RNase E Cleaves at Multiple Sites in Bubble Regions of RNA I Stem Loops Yielding Products That Dissociate Differentially from the Enzyme*

Vladimir R. Kaberdin‡, Yen-Huei Chao, and Sue Lin-Chao§

From the Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China

Earlier work has shown that RNase E cleaves RNAI, the antisense repressor of replication of ColE1-type plasmids, producing pRNA I, whose further decay is mediated by the poly(A)-dependent activity of polynucleotide phosphorylase and other 3’ to 5’ exonucleases. Using a poly(A) polymerase-deficient strain to impede exonucleolytic decay, we show that RNAI is additionally cleaved by RNase E at multiple sites, generating a series of decay intermediates that are differentially retained by the RNA binding domain (RBD) of RNase E. Primer extension analysis of RNAI decay intermediates and RNase E, mapping of the cleavage products of RNAI generated in vitro by affinity-purified RNase E, showed that RNase E can cleave internucleotide bonds in the bubble regions of duplex RNA segments and in single-stranded regions. Chemical in situ probing of a complex formed between RNAI and the RBD indicates that binding to the RBD destabilizes RNAI secondary structure. Our results suggest a model in which a series of sequential RNase E-mediated cleavages occurring at multiple sites of RNAI, some of which may be made more accessible to RNase E by the destabilizing effects of its RBD, generate RNA fragments that are further degraded by poly(A)-dependent 3’ to 5’ exonucleases.

In the bacterium Escherichia coli, RNA decay requires the coordinated activity of endonucleases and exonucleases (for review, see Ref. 1) and is usually initiated by RNase E and endonucleolytic cleavage(s) followed by additional RNase III or additional exonuclease. It has been shown that degradation of RNAI is retarded in E. coli strains deficient for poly(A) polymerase and consequently slowing 3’ to 5’ exonucleolytic decay, we detected other rne-dependent decay intermediates of pBR322 RNA I in addition to the previously described RNase E cleavage product, pRNA I. We report the characterization of these intermediates, and present a model for possible role of functional domains of RNase E in rne-dependent decay of RNA I.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Growth Conditions—The bacterial hosts used for studying the RNA I stability were E. coli K12 isogenic strains N3433 (lacZ43, relA, spoT1, thi1) and N3431 (rne-3071), whose further decay is mediated by the poly(A)-dependent 3’-exonucleolytic activity of RNase I. Also, RNase I-5 shows degradation by poly(A)ase or also is subjected to endonucleolytic cleavages in vivo, has not been elucidated. In the experiments reported here, by inactivating poly(A) polymerase and consequently slowing 3’ to 5’ exonucleolytic decay, we detected other rne-dependent decay intermediates of pBR322 RNA I in addition to the previously described RNase E cleavage product, pRNA I. We report the characterization of these intermediates, and present a model for possible role of functional domains of RNase E in rne-dependent decay of RNA I.

*This work was supported by an intramural fund from the Academia Sinica and by National Science Council of Republic of China Grant NSC-84/85-2311-B-001-094/104/Y (to S.L.-C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†Received a postdoctoral fellowship from the National Science Council of the Republic of China.

‡To whom correspondence should be addressed. Tel.: 886-2-789-9218; Fax: 886-2-782-6085; E-mail: mbsue@gcavx.sinica.edu.tw.

1The abbreviations used are: PNPase, polynucleotide phosphorylase; GGGRNA I, RNA I containing three additional guanosine residues from 3’-end; LMW, low molecular weight; RBD, RNA binding domain; ts, temperature sensitive.

