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An Efficient Shine-Dalgarno Sequence but Not Translation Is Necessary for *lacZ* mRNA Stability in *Escherichia coli*

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The 5′ ends of many bacterial transcripts are important in determining mRNA stability. A series of Shine-Dalgarno (SD) sequence changes showed that the complementarity of the SD sequence to the anti-SD sequence of 16S rRNA correlates with *lacZ* mRNA stability in *Escherichia coli*. Several initiation codon changes showed that an efficient initiation codon is not necessary to maintain *lacZ* mRNA stability. A stop codon in the 10th codon of *lacZ* increased mRNA stability. Therefore, ribosomal binding via the SD sequence but not translation of the coding region is necessary to maintain *lacZ* mRNA stability.

mRNA stability, as well as transcriptional and translational efficiency, plays an important role in regulating gene expression. The rates of decay of several bacterial transcripts, including *ompA*, *lacZ*, and *ermC*, are controlled by determinants located at the 5′ ends of the mRNAs (2, 3, 5, 12, 29, 30). Decay is initiated at the 5′ ends of some transcripts, for example, *lacZ* and *ompA* (6, 23, 24). However, no 5′-to-3′ exoribonuclease has been found in bacteria; therefore, the significance of 5′ stability determinants is unclear. One explanation is that ribosomes may provide protection to the transcript from attacking endoribonucleases. Therefore, translation initiation, which occurs at the 5′ end of mRNA and partly determines ribosomal density on the transcript, may also affect mRNA decay rates.

Several studies with bacteria suggest that ribosomes may be involved in protecting transcripts from ribonucleolytic action. Antibiotics that cause naked transcripts to form, such as puromycin and kasugamycin, result in rapid mRNA degradation (8, 11, 32, 36). Antibiotics that slow elongation, such as chloramphenicol and fusidic acid, lead to increased transcript stability (36). Unprotected tryptophan operon transcripts, downstream of nonsense codons, also decay rapidly (25). In these cases, transcripts that are unprotected by ribosomes decay rapidly.

Translation initiation frequency may also affect mRNA stability by altering ribosomal protection of transcripts. *lamB* mutations, which decrease translation initiation by sequestering the ribosome binding site within a secondary structure, accelerate the degradation of *lamB-lacZ* fusion transcripts (7, 40). The translation initiation frequency and mRNA stability of a fusion between *atpE* and the beta interferon gene are both decreased by secondary structure at the 5′ end of the transcript (14). The translation initiation region of the inefficiently translated *tetR* gene, fused to *lacZ*, results in destabilization of the chimeric mRNA (1). Translation initiation and transcript stability are also correlated in a Tn*10-lacZ* fusion (10). In the above-described cases, translation initiation frequency correlates with mRNA stability.

Ribosomal protection of only the 5′ ends of some transcripts is necessary to maintain mRNA stability. A translational stop at codon 56 of the β-lactamase gene does not destabilize the transcript (27). Translational induction of the *Bacillus subtilis ermC* transcript occurs by erythromycin-induced stalling of ribosomes in a short open reading frame within the 5′ leader (22, 26). This results in a 20-fold stabilization of the mRNA. Inducible stabilization of the *ermC* mRNA requires ribosomal stalling within the 5′ leader; however, translation of the coding region is not required for inducible stability (2, 18). Ribosomal protection of only the 5′ ends of some transcripts may be sufficient to protect the mRNAs from rate-limiting endonucleolytic cleavage.

In this study, the roles of the Shine-Dalgarno sequence (SD), the initiation codon, and translation in *lacZ* mRNA stability were investigated. The results suggest that protection of the 5′ end of the mRNA by efficient ribosomal binding, but not translation of the coding region, is necessary for *lacZ* mRNA stability.

