5 min. In A, rifampicin was added 10 min after infection (final concentration 175 µg/ml), and samples of cells were pulsed for 5 min and chased for 5 min. Rifampicin was added later at 30°C to compensate for differences in the rate of development between 30°C and 43°C. On the basis of the patterns of protein synthesis at the time of rifampicin addition (0 min), the infection appears to be at nearly the same stage of development. Labeled proteins were subsequently analyzed by SDS-PAGE with a 10% polyacrylamide gel. In B, the observed differences in amounts of labeled protein are not due to unequal loadings, because Coomassie Blue staining (not shown) indicated that the total amount of protein was essentially the same in each track. Some T4 gene products are indicated; the 23* represents the processed gene 23 protein (Vanderslice and Yegian 1974). In the *rne*⁻ strain at the 0 time point, additional protein bands are evident that are not visible in the *rne*⁺ strain; these are host proteins whose synthesis has not been completely shut off by the phage infection, presumably because of the slight delay in the infection of this mutant strain, which has been observed previously (Mudd et al. 1988). Because it takes several minutes for rifampicin to enter the cells and block transcription fully, this delay could partly explain the small increase in the levels of some of the T4 late gene products (e.g., 34, 7, and 37) observed between the 0- and 8-min time points after the addition of rifampicin. However, the delay in infection of the *rne*⁻ strain cannot explain the differences in functional stability in the two strains because these differences were also observed when rifampicin was added at a later stage of infection (not shown).

higher translational yields contribute more to the half-life estimate than those with lower yields. Many of the mRNAs are so highly stabilized in the *rne*⁻ strain that it is difficult to estimate their individual half-lives. For a few messages, protein synthesis even increases slightly at late times after the rifampicin treatment. This could reflect an increase in the number of ribosomes available for translation of these more stable messages as other messages decay. It is also apparent in Figure 1B that the various messages are not all stabilized to the same extent.

In a comparable experiment at the permissive temperature of 30°C (Fig. 1A), the estimated functional half-life for total T4 mRNA was 10–12 min in both the *rne*⁺ and *rne*⁻ strains, confirming that the stabilization observed at 43°C in the *rne*⁻ strain correlates with the inactivation of RNase E. In addition, the functional half-life of total T4 mRNA in a wild-type host strain at 37°C was 5 min (not shown), which is similar to that in the *rne*⁺ strain at 43°C, suggesting that other enzymes involved in T4 mRNA decay are functioning normally at 43°C.

To exclude the possibility that transcription initiation had not been blocked to equivalent extents by the rifampicin treatment in the mutant and wild-type strains, we measured the inhibition by rifampicin of the incorporation of [³H]uracil into trichloroacetic acid (TCA)-precipitable RNA in the two strains. In the phage-infected *rne*⁺ and *rne*⁻ cells (Fig. 2), in the absence of rifampicin, the

rate of incorporation of [³H]uracil into RNA falls initially, as observed previously (Young et al. 1980), and remains constant for the rest of the infection. After the addition of rifampicin, there is a rapid reduction in the incorporation of label, and the extent of this reduction is the same in both strains. The equivalent degree of inhibition of RNA synthesis in the two strains suggests that differences in rifampicin sensitivity are not the cause of the differences in protein synthesis patterns observed in Figure 1B.

The effect of RNase E on the chemical stability of T4 mRNAs

To quantitate the effect of the *rne* mutation on the chemical stability of newly synthesized T4 mRNA, host cells at 43°C were infected with T4 and pulse-labeled with [³H]uridine. The pulse was terminated by the addition of rifampicin and excess cold uridine, and at subsequent times, RNA was isolated from the cells and hybridized to filter-bound T4 DNA (Fig. 3). As estimated from the slope of the initial, linear portion of the curve for the *rne*⁺ strain, the half-life of the T4 mRNAs was 4 min. This is similar to the chemical half-life of 3.5 min estimated by Greene and Korn (1967), and 3.5–4 min estimated by Friesen (1969) for T4 mRNA at 37°C. The nonlinear portion of the curve at the later times after rifampicin treatment is likely to be due to classes of

