RNA Cleavage Linked With Ribosomal Action

Haruyo Yamanishi1 and Tetsuro Yonesaki2

Department of Biology, Graduate School of Science, Osaka University, Osaka 560-0043, Japan

Manuscript received February 24, 2005
Accepted for publication July 11, 2005

ABSTRACT

Ribonuclease LS in Escherichia coli is a potential antagonist of bacteriophage T4. When T4 dmd is mutated, this RNase efficiently cleaves T4 mRNAs and leads to the silencing of late genes, thus blocking T4 growth. We previously found that, when two consecutive ochre codons were placed in the open reading frame of T4 soc, RNase LS cleaved soc mRNA at a specific site downstream of the ochre codons. Here, we demonstrate that RNase LS cleaves soc RNA at the same site even when only a single ochre codon is present or is replaced with either an amber or an opal codon. On the other hand, disruption of the Shine-Dalgarno sequence, a ribosome-binding site required for the initiation of translation, eliminates the cleavage. These results strongly suggest that RNase LS cleaves in a manner dependent on translation termination. Consistent with this suggestion, the cleavage dependency on an amber codon was considerably reduced in the presence of amber-codon-suppressing tRNA. Indeed, two other cleavages that depend on translation of the region containing the target sites occurred farther downstream. Additional analysis suggests that an interaction of the ribosome with a stop codon might affect the site of cleavage by RNase LS in an mRNA molecule. This effect of the ribosome could reflect remodeling of the high-order structure of the mRNA molecule.

1 Present address: H & BC Research Center, Shiseido, Yokohama-shi, Kanagawa 236-8643, Japan.
2 Corresponding author: Department of Biology, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka-shi, Osaka 560-0043, Japan. E-mail: yonesaki@bio.sci.osaka-u.ac.jp

RIBONUCLEASE LS (late-gene silencing in bacteriophage T4) plays a role in Escherichia coli RNA metabolism (Otsuka and Yonesaki 2005), although its effect seems modest in comparison to that of RNase E (Kushner 2002). Remarkably, RNase LS is the first example, to our knowledge, of an RNase that antagonizes bacteriophage reproduction (see below). RNase LS preferentially cleaves RNA 3’ to pyrimidines, but exceptions are observed (Kai et al. 1996; Kai and Yonesaki 2002). Interestingly, this RNase triggers RNA degradation more efficiently when the target RNA is translatable than when it is untranslatable. Indeed, some cleavages by RNase LS are introduced only when the target is translatable, while others are independent of translation (Kai and Yonesaki 2002). Thus, the mechanism of RNA cleavage by RNase LS seems complicated and remains to be elucidated.

The dmd (discrimination of messages for degradation) gene of bacteriophage T4 is expressed immediately after infection (Kai et al. 1998) and is required for the regulation of mRNA stability in a stage-dependent manner during infection (Ueno and Yonesaki 2001). When dmd is defective, late genes are globally silenced because of rapid degradation of mRNAs by RNase LS (Kai et al. 1996). Thus, dmd is required for overcoming the RNase LS activity. In fact, in the in vitro cleavage reaction (Otsuka and Yonesaki 2005), Dmd inhibits the activity of RNase LS (Y. Otsuka and T. Yonesaki, unpublished results). During characterization of RNase LS activity after infection of a T4 dmd mutant, we found cleavages in T4 soc mRNA when two successive ochre codons were introduced into the open reading frame (Kai and Yonesaki 2002). Since the cleavages occurred proximally downstream of the premature termination codons, these cleavages could depend on translation termination. Here, we present a line of evidence indicating that the cleavages are tightly linked with translation termination and present insights into the selection of cleavage sites by RNase LS.

MATERIALS AND METHODS

Bacteria and phages, growth, and infection conditions: We used E. coli K12 MH1 (supF hsdR ΔlacX74 rpsL) as a non-permissive host and CR63 (supD) as a permissive host. TY0482 (MH1 rnlA2) was isolated previously (Otsuka et al. 2003; Otsuka and Yonesaki 2005). T4 amSF16 contains an amber mutation in dmd (Kai et al. 1996; Ueno and Yonesaki 2001) and T4 dK phage lacks dmd (Kai et al. 1998). T4 amSF16 soc-nel was described previously (Kai and Yonesaki 2002). Other T4 soc mutants were constructed in this study (see below). For the preparation of total RNA from T4-infected cells, MH1 cells were grown to a density of 4 × 10⁸ cells/ml in M9 minimal medium supplemented with 0.3% casamino acids, 1 μg/ml thiamine, and 20 μg/ml tryptophan and were infected at 30° with T4 at a multiplicity of 10.