2 RNase E cleaves RNAI, the antisense repressor of replication of ColE1-type plasmids, producing pRNA I, whose further decay is mediated by the poly(A)-dependent activity of polynucleotide phosphorylase and other 3’ to 5’ exonucleases. Using a poly(A) polymerase-deficient strain to impede exonucleolytic decay, we show that RNAI is additionally cleaved by RNase E at multiple sites, generating a series of decay intermediates that are differentially retained by the RNA binding domain (RBD) of RNase E. Primer extension analysis of RNAI decay intermediates and RNase E, mapping of the cleavage products of RNAI generated in vitro by affinity-purified RNase E, showed that RNase E can cleave internucleotide bonds in the bubble regions of duplex RNA segments and in single-stranded regions. Chemical in situ probing of a complex formed between RNAI and the RBD indicates that binding to the RBD destabilizes RNAI secondary structure. Our results suggest a model in which a series of sequential RNase E-mediated cleavages occurring at multiple sites of RNAI, some of which may be made more accessible to RNase E by the destabilizing effects of its RBD, generate RNA fragments that are further degraded by poly(A)-dependent 3’ to 5’ exonucleases.

In the bacterium Escherichia coli, RNA decay requires the coordinated activity of endonucleases and exonucleases (for review, see Ref. 1) and is usually initiated by RNase E and endonucleolytic cleavage(s) followed by additional RNase III or RNase P cuts (2–4). Resulting fragments of digested RNA are further degraded from the 3’-end by polynucleotide phosphorylase (PNPase), RNase T1, mapping of the cleavage products of RNAI generated in vitro by affinity-purified RNase E, showed that RNase E can cleave internucleotide bonds in the bubble regions of duplex RNA segments and in single-stranded regions. Chemical in situ probing of a complex formed between RNAI and the RBD indicates that binding to the RBD destabilizes RNAI secondary structure. Our results suggest a model in which a series of sequential RNase E-mediated cleavages occurring at multiple sites of RNAI, some of which may be made more accessible to RNase E by the destabilizing effects of its RBD, generate RNA fragments that are further degraded by poly(A)-dependent 3’ to 5’ exonucleases.

In the bacterium Escherichia coli, RNA decay requires the coordinated activity of endonucleases and exonucleases (for review, see Ref. 1) and is usually initiated by RNase E and endonucleolytic cleavage(s) followed by additional RNase III or RNase P cuts (2–4). Resulting fragments of digested RNA are further degraded from the 3’-end by polynucleotide phosphorylase (PNPase), RNase T1, mapping of the cleavage products of RNAI generated in vitro by affinity-purified RNase E, showed that RNase E can cleave internucleotide bonds in the bubble regions of duplex RNA segments and in single-stranded regions. Chemical in situ probing of a complex formed between RNAI and the RBD indicates that binding to the RBD destabilizes RNAI secondary structure. Our results suggest a model in which a series of sequential RNase E-mediated cleavages occurring at multiple sites of RNAI, some of which may be made more accessible to RNase E by the destabilizing effects of its RBD, generate RNA fragments that are further degraded by poly(A)-dependent 3’ to 5’ exonucleases.

In the bacterium Escherichia coli, RNA decay requires the coordinated activity of endonucleases and exonucleases (for review, see Ref. 1) and is usually initiated by RNase E and endonucleolytic cleavage(s) followed by additional RNase III or RNase P cuts (2–4). Resulting fragments of digested RNA are further degraded from the 3’-end by polynucleotide phosphorylase (PNPase), RNase T1, mapping of the cleavage products of RNAI generated in vitro by affinity-purified RNase E, showed that RNase E can cleave internucleotide bonds in the bubble regions of duplex RNA segments and in single-stranded regions. Chemical in situ probing of a complex formed between RNAI and the RBD indicates that binding to the RBD destabilizes RNAI secondary structure. Our results suggest a model in which a series of sequential RNase E-mediated cleavages occurring at multiple sites of RNAI, some of which may be made more accessible to RNase E by the destabilizing effects of its RBD, generate RNA fragments that are further degraded by poly(A)-dependent 3’ to 5’ exonucleases.