Figure 2. $[^3\text{H}]$ uracil incorporation in phage-infected rne^+ and rne^- *E. coli* at 43°C . At 10 min after T4 infection of rne^+ ($\square$) and rne^- ($\circ$) cells, the cultures were split into two aliquots: rifampicin ($100\ \mu\text{g}/\text{ml}$) was added to one (dotted line, solid symbols), and the other was untreated (solid line, open symbols). The $[^3\text{H}]$ uracil incorporated is plotted at the midpoint of each 2-min pulse-labeling, (e.g., 5 min represents labeling begun at 4 min and terminated at 6 min by the addition of ice-cold TCA). The mean counts per minute from duplicate TCA precipitations are plotted on a logarithmic scale against the time at which the cells were pulse-labeled.

RNAs that are more stable. In the rne^- strain, the estimated half-life of T4 mRNA was 15 min. This represents an approximately fourfold increase in the chemical stability of a large fraction of the newly synthesized T4 mRNAs, which is comparable to the sixfold increase in functional stability observed above. However, the 15-min chemical half-life is significantly less than the 42-min functional half-life estimated above for T4 transcripts in the rne^- strain.

The estimates of functional and chemical message stability need not agree precisely because the measurement of average functional stability depends on the efficiency of translation of each message in the population as well as the degree to which the rne^- mutation affects their stability. For instance, mRNAs that are highly stabilized in the rne^- strain and that also have high translational yields would contribute disproportionately to the estimate of functional half-life. In addition, the two measurements are not strictly comparable. Although the chemical measurement determines the rate of decay of messages synthesized in the pulse prior to the addition of rifampicin and excess cold uridine, the functional measurement follows the decay of all the messages in the infected cell. Because the inhibition of transcription upon rifampicin addition is not immediate (see Fig. 2), the functional measurement will be affected by the residual RNA synthesis following rifampicin addition,

whereas the chemical measurement is less sensitive to this effect because of the cold uridine chase.

To determine whether this general effect of stabilization of T4 mRNAs was uniform for all mRNAs, we examined the effects of the rne mutation on the chemical stability of specific T4 messages. Figure 4 shows Northern blots of total RNA isolated from phage-infected rne^+ and rne^- strains at various times after rifampicin treatment. A gene 43-specific probe hybridizes to a transcript of 2.9 kb in size. This is the expected size for a transcript encoding only the gene 43 product and could be either the monocistronic or the processed polycistronic gene 43 transcript (Guild et al. 1988). The approximate half-life of this transcript in the wild-type host is 4 min, whereas it is 11 min in the mutant strain. Once decay is initiated, the message presumably decays rapidly because discrete intermediates are not detected. The level of the 2.9-kb transcript at the 8-min time point after rifampicin addition is higher in the mutant than in the wild-type strain (see below). Figure 4B shows an example of a more complex but more typical T4 transcript pattern. The gene 32 RNA probe hybridizes to polycistronic and monocistronic transcripts, as well as their processed products and decay intermediates (Carpousis et al. 1989). Although it appears that most of the species are significantly more stable in the rne^- strain, the com-

Figure 3. The chemical decay of pulse-labeled phage transcripts from T4-infected rne^+ and rne^- *E. coli* at 43°C . The rne^+ ($\square$) and rne^- ($\bullet$) cells were infected for 8.5 min, pulsed with $[^3\text{H}]$ uridine for 3 min, and rifampicin and excess cold uridine were added for 3 min. RNA was isolated at the end of the chase (time 0) and at the subsequent times indicated. RNA ($5\ \mu\text{g}$) from each time point was hybridized in duplicate to filter-bound T4 DNA. The counts per minute plotted on the logarithmic scale are the mean values from the duplicate filters after subtraction of the background counts per minute for calf-thymus DNA filters. This background was ~ 20 cpm. The lines were drawn from a linear regression analysis of the data.