RNA techniques: Isolation of total RNA from T4-infected cells, primer-extension analysis of soc RNA, and analysis of cDNA products with polyclonal antibody gel electrophoresis were performed as described previously (Kai and Yonesaki 2002).

Construction of plasmid and phage soc mutants: Plasmids pTK40, pTK90, and pTK70, carrying the soc, soc-nls, and soc-nel
alleles, respectively, in a vector pBluescript II (Stratagene, La Jolla, CA) were described previously (Kai and Yonesaki 2002; Otsuka et al. 2003). Plasmids containing other soc alleles except for soc-nel+13b were constructed as follows. The soc alleles in pTK40, pTK60, and pTK70 are flanked with KS and SK primer sequences. A DNA fragment containing the 3’ or 5’ region of soc flanking the desired mutation was prepared by PCR with a primer for base substitutions and KS or SK primers. An appropriate template was chosen from the above three plasmids. Next, the amplified fragment was extended to contain the whole soc allele by PCR with another primer from KS and SK, using the same plasmid as a template. Each soc allele was cloned into an insertion/substitution vector pLS (Sellick et al. 1988) after digestion with BamHI and EcoRI. Table 1 lists the constructed soc alleles, the primers used for the first PCR, and the templates. The primer sequences complementary to pBluescript II are KS, 5’-CGCGTACCTGATATGTAACATG-3’ and SK, 5’-CCCGAGCTCTAGAACTAGTG-3’. To construct soc allele by PCR be-

<table>
<thead>
<tr>
<th>soc allele</th>
<th>First PCR</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>soc-hlf/ochre</td>
<td>KS, 5’-AATGAGGCGCCAGATTATTGTAGAAGTCGAG</td>
<td>pTK40</td>
</tr>
<tr>
<td>soc-nel/amber</td>
<td>SK, 5’-ACCGGAGTGACCTATGTAACATG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel/ochre</td>
<td>KS, 5’-TAACCCGGAGATCCTTAAAATCAG</td>
<td>pTK40</td>
</tr>
<tr>
<td>soc-nel/opal</td>
<td>SK, 5’-ACCGGAGTGACCTATGTAACATG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel/n1</td>
<td>SK, 5’-TTGATATTTAGATAACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel/n2</td>
<td>SK, 5’-TCTGCTCAAAATCTTTATTAGAT</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel1</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel2</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel3</td>
<td>SK, 5’-TACCCGGAGGCTTATTACATG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel4</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel5</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel6</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
<tr>
<td>soc-nel7</td>
<td>SK, 5’-ATGTTTTGATACCCGGAG</td>
<td>pTK70</td>
</tr>
</tbody>
</table>

immediately after the initiation codon of soc mRNA (Figure 1). With this allele, a prominent cleavage at nucleotide (nt) position 53, relative to the 5’-end of the transcript, was detected when dmd was defective but not when it was normal. Similarly, in soc-hlf (hadf), where two tandem ochre codons were placed in the middle of the open reading frame of soc, a cleavage at nt 153 was detected upon infection of a dmd mutant (Kai and Yonesaki 2002). Since these cleavages occurred concomitantly with the introduction of ochre codons, they could be related to translation termination. Alternatively, an alteration of the six-base sequence to generate two ochre codons might generate a target sequence for RNase L. To discriminate between these possibilities, we constructed several soc alleles derived from soc-nel (Figure 1) and investigated their effects on the cleavage. The cleavage in soc-nel occurred in the middle of 5’-AAUAUCAAAA and the site was named NE (for a cleavage specific to soc-nel) (Kai and Yonesaki 2002). soc-nel/ochre carries only the first ochre codon in soc-nel and thereby reduces base alterations to half of those of soc-nel. The effect of a single ochre codon on cleavage was examined by primer extension (Figure 2). In Figure 2, the bands marked by T (for truncation by RNase E; see below) as well as by NE were detected. Previous work had revealed that band T was observed regardless of whether soc was wild type or soc-nel or whether dmd was normal or defective (Kai and Yonesaki 2002). More recently, we showed that this band corresponded to 5’-truncated transcripts produced by RNase E cleavage (Otsuka et al. 2003). In the present experiment, cleavage at NE was detected in soc-nel/ochre as well as in soc-nel/RNA (lane 7). Similar to soc-nel, cleavage at NE occurred in soc-nel/ochre when dmd was defective (lane 5) but not when it was normal (lane 6). We also observed that a single ochre codon was sufficient for cleavage at nt 153 in soc-hlf when dmd was defective (data not shown).