Received for publication, January 19, 1996

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 271, No. 22, Issue of May 31, pp. 13103–13109, 1996
Printed in U.S.A.
Northern Blot Analysis—Cells were grown exponentially in M9/glucose medium at 33 °C or alternatively shifted to 43 °C (rne<sup>ts</sup> strain) for 30 min prior to RNA extraction. The total cellular RNA isolated as described (23) at various times after rifampicin (0.5 mg/ml) was added is logarithmic phase cultures (OD<sub>460</sub> 0.4) and was separated on an 8% polyacrylamide sequencing gel, and 1–5 pmol of it was uniformly labeled GGGRNA I were incubated separately with/without RNase E-dependent RNA Decay Pathway.

Characterization of 70-mer, 67-mer, 41-mer, and 29-mer by RNase T1 Cleavage of GGGRNA I in Vitro—The template for in vitro synthesis of GGGRNA I by T7 RNA polymerase was generated as polymerase chain reaction amplification of a corresponding segment of pCML108 plasmid encoding RNA I and using primers 5'-GGTACCCTTAATACGACTCACTATAGGGGACAGTATTTGGTATC-3' and 5'-ACAAAAAAACCCGCCTACCG (the sequence encoding a T7 promoter is in bold) and 5'-ACAAAAAAACCCGCCTACCC. Continuously labeled GGGRNA I was purified in an 8% polyacrylamide sequencing gel, and 1-5 pmol of it was incubated with 50 ng of full-length RNase E (affinity purified under denaturing conditions and refolded) in 50 mM Tris-HCl (pH 8.0), 5 mM magnesium chloride, 100 mM sodium chloride, 5% glycerol, 0.1% Triton X-100, 0.1 mM dithiothreitol. Both protein preparations were kindly provided by McDowall and Cohen (Stanford University). Aliquots (10 pmol) were removed from the reaction after 1, 3, 10, and 30 min, phenol extracted, mixed separately with 8 pmol of sequencing stop buffer, denatured by incubating at 85 °C for 3 min, and electrophoresed with molecular weight standards. Standards were prepared by incubation of 5 pmol of terminally labeled GGGRNA I in 10 mM Tris-HCl (pH 8.0), 100 mM sodium chloride, 5 mM magnesium chloride with 5 units of RNase T<sub>1</sub> at 37 °C for 10 min (RNase T<sub>1</sub> digest) and in 1 mM EDTA, 50 mM sodium carbonate (pH 9.2) at 40 °C for 20 min (1-nucleotide alkaline hydrolysis ladder).

Results—Inactivation of PolyA Polymerase Results in Accumulation of Several rne-dependent Degradative Intermediates of RNA I—Northern blot hybridization to a 32P-labeled riboprobe complementary to RNA I was used to detect full-length RNA I and its degradative products in preparations of total cellular RNA isolated from wild-type E. coli and from strains mutated in PNPase (pnp<sup>−</sup>), RNase E (rne<sup>−</sup>), or poly(A) polymerase (pcnB<sup>−</sup>). As seen in Fig. 2A, all RNA samples except the one isolated from a pcnB<sup>−</sup> strain mainly contain full-length RNA I, pRNA I<sub>5</sub>, and their polyadenylated products (bands that are positioned above the bands corresponding to RNA I and pRNA I<sub>5</sub> species). These data are consistent with the earlier evidence that RNase E cleavage of RNA I, together with polyadenylation, initiates its further degradation by PNPase or Rnase II (5). However, in the pcnB mutant (YHC3393), we also detected other low molecular weight (LMW) degradative intermediates (Fig. 2, A-C) in cells containing the pCML108 plasmid, which encodes full-length RNA I (Fig. 2B), or the pCML103 plasmid (Fig. 2C), which encodes ppRNA I<sub>5</sub>. The preferential accumulation of LMW products in a strain deficient for poly(A) polymerase suggests that RNA I decay in wild-type E. coli may involve multiple endonucleolytic cleavages producing short RNA I fragments that are made unstable by polyadenylation and consequently undergo rapid digestion by PNPase, Rnase II, or other nucleases. To determine whether mutations in the rne gene affect formation of these degradative products, we performed Northern blot analysis of total RNA isolated from the pcnB/ rne<sup>−</sup> double mutant (LK01) at permissive and non-permissive temperatures after rifampicin treatment. As shown in Fig. 2D, after shift of cells to 43 °C, which results in inactivation of RNase E, the concentration of LMW intermediates is decreased, whereas the same temperature shift in pcnB rne<sup>−</sup> strain does not affect the degradation pattern even after a longer incubation time (Fig. 2E). Some
additional bands located between LMW intermediates and RNA I (Fig. 2D) cannot be detected by primer extension analysis (see below) and are likely pRNA I 25 and pppRNA I 25 species truncated from the 3' end (3'-RNA I (I 25)) by the action of PNPase (or RNase II). This implies that the conversion of RNA I and pRNA I 25 into smaller RNA I degradative products is retarded by the rne mutation and thus that RNase E is the enzyme responsible for their formation.