MATERIALS AND METHODS

Plasmid construction. The parent plasmid, pCU, was constructed by cloning a *lacZ* fragment into a pKK223-3 derivative as described previously (38). Features of pCU include a translation initiation region that differs from that of wild-type *lacZ* in the following respects: it contains only one AUG initiation codon, a synthetic *tac* promoter containing a *Sal*I site, an *Eco*RI site between the SD and the initiation codon, and a *Hin*dIII site at the 5th codon of the *lacZ* gene. Mutations in the SD and codons 1 to 10 were made by cloning oligonucleotides into the *Sal*I, *Eco*RI, and *Hin*dIII sites. The sequence of the translation initiation region of pCU is 5′. .CAGGAAACA GAATTCACCATGATTACGCUAAGCTTGGCACTGG. . *lacZ*. The sequences from the SD to the initiation codon of the plasmids pCU-AGGGG and pCU-AGC-CUG are 5′. .AGGG GAATTAATAATG. .*lacZ* and 5′. . .AGCAAACAGAATTC CTG. .*lacZ*, respectively. The sequence of the HIII spacer which contains the stop codon is 5′AGCTTAGCTCGAG CATAAGTCGACGGTACCTCTCGAGATCT3′. The *Escherichia coli* strain used was K-12 SU1675 Δ(*pro-lac*) *recA56* F′ *lacI*^{q}.

β-Galactosidase assays and functional mRNA half-lives. Overnight cultures were diluted 1:50 in M9 minimal medium plus 0.2% glucose, 0.4% Casamino Acids, 0.01% thiamine, 2

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TABLE 1. Plasmid constructs and gene expression data

Construct	Sequence[a]	β-galactosidase level[b] (U)	Half-life[c] (min)
pCU	5′--AGGA--AUG----*lacZ*	14,406 ± 1109	1.3 ± 0.12
pCU-AGGGG	5′--AGGGG- AUG----*lacZ*	20,300 ± 1827	2.8 ± 0.26
pCU-GGG	5′--GGGA--AUG----*lacZ*	12,432 ± 430	1.3 ± 0.16
pCU-AAG	5′--AAGA--AUG----*lacZ*	4,591 ± 450	1.1 ± 0.20
pCU-AGC	5′--AGCA--AUG----*lacZ*	4,253 ± 221	0.8 ± 0.14
pCU-GUG	5′--AGGA--GUG----*lacZ*	9,420 ± 245	1.3 ± 0.12
pCU-UUG	5′--AGGA--UUG----*lacZ*	7,232 ± 571	1.0 ± 0.10
pCU-CUG	5′--AGGA--CUG----*lacZ*	344 ± 7.9	1.5 ± 0.48

[a] The SD and initiation codon are shown. Changes from pCU are boxed.
[b] Steady-state β-galactosidase units and standard deviations are shown.
[c] Functional mRNA half-lives and 95% confidence levels are shown.

mM IPTG (isopropyl-β-D-thiogalactopyranoside), and 100 μg of ampicillin per ml and grown at 37°C to log phase (0.5 U of optical density at 600 nm). Assays were as described by Weiss et al. (39). Determination of functional half-lives was based on the procedure of Kepes (21). Cultures were induced for 1 min before the addition of rifampin to 200 μg/ml. Aliquots were taken from the culture at time points from 2 to 20 min. After induction, chloramphenicol was added to 10 mg/ml, and the tubes were placed on ice. Half-lives and standard errors were estimated by a nonlinear regression analysis using a maximum-likelihood program from BMPD Statistical Software Inc. The equation $y = a_1 + a_2e^{-a_3x}$ describes the function, where a_3 is the slope of the curve. Standard errors were converted into 95% confidence levels.

RNA preparation, primer extension, and chemical mRNA decay. RNA isolation and primer extension were done as described previously (38). The primer used for Fig. 2, 4, and 6, 5′TTAAGTTGGGTAACGCCA3′, anneals to bases 104 to 122 of the pCU *lacZ* mRNA. A primer which anneals to the 5′ end of the lipoprotein gene (*lpp*) and one which anneals to the 5′ end of 5S rRNA were also used as controls. A mixture of radiolabelled and unlabelled *lpp* or 5S rRNA primers was used to achieve the desired counts per minute. Chemical mRNA decay measurements were performed on log-phase cultures at 0.5 U of optical density at 600 nm. Rifampin was added to 200 μg/ml at 0 min, and aliquots were taken at various time points and placed directly on ice. The culture was pelleted gently at 4°C and resuspended in 1 ml of ice-cold water, and the RNA was immediately isolated. Quantitation was done on a Molecular Dynamics phosphoimager or densitometer, and the amount of RNA loaded was normalized to the *lpp* or the 5S rRNA control band. A half-life is not calculated because chemical decay of the 5′ ends of *lacZ* transcripts is exponential only at the early time points. Variation in in vivo mRNA decay, due to temperature variation and unknown factors, was minimized by determining all half-lives that are shown within a single figure on the same day.