The above results prompted us to investigate whether or not other nonsense codons can induce the cleavage.
In two additional alleles, *soc-nel/amber* and *soc-nel/opal*, the ochre codon (UAA) in *soc-nel/ochre* was replaced with an amber (UAG) or an opal (UGA) codon, respectively (Figure 1). As shown in Figure 2, primer extension with RNAs of these alleles showed that cleavage was induced regardless of the type of nonsense codon (lanes 1 and 3) but only in a *dmd* mutant background (compare with lanes 2 and 4). *E. coli rnlA* is essential for the activity of RNase LS both in vivo and in vitro (Otsuka et al. 2003; Otsuka and Yonesaki 2005). When a *dmd* mutant infected cells carrying a nonsense mutation in *rnlA*, the cleavage at NE was not detected for *soc-nel*, *soc-nel/amber*, *soc-nel/ochre*, or *soc-nel/opal* (Otsuka et al. 2003; data not shown), confirming that the cleavage at NE was attributable to RNase LS activity. Together, these results suggest that the cleavage at NE by RNase LS depends on a termination codon and is independent of a specific sequence generated by base alterations.

**Requirement for a SD sequence:** If the cleavage at NE depends on translation termination, then initiation of translation must be a prerequisite for cleavage. To examine this prediction, we eliminated the SD sequence in *soc-nel*. Because the SD sequence is required for ribosome binding, initiation of translation should be blocked in its absence. *soc-sls* (SD sequence less) has three base changes in the SD sequence from 5'−AAGGAG to 5'−AAGCTT (Kai and Yonesaki 2002), rendering this allele untranslatable. In fact, T4 phage carrying *soc-sls* cannot synthesize the Soc protein (data not shown). We constructed *soc-nel/sls* (Figure 1) by introducing the same base changes into the SD sequence in *soc-nel*. As shown in Figure 3, the cleavage at NE was observed for *soc-nel* (lane 1) but not for either *soc-nel/sls* (lane 3) or *soc-sls* (lane 2). This result clearly demonstrates that the SD sequence is essential for cleavage.

**Effects of amber-codon-suppressing tRNA on cleavage:** Plasmid pBSPLO_c255_carries supF encoding an amber-codon-suppressing tRNA (Selick et al. 1988). Because the supF tRNA can mediate the insertion of a tyrosine at an amber codon, translation does not terminate. Therefore, use of supF tRNA allows us to further investigate the correlation between cleavage at NE and translation termination. For this purpose, we could not use amSF16 phage as a dmd mutant, because supF tRNA
suppresses the mutation. Instead, we used T4 dK phage, in which the dmd gene is deleted (Kai et al. 1998). In Figure 4, the band marked by F corresponds to full-length soc transcript. A band marked by TU (translation uncoupled) corresponds to a dmd mutant-specific cleavage by RNase LS (Otsuka and Yonesaki 2005) at nt 207 and this cleavage is independent of translation (Kai and Yonesaki 2002). When dK-infected cells did not carry supF tRNA, the cleavage at NE in soc-nel/amber (lane 3) was detected as prominently as in soc-nel (lane 4). However, the cleavage in soc-nel/amber was considerably reduced when dK-infected cells carried supF tRNA from pBSPLO/C255 (lane 2). In this case, other bands marked by TC1 (translation-coupled) and TC2 corresponding to the cleavages by RNase LS (Otsuka and Yonesaki 2005) at nt 172 and 185 were also discernible. Remarkably, these cleavages were observed for wild-type soc (lane 1) but not for soc-nel/amber in the absence of the supF tRNA (lane 3). Therefore, the presence of supF tRNA exhibited opposite effects on the cleavage at NE and on the cleavages at TC1 and TC2 in soc-nel/amber, suggesting a tight linkage between these cleavages and ribosomal action (see discussion).