RNase E Cleaves Multiple Internucleotide Bonds in Bubble Regions of RNA I Duplex Segments in Vivo and in Vitro—As shown in Fig. 3, incubation of in vitro prepared GGRNA I, which has the same secondary structure and substrate properties as native RNA I (27) with affinity-purified RNase E, leads to the formation of 70-mer, 67-mer, 41-mer, and 29-mer in addition to pRNA I 25. To determine the nature of these species, we subjected GGRNA I to RNase E treatment, and the resulting products were dephosphorylated with alkaline phosphatase and 5'-end labeled with 32P. Then, the terminally labeled 70-mer, 67-mer, 41-mer, and 29-mer species were individually isolated and analyzed by RNase T1 digestion as described under "Materials and Methods." The corresponding host strain used for the individual experiment is indicated above each lane. Cells were grown exponentially in M9/glucose medium at temperature shown beneath of each lane to an OD 600 of 0.4 followed by addition of rifampicin (0.5 mg/ml). Total cellular RNA was isolated from aliquots withdrawn at successive times, as indicated in minutes at the top of each lane, after rifampicin addition. Equal amounts of total RNA were separated on an 8% polyacrylamide sequencing gel, transferred to membrane, and the procedures described under "Materials and Methods." The RNase T1 partial digestion and partial alkaline hydrolysate of GGRNA I, as shown in Fig. 4 and Table 1, the first base of 70-mer was A6 and the last one is A 75. The 67-mer also had
A$_6$ as the first base, whereas the last one is C$_{72}$. Formation of these degradative intermediates can be explained by RNase E cleavage of pRNA I-5 in stem-loop III at two different sites: after A$_{75}$ (70-mer) and after C$_{72}$ (67-mer). A more complicated situation was observed in the case of the 41-mer and 29-mer. The RNase T$_1$ digestion patterns of these oligoribonucleotides do not correspond to any definite fragment of RNA I; that is, the 29-mer and 41-mer appear to be a mixture of two or more oligoribonucleotides. On the other hand, the presence of fragments of 29 and 41 nucleotides in length in an RNase E digest of the 70-mer (data not shown), plus the existence of an RNase E cleavage of pRNA I in a bubble region of a duplex segment of GGGRNA I between G$_{34}$ and U$_{35}$ (Fig. 5A primer extension analysis; the RNase E cleavage sites are indicated in lanes 6 and 7), suggests that the 29-mer and the 41-mer contain RNA species 29-mer* and 41-mer*, respectively (Fig. 4A). These species correspond to the degradative products of the 70-mer. However, the nature of other components of these mixtures could not be identified unambiguously, and thus these species were not investigated further.