RESULTS

30S subunit binding affects *lacZ* mRNA stability. The SD is a consensus sequence found in the 5′ leader of mRNA, 5 to 14 bases upstream of the initiation codon (33, 34). The SD interacts with the 30S subunit of the ribosome by base pairing with the 3′ end of 16S rRNA (the anti-SD) (19, 20) and can enhance ribosomal binding more than 10-fold (4, 19). The binding of the 30S ribosomal subunit to the mRNA via the 16S rRNA-SD interaction is referred to as ribosomal binding throughout this paper. To determine the effect of ribosomal binding on *lacZ* mRNA half-life, several SD variants were constructed from the *lacZ* plasmid pCU. pCU contains a *lacZ* gene that is different from the wild-type gene in the following respects: a pTAC promoter replaces the *lac* promoter, only one AUG initiation codon is present, and several restriction sites are at the 5′ end of the transcript. The *lacZ* SD, AGGA, was changed to AGGGG, GGGA, AAGA, and AGCA (Table 1). The pCU-AGGGG construct also has an enriched A-U content in the region between the SD and the initiation codon. The role of this A-U-rich region is to decrease occluding secondary structures within the translation initiation region and therefore to enhance translation initiation (9, 31, 34). All other SD mutations are one- or two-base changes in the natural *lacZ* SD. The AGGGG SD contains 5 bases capable of pairing with the anti-SD, UCCUCC, if Watson-Crick base pairs and G-U base pairs are included. The SD sequences AGGA and GGGA both contain 4 bases complementary to the anti-SD. The SD sequences AAGA and AGCA contain only 3 bases complementary to the anti-SD. The steady-state β-galactosidase level of the strain containing the pCU-AGGGG plasmid increased to 20,300 U from the level of 14,406 U in the strain containing the parent plasmid, pCU. The pCU-GGG plasmid produced β-galactosidase levels of 12,432 U. The pCU-AAG plasmid caused a decrease in β-galactosidase levels to 4,591 U, and the pCU-AGCA plasmid produced β-galactosidase levels of 4,253 U (Table 1). Decreased complementarity of the SD to the anti-SD correlates with lower β-galactosidase activity, probably because of decreased ribosomal binding to the transcript. Altered ribosomal binding to the SD affects gene expression in the *lacZ* SD mutants by decreasing translation initiation frequency; however, changes in mRNA stability also affect the level of gene expression.

The efficiency of the SD, as measured by β-galactosidase levels and complementarity to the anti-SD, is correlated with the functional half-life of the transcript. The pCU-AGGGG construct produced *lacZ* mRNA with a half-life of 2.8 min, approximately twofold higher than the pCU *lacZ* transcript's 1.3-min half-life (Table 1 and Fig. 1). pCU and pCU-GGG transcripts had half-lives of 1.3 min, similar to the published value of 1.5 min for the wild-type *lacZ* half-life (5). pCU-AAG transcripts had a shorter half life of 1.1 min, and pCU-AGCA *lacZ* mRNA also exhibited a decreased half life of 0.8 min (Table 1 and Fig. 1). Ribosomal binding to the SD correlates

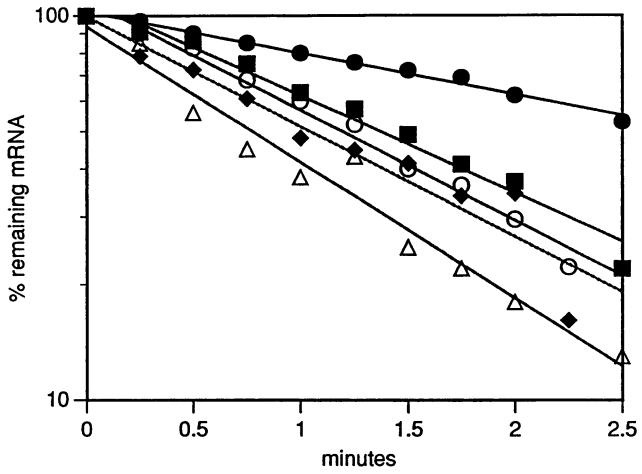

FIG. 1. Functional decay of mRNA containing wild-type and mutant SDs. Data were obtained by the method of Kepes (21). Solid circles, pCU-AGGGG; squares, pCU; open circles, pCU-GGG; diamonds, pCU-AAG; triangles, pCU-AGC.