**Selection of cleavage sites:** We have detected >30 cleavage sites of RNase LS in mRNAs of gene 23 (Kai et al. 1996), soc (Kai and Yonesaki 2002), and uisY (T. Kaneko and T. Yonesaki, unpublished results). Collective information about the cleavage sites suggests no preference for a specific base sequence and instead reveals that RNase LS preferentially cleaves 3' to a pyrimidine. However, the fact that only a few pyrimidines are targets for cleavage suggests that this RNase selects a target depending on some condition in addition to the base preference. To investigate the basis for the selection of the NE site, we compared cleavage between soc-nel and its derivatives. First, we constructed two alleles, soc-nel/ns1 (no stem; see below) and soc-nel/ns2 (Figure 1), to examine the effects of base changes in

**Figure 2.—Effects of termination codons on the cleavage at NE.** MH1 cells were grown to 4 × 10⁶ cells/ml and infected with T4 wild type (+) or dmd mutant (−) carrying soc-nel/amber, soc-nel/opal, soc-nel/ochre, or soc-nel as indicated, according to the method described in MATERIALS AND METHODS. Total RNAs were extracted at 20 min after infection and used as templates for primer extension as described (Kai and Yonesaki 2002). A set of sequence ladders for soc-nel obtained by dideoxy-sequencing method is presented.

**Figure 3.—Effect of the SD sequence on the cleavage at NE.** Primer extension analysis for total RNA from T4-infected cells was performed as in Figure 2. T4 phage used for infection was wild type (+) or dmd mutant (−) carrying soc-nel, soc-sl, or soc-sl/nel as indicated.

**Figure 4.—Effects of amber-codon-suppressing tRNA on the cleavage of soc RNA.** The T4 phage used for infection was dK, in which the dmd was deleted (Kai et al. 1998). T4 dK carrying a wild-type soc gene, soc-nel/amber, or soc-nel was used for infection of MH1 cells with (+) or without (−) pBSPLO−. Primer extension analysis was performed as in Figure 2.
the vicinity of the NE site on cleavage at NE. As shown in Figure 5, the base changes 5’ to the NE site in soc-nel/ns1 did not affect the cleavage (lane 3). In contrast, the cleavage was much reduced in soc-nel/ns2 (lane 4), suggesting that a sequence in the vicinity of a cleavage site is important for selection of a target.

The base changes introduced into soc-nel/ns1 and soc-nel/ns2 were originally aimed to disrupt a putative stem formed by AUGUU (nt 44–48) and AACAU (nt 57–61). We also constructed an allele that had all base changes in soc-nel/ns1 and soc-nel/ns2, in which a putative stem could be formed again. The cleavage at NE in this allele was reduced as much as that in soc-nel/ns2 (data not shown). This result, together with the above results, suggested that the putative stem, even if formed, was not involved in the selection of a target.

**Effect of deletion of the NE site on cleavage:** The cleavage at NE is 3’ of a uracil 25 nt downstream of the first ochre codon introduced into soc-nel and followed by four consecutive adenines (Figure 1). Because RNase LS prefers pyrimidines as noted above and the adenine cluster may disfavor a cleavage site for RNase LS, deletion of the 5’-AUC surrounding the NE site might eliminate cleavage in this region. The soc-nelΔ4 lacks this 5’-AUC and was examined for cleavage in a dmd mutant (Figure 6A). As expected, cleavage at a nt position corresponding to NE was reduced. Instead, two new cleavages appeared. These cleavages are 3’ of uracils 17 and 18 nt downstream of the first ochre codon and the sequences containing the sites are 5’-GUU and 5’-UUA. This result suggests that, when a primary cleavage site was deleted, secondary sites were selected in the vicinity of the primary site and also that a specific sequence did not define a target of RNA cleavage in a termination codon-dependent manner.