Primer extension analysis of total cellular RNA I isolated from a pcnB mutant showed that, in addition to the expected cleavage site at the 5'-position (the 5'-end of pRNA I-5), another cleavage occurred between G$_{34}$ and U$_{35}$ (Fig. 5B, lane 8). This cleavage was reduced sharply in an isogenic strain carrying the pcnB/rne double mutations under conditions inactivating RNase E (Fig. 5B, lane 1 versus lane 2). These results indicate that RNase E also cleaves the internucleotide bond between G$_{34}$ and U$_{35}$ in vivo. Using an RNase E digest of GGGRNA I as a size marker, we determined by Northern blot analysis that two major LMW intermediates approximately 71 and 73 nucleotides in length hybridized to a $^{32}$P-labeled riboprobe complementary to the 3'-end of RNA I (data not shown). Our data indicate that both LMW intermediates observed in pcnB mutant are decay intermediates that correspond to the 3'-end of pRNA I-5 and are generated by RNase E cleavage of an internucleotide bond in the bubble region of stem-loop I (shown in Fig. 1); the 2-nucleotide difference in the length of these intermediates is due to heterogeneity at the 3'-end (8). Thus, in addition to the cleavage in single-stranded region at the 5'-end of RNA I, RNase E can cleave in the bubble regions of duplex segments of RNA I in vivo and in vitro.

The Catalytic Domain of RNase E Is Responsible for the Multiple Cleavages in RNA I Resulting in Formation of Decay Intermediates, Which Have Different Affinity to the RBD of the Enzyme—Assay of the enzymatic activity of the affinity-purified catalytic domain of RNase E (N-terminal half of the enzyme) showed that it reproducibly generates the same species
of degradative intermediates as full-length RNase E (Fig. 6) plus additional products (bands located between pRNA I-5 and 70-mer). We do not know whether an appearance of the additional bands reflects slightly broader substrate specificity of the truncated enzyme because of absence of its C-terminal fragment or because minimal distortions in the enzyme’s structure appeared during its purification.

As found by McDowall and Cohen (14), the affinity of the catalytic domain to RNA I, in comparison with the affinity of an arginine-rich RBD located near the center of the Rne protein, is negligible. To understand better the role of the RBD, we examined the effect of the RBD on RNA I structure and the accessibility of its internucleotide bonds to nuclease attack using footprinting analysis with chemical nuclease. Comparison of

**DISCUSSION**

While pRNA I-5 is the principal product of RNase E cleavage of RNA I (22, 23, 29, 30), the detection of an RNase E-dependent cleavage product about 70 nucleotides in length in vivo (23, 30) and in vitro (30) suggests that additional RNase E cleavages play a role in the decay of RNA I. Analysis of the decay of RNA I encoded by pCM108, a pSC101-derived non-ColE1-type plasmid, in a strain deficient for poly(A) polymerase (pcnB mutant) revealed several degradative products not detected in a pcmB+ strain (Fig. 2A), indicating that polyadenylation, which accelerates the decay of RNA I and other RNA species (6–8), can prevent detection of RNA I degradative intermediates in pcmB+ bacteria under wild-type conditions. However, these products were detected in a pcmB+ strain harboring pBR322-derived plasmid (23, 30), suggesting that complementary RNA II affects RNA I decay, possibly by binding to RNA I degradative products. Our results show that the accumulation of these decay intermediates depends directly on RNase E activity and decreases in a rneE strain at a non-permissive temperature. In vitro studies confirmed that GGGRNA I, and
consequently RNA I, is cleaved at multiple sites (Fig. 3). The characterization of these degradative products by RNase T1 mapping and primer extension analysis indicates that RNase E makes cuts preferentially in bubble regions of stem-loop segments of substrate RNA (namely, after G34, A75), as well as in single-stranded regions.

While rne-dependent in vitro cleavage in a bubble region of RNA I encoded by plasmid pACYC184 was noted previously (30), the preparation of RNase E used in that study was partially purified and therefore potentially contained other proteins now known to be associated with RNase E (15–18). Our finding that multiple cleavages are produced by affinity-purified enzyme provides direct evidence that cleavage of internucleotide bonds within structured regions of substrate RNA is due to RNase E itself. Thus, the cleavage potential of this enzyme may be broader than was previously thought.