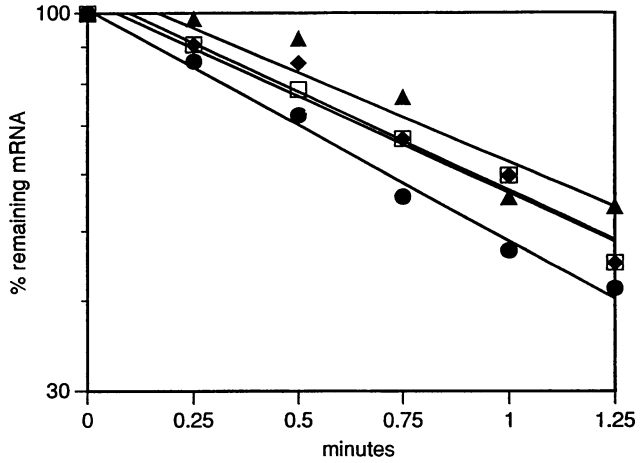

FIG. 3. Functional decay of mRNAs containing wild-type and mutant initiation codons. Data were obtained by the method of Kepes (21). Squares, pCU; diamonds, pCU-GUG; circles, pCU-UUG; triangles, pCU-CUG.

with *lacZ* mRNA stability, probably because of ribosomal protection of the transcript from RNases.

The wild-type *lacZ* transcript decays in a 5′-to-3′ direction (6). Therefore, chemical decay of the 5′ ends of *lacZ* mRNA is expected to be similar to functional decay. Chemical decay of the 5′ ends of pCU mRNA and transcripts derived from the SD mutant, pCU-AAG, was measured by primer extension of *lacZ* mRNA at various time points after transcriptional inhibition. 5S rRNA was also reverse transcribed in the same reaction as an internal control (Fig. 2). The chemical decay pattern of pCU-AAG mRNA was more rapid than that of pCU transcripts (Fig. 2). The chemical decay pattern of pCU-AGC transcripts was also more rapid than pCU mRNA decay when measured by the same method (37). The relative chemical decay rates of mRNAs containing mutant or wild-type SDs is similar to the functional decay rates of these transcripts.

Initiation codon identity does not affect *lacZ* mRNA stability. AUG, the most efficient initiation codon, is present in greater than 90% of *E. coli* transcripts and is complementary to the anticodon, CAU, of formylmethionyl-tRNA. The less-efficient initiation codons GUG and UUG are rarely used to initiate protein synthesis in *E. coli* (13). In all known cases, formylmethionine is the amino acid inserted at the amino-terminal end of the protein. The initiation codon AUG was altered to GUG, UUG, and CUG to determine the effect of inefficient initiation codons on *lacZ* mRNA half-life (Table 1). These plasmids were named pCU-GUG, pCU-UUG and pCU-CUG, respectively. The steady-state β-galactosidase level of the strain containing the pCU-GUG plasmid decreased to 9,420 U from the level of 14,406 U produced by the plasmid containing an AUG initiation codon, pCU. Strains containing the pCU-UUG and the pCU-CUG plasmids produced 7,232 and 344 U of β-galactosidase, respectively. The functional half-lives of the pCU-GUG and pCU-UUG transcripts were 1.3 and 1.0 min, respectively. Although the functional half-life of pCU-UUG transcripts decreased relative to that of the pCU transcript, there was no overall trend towards decreased functional half-life with decreased initiation codon efficiency. For example, a change in the initiation codon from AUG to CUG decreased β-galactosidase levels by more than 40-fold. However, the functional half-life of pCU-CUG transcripts was 1.5 min, which is statistically unchanged from the pCU transcript half-life (Table 1 and Fig. 3). The efficiency of the initiation codon does not correlate with the functional half-life of *lacZ* mRNA.