**Distance between a stop codon and a cleavage site:** A structural feature of the ribosome bound to mRNA suggested that the distance between a termination codon and a cleavage site might be important. To investigate this possibility, we constructed the soc-nel+3b, soc-nel+6b, and soc-nel+13b alleles. These alleles have insertions of 3, 6, and 13 bases downstream of the ochre codons, thereby extending the distance between the first ochre codon and the cleavage site of NE (Figure 1). Interestingly, new cleavages as well as the cleavage at NE were introduced in soc-nel+3b and soc-nel+6b RNAs (Figure 6B) but no cleavage other than the RNase E-dependent cleavage at site T and the RNase LS-dependent cleavage at site TU was observed in soc-nel+13b RNA (data not shown). The new cleavages are 3’ of uracils 18 and 21 nt downstream of the first ochre codon in soc-nel+3b and soc-nel+6b RNAs, respectively. We also constructed soc-nelΔ2 and soc-nelΔ3 in which the distance between first ochre codon and the NE site was shortened by 9 nt (see Figure 1 legend). In these alleles, the cleavage at NE did not take place and the cleavage at site T, so far attributed to RNase E, was strengthened (data not shown), suggesting that cleavage by RNase LS is dependent cleavage at site T and the RNase LS-dependent cleavage at site TU was observed in soc-nel+13b RNA (data not shown).

**Discussion:** The results suggest that the distance from a nonsense codon is not a strict determinant for the selection of a cleavage site by RNase LS (see discussion).
Recent studies have revealed that endonucleolytic cleavage is a fundamental aspect of nonsense-mediated mRNA decay in eukaryotes (Stevens et al. 2002; Gatfield and Tzaurralde 2004) and transl-translation in prokaryotes (Sunohara et al. 2004). In these cases, the presence of a stop codon somehow promotes endonucleolytic cleavages of mRNA. Similarly, RNase LS in E. coli was suggested to cleave mRNA, depending on a stop codon to accelerate mRNA degradation (Kai and Yonesaki 2002). In this study, we investigated the stop codon-dependent cleavage of T4 soc mRNA by RNase LS and found several characteristic features of the cleavage. First, any stop codon, amber, ochre, or opal, was effective for inducing cleavage at NE. Second, the initiation of translation was required for cleavage, which did not occur when the Shine-Dalgarno sequence was eliminated. Third, cleavage depending on an amber codon was significantly reduced by the presence of amber-codon-suppressing tRNA. All of these results strongly suggest an intimate linkage between cleavages by RNase LS and translation termination.

An amber-codon-suppressing tRNA helps a ribosome to continue translation beyond an amber codon. In this study, it was effective in reducing cleavage at NE. This result suggests that cessation of ribosomal movement would be a prerequisite for the action of RNase LS. Cleavages at TC1 and TC2 take place at sites when a region containing the cleavage sites is translatable (Kai and Yonesaki 2002). Indeed, when an amber codon was placed upstream of TC1 and TC2 sites in soc-amber, these cleavages did not occur. However, these cleavages did occur in the same allele when an amber-codon-suppressing tRNA was supplied. This observation strongly suggests that these cleavages depend on peptide-chain elongation. As mentioned above, cessation of ribosomal movement would facilitate translation-termination-dependent cleavage by RNase LS. Similarly, peptide-chain-elongation-dependent cleavage might require pausing of ribosomal movement during translation. A pause during peptide-chain elongation can be caused by certain nascent polypeptides (Nakatogawa and Ito 2002). A pause is also possible in the presence of rare codons and with mRNA secondary structures. This notion is strengthened by a recent observation: bla mRNA, whose degradation is stimulated by rare codons (Deana et al. 1996), is stabilized by the rnlA2 mutation (Otsuka and Yonesaki 2005). From these considerations, we propose that RNase LS cleaves mRNA when migration of a bound ribosome ceases or slows down.