Despite RNase E cleavage of GGGRNA I in vitro in both the stem-loop I and stem-loop III regions, Northern blot analysis of RNA derivatives accumulating in vivo showed products cleaved only in stem-loop I (71-mer and 73-mer). The absence of a detectable stem-loop III cleavage product in vivo may reflect difference in lability and, consequently, in abundance of different LMW intermediates in vivo. On the other hand, preferential formation in vivo of RNA I fragments (71-mer and 73-mer) containing intact stem-loop III also can be explained by an inaccessibility to nuclease of the internucleotide bonds located in stem-loop III, possibly because of their protection by other proteins associated with RNase E in vivo. A possible model for RNA I decay reflecting variations in the products generated by RNase E in vivo and in vitro is outlined in Fig. 9.

RNase E has been demonstrated to have separate catalytic (N-terminal half of enzyme) and RNA binding (middle part of protein) domains (14). While assaying the enzymatic activity of RNase E-dependent RNA Decay Pathway

Fig. 7. Footprinting analysis of RBD-GGGRNA I complex. Free and bound 5'-end-labeled GGGRNA I were separated in native 4% polyacrylamide gel and subjected in situ (in gel) exposure to the relatively unspecific activity of the 2:1 1,10-phenanthroline-cuprous complex, followed by elution of partially cleaved RNA and subsequent sizing in 8% polyacrylamide sequencing gels (lanes 3 and 4, respectively) for 2 (left panel) and 4 (right panel) h. Band assignment was facilitated by coelectrophoresing a partial RNase T1 digest (lane 1) and a partial alkaline hydrolyzing (lane 2) of the terminally labeled RNA employed in the assay. The loop regions of GGGRNA I are shown with brackets.

Fig. 8. Binding the RNA I and its rne-dependent degradative intermediates to RBD. Free GGGRNA I or its degradative intermediate (labeled as 1) and their individual mixtures with RBD-containing protein (labeled as 2) were electrophoresed in non-denaturing 4% polyacrylamide gel. The synthesis of GGGRNA I, its cleavage in vitro by RNase E, purification of corresponding cleavage products from gel, and gel retardation assay were performed according to procedures described under “Materials and Methods.”
the affinity-purified catalytic domain, we showed that it cleaves RNA I, yielding not only pRNA I by full-length RNase E. This observation is consistent with findings that the middle part of RNase E, which contains the enzyme’s RNA binding domain, does not participate in cleavage directly. Rather, this region of RNase E, because of its high affinity to RNA I in comparison with the affinity of catalytic domain (14), may instead promote targeting of the enzyme to the substrate.

Chemical in situ probing of the effect of the RBD on the structure of RNA I (Fig. 7) indicates that regions of secondary structure within the substrate are destabilized by formation of the RBD-RNA I complex. Thus, the action of the RBD in vivo may increase accessibility of the internucleotide bonds of RNA I to the catalytic domain of RNase E and/or other RNA processing enzymes associated with RNase E in the “processosome.” Moreover, the major products produced by the cleavages of RNA I by RNase E are different in their affinity to RBD (Fig. 8). The affinity reduces with the decrease in number of RNase E cleavage sites in intermediate, and is minimal in case of 29-mer and 41-mer. These observations imply that, during sequential digestion of substrate RNA, RNase E can differentially release degradative products in vivo.

Collectively, our findings suggest a model for RNase E-dependent RNA degradation. We propose that the RBD of the enzyme initiates the recognition and binding of substrate RNA, destabilizing RNA tertiary and secondary structures, and then retains the target RNA until the catalytic domain makes multiple cuts. The cleaved fragments of substrate RNA generated by RNase E, which are differentially released by the enzyme, are further degraded by poly(A)-dependent 3′-exonucleolytic activity of PNPase or by RNase II.