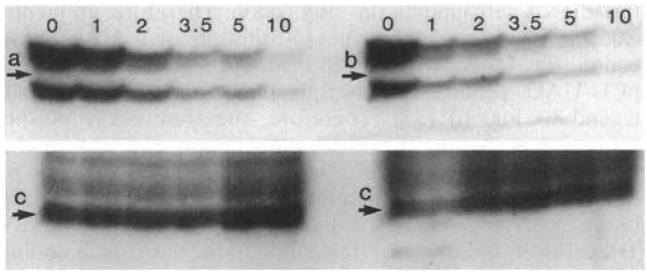

FIG. 2. Decay of 5′ ends of *lacZ* mRNAs containing wild-type and mutant SDs. Primer extension analysis of mRNA isolated at various time points after transcriptional inhibition by rifampin is shown. Minutes after rifampin addition are shown above the lanes. The upper doublet is due to the multiple 5′ ends that occur naturally in all *lacZ* transcripts. Arrows: a, pCU; b, pCU-AAG; c, 5S rRNA control which was reverse transcribed in the same reaction (smearing of this control band may be due to overloading or to several populations of 5S RNA molecules). The percentages of mRNA remaining, calculated from densitometric measurements and normalized to the control value, are as follows. pCU: 0 min, 100%; 1 min, 76%; 2 min, 37%; 3.5 min, 18%; 5 min, 10%. pCU-AAG: 0 min, 100%; 1 min, 34%; 2 min, 13%; 3.5 min, 7%; 5 min, 5%.

Chemical decay of the 5′ ends of pCU and pCU-UUG transcripts was measured by primer extension of *lacZ* mRNA at various time points following transcriptional inhibition. The 5′ ends of transcripts derived from pCU and pCU-UUG decayed at similar rates (Fig. 4). The 5′ ends of mRNAs derived from pCU and pCU-CUG also decayed at similar rates when measured by the same method (37). These results show that although the SD is involved in *lacZ* mRNA stability, efficient translation of the coding region of the transcript is not necessary to maintain *lacZ* mRNA half-life.

Stability of transcripts with mutations in both the SD and the initiation codon. Ribosomal protection via SD binding may provide an explanation for the unaltered stability of *lacZ* mRNA with altered initiation codons. Alternatively, a base

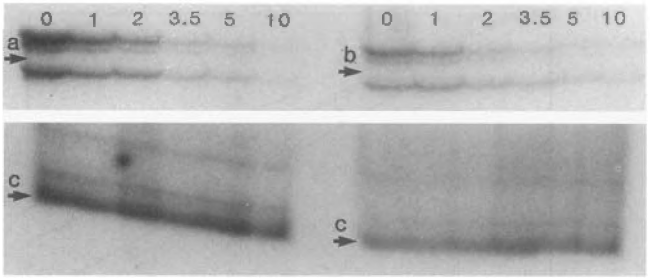

FIG. 4. Decay of 5′ ends of *lacZ* mRNAs containing wild-type and mutant initiation codons. Primer extension analysis of mRNA isolated at various time points after transcriptional inhibition by rifampin is shown. Minutes after rifampin addition are shown above the lanes. Arrows: a, pCU; b, pCU-UUG; c, *lpp* transcript which was reverse transcribed in the same reaction as a control. The percentages of mRNA remaining, calculated from densitometric measurements and normalized to the control value, are as follows. pCU: 0 min, 100%; 1 min, 56%; 2 min, 34%; 3.5 min, 17%; 5 min, 9%. pCU-UUG: 0 min, 100%; 1 min, 69%; 2 min, 38%; 3.5 min, 17%; 5 min, 15%.

change in the initiation codon could be destroying an RNase site. If an RNase site is destroyed by altering the initiation codon, transcripts could exhibit increased stability despite a lack of ribosomal protection. To distinguish between these two possibilities, a double mutant containing both an AGCA SD and a CUG initiation codon was constructed. The resulting plasmid was named pCU-AGC-CUG and produced trace amounts of β-galactosidase (less than 50 U). Because of the low β-galactosidase activity of the double mutant, a chemical half-life determination was performed by primer extension at various time points following transcriptional inhibition. The decay of transcripts derived from the pCU-AGC-CUG plasmid was slightly more rapid than pCU-AGC mRNA decay (Fig. 5). This suggests that the initiation codon is not part of an RNase site which is sensitive to ribosomal binding.