Figure 4. Northern blot analysis of RNA isolated from phage-infected *rne*⁺ and *rne*⁻ host cells. Cells were infected with T4 at 43°C for 6 min before the addition of rifampicin (time 0). Total RNA was isolated at the times shown, and the same RNA preparations were used in A and B. Equal amounts of RNA (6 µg) were loaded in each lane of the 1% agarose/6% formaldehyde gels and, after electrophoresis, were transferred to nylon membranes by electroblotting (A) or capillary transfer (B). The probes used in A and B, respectively, were ³²P-labeled gene 43 plasmid DNA (Table 1) and gene 32 RNA from plasmid pTAK64 (see Materials and methods). (A) rRNA size markers are indicated at left. (B) Lane M represents RNA size markers prepared in vitro; the sizes (kb) of these markers and rRNA markers are indicated at right. The 5.4-kb and 0.4-kb in vitro transcripts are only visible on a longer exposure of the blot than the one shown. The identities of some of the gene 32 transcripts in B are indicated at left (see Carpousis et al. 1989). The numbers represent the locations of the 5' ends of either polycistronic (-2874 and -2020) or monocistronic (-200 and -71) transcripts with respect to the first nucleotide of the gene 32 initiation codon at +1. A1 and A2 may be decay intermediates of the polycistronic transcripts. (B) The *rne*⁻ rifampicin 0 time point was diluted as shown (1/2, 1/4, and 1/8) for quantitation purposes. In both A and B, the blots were exposed to film at -70°C with intensifying screens. The RNA samples were from the same infection in which the proteins shown in Fig. 1B were labeled.

plexity of the pattern makes quantitative estimates of the half-lives of individual species difficult. The level of gene 32 transcripts is approximately twofold higher in the *rne*⁻ strain at the 0 time point (as estimated by comparison to densitometric scans of the diluted samples in Fig. 4B). Multiple transcripts were also observed in Northern blots when the gene 39 and *rIIA/rIIB* probes were used, and, again, the levels and stability of these various transcripts were higher in the *rne*⁻ strain (not shown). The observed increases in transcript levels in the *rne*⁻ strain could reflect either increased message stability or a delay in the infection of this strain, as discussed below.

Because of the difficulties in estimating half-lives of messages that are represented by more than a single species by Northern blot analysis, the effect of the *rne* mutation on the chemical stability of specific, newly synthesized transcripts was examined by hybridization to filter-bound plasmid DNA. In this method, only the stabilities of transcripts that specifically hybridize to the filter-bound DNA are analyzed because pancreatic RNase treatment removes the portions of transcripts that extend beyond the filter-bound probes. Figure 5A shows the results of hybridizing the same RNA preparations used for the experiment shown in Figure 3 to filter-bound gene-specific probes (Table 1). The approximate half-lives of the transcripts estimated from the slopes of the curves are shown. There is a 24-fold stabilization of gene 37 transcripts and a similar 19-fold stabilization of gene 23 transcripts in the *rne*⁻ strain. The gene 39 transcripts are stabilized almost 6-fold, and the smallest effect was observed with the gene 43 transcript that was stabilized 2.5-fold in the mutant strain. This small but significant effect on the gene 43 transcript agrees with the estimated effect of the mutation on the

stability of this transcript from the Northern blot analysis (Fig. 4A). The degree of chemical stabilization of newly synthesized transcripts in the RNase E-deficient strain therefore varies with different mRNAs.

To determine whether the effect of the *rne* mutation depends on the stage of phage development (i.e., the length of time after infection at which the pulse chase was carried out), RNA samples labeled at an earlier time in infection than those in Figure 5A were used for the hybridizations shown in Figure 5B. With a gene 32 probe, there is a 28-fold stabilization of the mRNAs in the *rne*⁻ strain compared with the *rne*⁺ strain. The *rIIA/rIIB* transcripts are stabilized almost 4-fold in the *rne*⁻ strain, and a similar effect is observed for gene 39 transcripts. The gene 43 transcript is only stabilized by 1.5-fold in the mutant strain. For the gene 39 and gene 43 probes, the extent of the stabilization is similar to that observed when the RNA was labeled at a later time in infection (cf. Fig. 5A). At least for these species, the stage of the phage infection does not appear to be a variable in the chemical stabilization of T4 mRNAs in the *rne* mutant strain.