Acknowledgments—We thank K. J. McDowall and S. N. Cohen for providing plasmids and strains and for helpful discussion during this study. We also thank M.-C. Kan for construction of LK01 strain.

REFERENCES
1. Belasco, J. G., and Higgins, C. F. (1988) Gene (Amst.) 72, 15–23
2. Alifano, P., Rivellini, F., Piscitelli, C., Arraiolo, C. M., Bruni, C. B., and Carlonomagno, M. S. (1994) Gene & Dev. 8, 3021–3031
3. Hajsínský, E., Steier, O., Coscuy, L., Teyssset, L., and Regnier, P. (1994) EMBO J. 13, 3368–3377
4. Hajsínský, E., Carposíus, A. J., and Regnier, P. (1994) J. Mol. Biol. 239, 439–454
5. Xu, F., and Cohen, S. N. (1995) Nature 374, 180–183
6. O’Hara, E. B., Chekanova, J. A., Ingle, C. A., Kushner, Z. R., Peters, E., and Kushner, S. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1807–1811
7. Hajsínský, E., Braun, F., Hauget-Nielsen, J., and Regnier, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3973–3977
8. Xu, F., Lin-Chao, S., and Cohen, S. N. (1993) Proc Natl Acad Sci. U. S. A. 90, 6756–6760
9. He, L., Soderbom, F., Wagner, E. G. H., Binnie, U., Binns, N., and Masters, M. (1993) Mol. Microbiol. 9, 1131–1142
10. Cohen, S. N. (1995) Cell 80, 829–832
11. Carposíus, A. J., Mudd, E. A., and Krisch, N. M. (1989) Mol. & Gen. Genet. 219, 39–48
12. Mackie, G. A. (1991) J. Bacteriol. 173, 2488–2497
13. Kuwano, M., Ono, M., Endo, H., Hori, K., Nakamura, K., Hirota, Y., and Ohnishi, Y. (1977) Mol. & Gen. Genet. 154, 279–285
14. McDowall, K. J., and Cohen, S. N. (1996) J. Mol. Biol. 255, 349–355
15. Schildberg, B., Lunding, U., Hartl, F.-U., and von Gabain, A. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 277–281
16. Carposíus, A. J., Van Houwe, G., Ehretsmann, C., and Krisch, H. M. (1994) Cell 76, 889–900
17. Py, B., Cauton, H., Mudd, E. A., and Higgins, C. F. (1994) Mol. Microbiol. 14, 717–729
18. Miczak, A., Kaberdin, V. R., Wei, C.-L., and Lin-Chao, S. (1996) Proc Natl Acad Sci U.S.A. 93, 3884–3889
19. Miczak, A., Srivastava, R. A. K., and Apirion, D. (1991) Mol. Microbiol. 5, 1801–1810
20. Goldblum, K., and Apirion, D. (1981) J. Bacteriol. 146, 128–132
21. Lopilato, J., Bortner, S., and Beckwith, J. (1986) Mol. & Gen. Genet. 205, 285–290
22. Lin-Chao, S., Chen, W.-T., and Wong, T.-T. (1992) Mol. Microbiol. 6, 3385–3393
23. Lin-Chao, S., and Cohen, S. N. (1993) J. Biol. Chem. 269, 10797–10803
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 1.33–1.35, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
25. Lin-Chao, S., and Bremer, H. (1986) Mol. Microbiol. 24, 287–290
26. Lin-Chao, S., and Bremer, H. (1986) Mol. Microbiol. 7, 185–188
27. Lin-Chao, S., Chen, W.-T., and Wong, T.-T. (1992) Mol. Microbiol. 6, 3385–3393
28. McDowall, K. J., Kaberdin, V. R., Wu, S.-W., Cohen, S. N., and Lin-Chao, S. (1995) Nature 374, 287–290
29. Tamm, J., and Polisky, B. (1983) Nucleic Acids Res. 11, 6381–6397