The NE site is 23 nt downstream of the premature stop codon in soc-amber. The NE site could be cleaved even when the distance from a stop codon was extended by 3 or 6 nt. In these cases, an additional cleavage was detected 3′ of a U closer than NE to the stop codon. The distances of these cleavage sites from a stop codon are 18 nt in soc-amber and 21 nt in soc-nel+6b, and those of the NE site are 26 and 29 nt, respectively. Furthermore, cleavages occurred 3′ of uracils 17 and 18 nt downstream of the stop codon in soc-nel+4. These facts suggest that a potential site can be cleaved without a strict constraint of distance from a stop codon. However, the NE site was not cleaved when its distance from a stop codon was further extended to 36 nt by insertion of 13 nt in soc-amber+13b or shortened to 5 nt in soc-nel+1. A computer search with the Genetyx program (Software Development, Tokyo) revealed a stable stem-loop structure in the region between 16 and 29 nt downstream of the first ochre codon in soc-nel+4 and between 15 and 29 nt in soc-amber+13b (ΔG = −2.30 and −3.30 kcal/mol, respectively). This observation suggests that a single-stranded structure is necessary for cleavage.
Another feature is also required for the selection of a cleavage site. When a trinucleotide including the NE site was deleted, cleavages occurred 3' of uracils 17 and 18 nt downstream of the stop codon in soc-nelA-4. Although these sites are located at the same distances from a stop codon, their cleavages could not be observed in soc-nel. Moreover, although there are 6–8 pyrimidines within 17–29 nt downstream of a stop codon in the alleles used in this study, a few prominent cleavages other than that at NE could be detected within this range. These facts and the results from base substitutions in soc-nel/ns1 and soc-nel/ns2 may suggest that RNase LS can recognize sequences 3' of pyrimidines.

Translation-termination-dependent cleavage of mRNA indicates that a ribosome bound to mRNA helps an endoribonuclease to select a target during a termination process. As revealed in this study, the cleavages depend on a stop codon in soc-nel relatives were 3' to a pyrimidine. This feature is the same as that of other cleavages by RNase LS, suggesting that a basic property of this RNase is also conserved in translation-termination-dependent cleavage. In addition, the cleavage sites were located between 17 and 29 nt from a stop codon. Considering that cleavage in soc-hlf occurs 6 nt downstream of a stop codon (Kai and Onesaki 2002), RNase LS can select a site within a relatively wide range downstream of a stop codon. Accordingly, an interaction of the ribosome with a stop codon might affect the selectivity of RNase LS over a distance in an mRNA molecule. This effect could result from remodeling of high-order structure of the mRNA molecule. In fact, the mfold program (http://www.bioinfo.rpi.edu/applications/mfold/old/rna/form1.cgi) predicts that 80% of the region of soc RNA forms secondary structures. Binding of a ribosome may locally unfold such secondary structures, and stalling of a ribosome during termination may remodel the RNA structures and expose a target for RNase LS.

Linkage of translation and mRNA cleavage is reported for a bacterial toxin RelE. This protein can cleave mRNA positioned at the ribosomal A site between the second and third base of the codon, although it does not cleave free RNA (Pedersen et al. 2003). RelE is activated when cells are starved for amino acids, and mRNA cleavage by cooperation between RelE and the ribosome may not function when cells divide actively. Under normal growth conditions, it has been widely accepted that ribosomes compete with RNase for mRNA and that actively translating ribosomes protect mRNA from RNase attack (Joyce and Dreyfus 1998; Vyttvytyska et al. 2000; Baker and Mackie 2003). RNase LS is constitutively expressed in normally growing cells (Otsuka and Onesaki 2005). Therefore, the idea that this endonuclease cleaves mRNA in linkage with ribosomal action challenges the previous view.

We cordially thank John W. Drake at the National Institute of Environmental Health Sciences for invaluable help with the manuscript. We are grateful to anonymous reviewers for valuable suggestions and we thank the staff of the Radioisotope Research Center at Toyonaka, Osaka University, for the facilitation of our research, since all of our experiments using radioisotopes were carried out at the center. This work was supported in part by a grant from the program Grants-in-aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

LITERATURE CITED


Kai, T., and T. Onesaki, 2002 Multiple mechanisms for degradation of bacteriophage T4 soc mRNA. Genetics 160: 5–12.


Communicating editor: G. R. Smith

Ribosomal Action and RNA Cleavage 425