The estimated half-lives of between 3 and 4 min for specific T4 messages in the *rne*⁺ strain (Fig. 5) are very similar to the estimated chemical half-life of 4 min for total T4 mRNA (Fig. 3). The exception was the gene 43 mRNA, which had a half-life of 6–6.5 min in the *rne*⁺ strain, and it is interesting to note that the stability of this mRNA was also the least affected by the *rne* mutation. The chemical half-life of 4.5 min for the gene 32 mRNA in the *rne*⁺ strain is surprisingly short. The functional half-life of gene 32 mRNA had been estimated previously as 15–30 min at 30°C (Russell et al. 1976). However, we have found that the chemical stability of gene 32 mRNA is highly temperature-dependent (A.J.).

Figure 5. The chemical decay of specific pulse-labeled transcripts in T4-infected rne^+ and rne^- *E. coli* at 43°C. The experimental conditions are the same as in the legend to Fig. 3, except that in B, the labeling was begun at 3.5 min after infection and the chase was added at 6.5 min. The specific filter-bound T4 plasmid DNAs (Table 1) were hybridized with [3 H]uridine-labeled RNA from the infected rne^+ (□) and rne^- (●) cells. pBR322 DNA filters were included in each hybridization, and this background (~20–70 cpm) was subtracted. Samples with <20 cpm after correction for background were not included.

Carpousis, unpubl.): The message is considerably less stable at 42°C than at 30°C.

It is evident from Figure 5 that there are differences in the initial levels of newly synthesized transcripts in the two host strains, depending on the transcripts examined. The observed differences in levels are consistent with a delay in the infection of the rne^- cells or a reduced rate of phage development in this host. The stage of phage development at which transcript levels are measured is important because transcription of "early" genes is turned off as "late" gene transcription becomes predominant. In Figure 5A, a possible explanation for the higher starting levels of the late transcripts (genes 37 and 23) in the rne^+ strain is that the infection is more advanced than in the rne^- strain, and for this reason as well, the early transcripts (genes 39 and 43) are already decreasing in the rne^+ strain compared with the rne^- strain. Even at the earlier labeling time used in Figure 5B, the early transcription (genes *rIIA/rIIB*, 39 and 43) is already being reduced in the rne^+ strain. Gene 32 transcripts are synthesized throughout infection (Belin et al. 1987), but the exact contribution of the various early and late transcripts to the overall transcription rates here is not known. As described above, many transcripts showed increased starting levels in the rne^- strain by Northern blot analysis. For the early transcripts, this effect could

again be explained by the delay in the infection of the rne^- strain, but it could also be due, in part, to the increased stabilities of the messages. However, there may not be a simple relationship between mRNA stability and mRNA levels in a T4 infection, because it is unlikely that a steady-state balance between synthesis and decay is achieved during the infectious cycle. Regardless of differences in the stage of infection, the results shown in Figure 5 clearly demonstrate that there are significant differences in the chemical stabilities of specific T4 mRNAs in the rne^+ and rne^- cells.

The number of phage progeny produced per infected bacterium is significantly reduced in the rne^- strain

The synthesis of T4 proteins is slightly delayed in the rne^- host (Mudd et al. 1988), and densitometric scans of gene 34, 7, and 37 tail fiber structural proteins indicate that the rate of synthesis of these proteins is reduced 1.3-fold compared to that in the rne^+ strain (not shown). We were therefore interested in determining the effect of the *rne* mutation on the production of viable progeny phage. In a typical wild-type phage infection at 37°C, ~200 phage progeny per bacterium can be released upon spontaneous lysis of the infected cells (Guttman and Kutter 1983). Figure 6 shows that in a wild-type infec-

Table 1. *Plasmids used as DNA probes*

Plasmid name	Insert site	Insert size (kb)	Known translated regions of inserts	References for DNA sequence
pVH721 ^a (gene 23)	<i>Bgl</i> III– <i>Eco</i> RI	2.4	2.2 kb of gene 23 (head subunit), carboxy-terminal 0.26 kb of gene 22 (head assembly core)	Parker et al. (1984)
pVH653 ^b (gene 37)	<i>Eco</i> RI	3.1	2.6 kb internal to gene 37 (tail subunit) and amino-terminal 0.48 kb of gene 38 (tail assembly)	Oliver and Crowther (1981)
pVH626 ^b (gene 39)	<i>Eco</i> RI	3.9	all of gene 39 (DNA topoisomerase; 1.58 kb)	Huang (1986)
pVH622 ^b (gene 43)	<i>Eco</i> RI	1.5	internal to gene 43 only	Spicer et al. (1988)
pAC67 ^c (gene 32)	<i>Cla</i> I– <i>Hind</i> III	1.1	all of gene 32 (helix-destabilizing protein) (1.1 kb), carboxyl terminus of orf C (0.08 kb)	Krisch and Allet (1982)
pVH632 ^{a,d} (<i>r</i> IIA/B)	<i>Hind</i> III	0.9	carboxy-terminal 0.42 kb of <i>r</i> IIA, amino-terminal 0.41 kb of <i>r</i> IIB (membrane proteins)	Pribnow et al. (1981)

The vector used for all of the plasmid constructs was pBR322. The plasmid pSP64TAK, used as the template for the preparation of the cRNA probe, contains the gene 32 leader region (from –343 to +3) cloned in the SP6 vector (Belin et al. 1987).

^aG. van Houwe (pers. comm.).

^bYoung et al. (1980).

^cThis report.

^dSelzer et al. (1978).

tion of the *mne*⁺ strain at 43°C, the number of viable progeny phage produced per bacterium is ~250. However, in the *mne*[–] strain, there several minutes of delay, a slower rate of progeny production, and a reduction in final phage yield of ~50%. In a comparable experiment at 30°C, there were no significant differences in the number or timing of phage progeny produced (not shown). The observed differences at 43°C therefore correlate with the inactivation of RNase E.

Discussion

Inactivation of host RNase E in T4-infected cells results in marked stabilization of many T4 mRNAs, both chemically and functionally. This suggests that the decay of these messages is mediated by RNase E. The RNase E deficiency also resulted in a significant reduction in the number of phage progeny produced in each infected bacterium. This result was expected because we observed reduced levels of synthesis of some late proteins, which are required for assembling new phage, in the *mne* mutant strain. The deleterious effect of the *mne* mutation on the production of progeny phage may be linked to the observed effects on mRNA stability. For example, reduced degradation of early T4 messages could interfere with late gene expression. Even small alterations in the level of expression of certain T4 genes could have a significant effect on the production of viable phage.

The simplest interpretation of our results is that RNase E acts directly in the degradation of T4 mRNAs. Nevertheless, it is possible that the effect is indirect.

Some RNA species that accumulate in vivo in the RNase E-deficient strain are apparently not processed in vitro by partially purified RNase E (Pragai and Apirion 1982; Gurevitz et al. 1983). Although there are several possible explanations of these results, Apirion and collaborators prefer models in which RNase E is part of a processing enzyme complex and the RNase E mutation leads to disruption of the efficient function of other nucleases in the complex. Regardless of whether RNase E acts directly or indirectly, this is the first demonstration of a role for RNase E in mRNA degradation, and the first identified endonuclease involved in mRNA functional decay.

In our experiments, the RNase E temperature-sensitive enzyme was inactivated by incubating the bacteria at 43°C for 10 min prior to infection. It is unlikely, however, that the increased stability of the T4 phage mRNA in these cells was due to an indirect effect of RNase E inactivation on *E. coli* gene expression. Infection of the *mne* temperature-sensitive host at 30°C results in the shutoff of host gene expression, yet subsequent inactivation of RNase E by a shift to 43°C still leads to stabilization of T4 mRNAs (not shown). Because the effect of RNase E inactivation on T4 message stability was apparent within a few minutes of the shift to the nonpermissive temperature, the activity of any host or phage mediator would have to be tightly coupled to that of RNase E.

Mechanisms of prokaryotic mRNA decay have been debated extensively (for reviews, see Kennell 1986; King et al. 1986; Brawerman 1987, 1989; Belasco and Higgins

Figure 6. Time course of the number of progeny phage produced per infected *rne*⁺ and *rne*⁻ host cell at 43°C. The *rne*⁺ (□) and *rne*⁻ (●) cells were infected with T4 for 3 min, at which time T4 antibody was added to neutralize unadsorbed phage. At 8 min after infection, the cells were diluted 1/1000 in M9S medium to prevent readsorption of any remaining free phage, and the numbers of infected bacteria were titered. At subsequent times after infection, the numbers of progeny phage produced were titered after lysing the cells prematurely with chloroform. Titers were average values from duplicate platings. The number of progeny phage per infected bacterium was calculated by dividing the phage titers at the subsequent time points by the number of infected bacteria at 8 min; this number was plotted against the time after infection.

1988). One model for mRNA decay proposes that endonucleases initiate decay by cleaving mRNAs, thereby creating 3' ends that are then processively degraded by 3' → 5' exonucleases. Several such events lead to the decay of entire messages (for a recent discussion of this model, see Belasco and Higgins 1988). The endonucleases that initiate this decay have not yet been identified. RNase E could directly affect T4 mRNA decay by cleaving the messages endonucleolytically, thereby creating sites for the entry of 3' → 5' exonucleases, which then processively degrade them. This interpretation is supported by our previous studies of RNase E-dependent mRNA processing at the -71 and -1340 sites in the gene 32 transcription unit (Mudd et al. 1988; Carpousis et al. 1989), in which the mRNA upstream of the cleavage site was rapidly degraded. In addition, we have recently identified an RNase E-dependent cleavage site

at position +831 within the gene 32 message (A.J. Carpousis, unpubl.), which is one of the messages that is highly stabilized in the RNase E-deficient strain. All three of the RNase E-dependent cleavage sites within the gene 32 transcription unit show some similarity in sequence at the cleavage (5'-Pu ↓ A ↓ U-U-3') and in the potential to form RNA secondary structure just downstream of the site.

The RNase E deficiency does not affect T4 message degradation uniformly. The principle decay pathway for messages such as the gene 37, 23, and 32 RNAs appears to be almost completely blocked in the RNase E-deficient strain. However, additional degradation pathways apparently exist because some T4 mRNAs, such as gene 43 and *rIIA/rIIB* RNAs, are still rapidly degraded in the RNase E-deficient strain. We have found that *E. coli* RNase III does not have a significant role in the decay of total T4 mRNAs because the functional stabilities of these mRNAs were very similar in isogenic RNase III⁺ and RNase III⁻ strains (not shown). Factors that could affect the degree to which mRNA decay is RNase E-dependent include the number of RNase E-sensitive cleavage sites, the degree of ribosomal loading, the susceptibility of the messages to decay mediated by other endonucleases, and the vulnerability of their 3' ends to exonuclease digestion (see Higgins et al. 1988).

We have presented evidence that an *E. coli* RNA processing enzyme is involved in the degradation of T4 mRNAs, and for many of the mRNAs examined, the activity of this enzyme appears to be the major determinant of their decay. These messages are apparently degraded by use of a pathway that differs from the pathway for degradation of bulk *E. coli* mRNA, although it is possible that the decay of some *E. coli* messages is RNase E-mediated. Future studies will be aimed at identifying additional RNase E-mediated cleavage sites and determining whether they have sequence and/or structure in common with the sites already mapped. It will be interesting to discover whether these cleavages are the limiting step in the degradation of the messages that are highly stabilized in RNase E-deficient *E. coli*.

Materials and methods

Growth of bacterial strains and infection conditions

The conditions used for growth and phage infections of the isogenic *rne*⁺ (N3433) and temperature-sensitive *rne*⁻ (N3431) strains (Goldblum and Apirion 1981) were as described (Mudd et al. 1988). Wild-type phage (T4D⁺ from the Geneva collection) was used for all infections. Bacteria were grown in M9S medium containing 0.2% casamino acids (Champe and Benzer 1962) at 30°C to 5 × 10⁷ cells/ml, centrifuged, and resuspended at 4 × 10⁸ cells/ml. After a 10-min preincubation at 43°C, the cells were infected with a multiplicity of 20 phage per bacterium. The percentage of surviving bacteria 2 min after infection was generally ~20% for the *rne*⁺ strain and 30% for the *rne*⁻ strain.

Pulse-labeling of proteins and RNA isolation

The methods for labeling proteins with ¹⁴C-labeled amino acids and for RNA isolation have been described (Mudd et al. 1988).

[³H]uracil labeling of RNA and TCA precipitation

One-milliliter samples of cultures were removed and pulse-labeled with 20 μ Ci [³H]uracil (NEN; 26 Ci/mmol) for 2 min at 43°C. Duplicate 0.25-ml samples of the labeled culture were then added to 2.5 ml of ice-cold 5% TCA, which lyses the bacteria. Carrier bacteria were mixed with the TCA samples, which were then filtered onto GF/C glass fiber filters (Whatman) and washed twice with 5% TCA and once with absolute ethanol. The filters were dried and counted in scintillant (toluene/PPO/POPOP).

[³H]uridine pulse-chase labeling of RNA and hybridization to DNA filters

Cells were labeled with 40 μ Ci/ml [5-³H]uridine (Amersham; 27 Ci/mmol) for 3 min, followed by a 3-min chase with a mix of excess cold uridine (100 μ g/ml) and rifampicin (200 μ g/ml). RNA was isolated from 2-ml samples at subsequent time points. The specific activity before rifampicin treatment was ~40,000–60,000 cpm/ μ g RNA.

DNA filters were prepared and hybridized, without formamide, by using 5 μ g of labeled RNA, as described (Young et al. 1980). The amounts of DNA bound per whole filter were 16 μ g of calf thymus, 32 μ g of whole T4, and 3.2 μ g of plasmid. One-quarter of each filter was used per hybridization. After digestion with pancreatic RNase and washes to remove nonhybridized RNA, the filter quarters were dried and counted in scintillant. The number of counts per minute that hybridized depended on the concentration of labeled RNA used, showing that the T4 DNA was in excess to the mRNAs (not shown).

Northern blot analysis of RNA

RNA samples were separated by 1% agarose gel electrophoresis in the presence of formamide/formaldehyde, transferred to GeneScreen nylon membranes (NEN), stained with methylene blue to verify the quality of the transfer, and hybridized with DNA or RNA probes following the procedure of Khandjian (1986). The DNA probe was prepared by nick-translation of plasmid DNA (Rigby et al. 1977), and the RNA probe was prepared in vitro from the plasmid template pSP64TAK, which contains the gene 32 leader region (from –343 to +3) cloned in the SP6 vector (Belin et al. 1987). The endogenous *E. coli* 23S and 16S rRNA, as well as labeled RNAs of known size prepared in vitro using the T7 system, were used as size standards.

Half-life estimations from autoradiographs

Autoradiographs were scanned with a GS300 scanning densitometer [Hoefer Scientific Instruments (HSI)], and the appropriate peak areas, or total peak areas from scans of whole lanes, were determined by integration with the HSI program GS-370. Half-lives were estimated from semilog plots of peak areas versus the time after rifampicin addition.

Estimation of the number of phage progeny released per infected bacterium

For experimental details on the estimation of the number of phage progeny released, see the legend to Figure 6. Equal volumes of bacteria and phage, both at 2×10^8 /ml, were mixed at 43°C. Three minutes after the T4 antibody treatment, the titer of unabsorbed phage was reduced 100-fold. The number of infected bacteria immediately after dilution was $\sim 2 \times 10^4$ /ml. Phage and infected bacteria were titered on the *E. coli* strain S/6 by using standard techniques (Steinberg and Edgar 1962).

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