5′ proximal stop codons do not decrease mRNA stability. The results presented above show that 30S ribosomal subunit binding to the SD but not efficient translation initiation is important for functional and chemical stability of *lacZ* mRNA. If efficient translation of the coding region is not necessary for

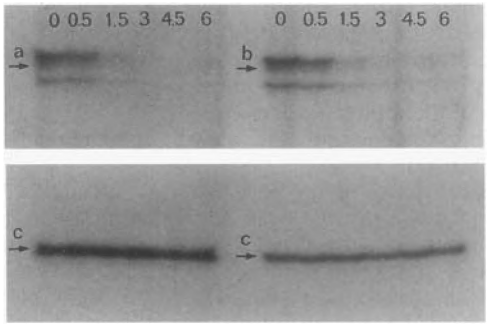

FIG. 5. Decay of 5′ ends of *lacZ* mRNAs containing a mutant SD or both a mutant SD and a mutant initiation codon. Primer extension of pCU-AGC-CUG (arrow a) and pCU-AGC (arrow b) shows mRNA decay at various time points after rifampin addition. Time points, in minutes, are shown above the lanes. A lighter exposure of *lpp* transcripts from the same reactions is shown as a control (arrows c). The percentages of mRNA remaining are as follows. pCU-AGC-CUG: 0 min, 100%; 0.5 min, 49%; 1.5 min, 8%. pCU-AGC: 0 min, 100%; 0.5 min, 63%; 1.5 min, 21%.

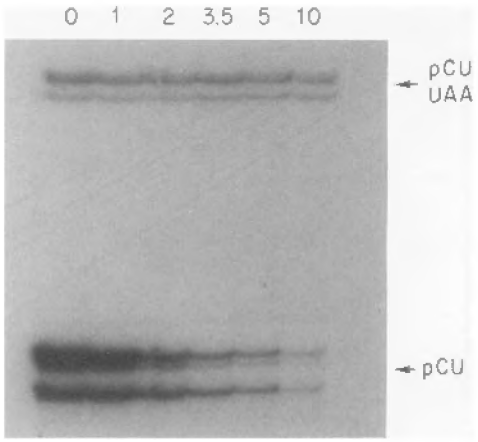

FIG. 6. Decay of 5′ ends of translated and untranslated *lacZ* mRNAs. Log-phase cultures containing pCU and pCU-UAA plasmids were mixed prior to rifampin addition. Primer extension of pCU-UAA and pCU shows mRNA decay at various time points after rifampin addition. Time points, in minutes, are shown above the lanes. The primer anneals downstream of the stop codon. The percentages of mRNA remaining are as follows. pCU: 0 min, 100%; 1 min, 83%; 2 min, 41; 3.5 min, 18%; 5 min, 7%. pCU-UAA: 0 min, 100%; 1 min, 80%; 2 min, 80%; 3.5 min, 73%; 5 min, 80%; 10 min, 51%. pCU-UAC: 0 min, 100%; 1 min, 30%; 2 min, 11%; 3 min, 5%; 5 min, 8%; 10 min, 6%.

mRNA stability, then a stop codon in the 5′ proximal coding region should not decrease mRNA stability. This hypothesis was tested by cloning an oligonucleotide containing a translational stop at codon 10 (see Materials and Methods) into a restriction site at codon 5 of the pCU plasmid. The resulting plasmid was named pCU-UAA and produced trace amounts of β-galactosidase (less than 50 U). pCU-UAA transcript decay relative to pCU mRNA decay was assessed by primer extension analysis at various time points after transcriptional inhibition (Fig. 6). The 5′ ends of the untranslated pCU-UAA transcripts decayed relatively slowly; more than half of the transcripts remained at 10 min after rifampin addition. The slow decay of pCU-UAA transcripts could be due either to the presence of a stop codon or to an undetermined sequence in the mRNA surrounding the stop codon. Therefore, an identical transcript, except for the substitution of a UAC sense codon for UAA, was constructed. The resulting plasmid, pCU-UAC, produced transcripts that decayed rapidly (see legend to Fig. 6) (37). Therefore, the slow decay of this transcript is due to the UAA stop codon and not to the presence of the insert. Furthermore, increased stability of full-length transcripts containing 5′ stop codons has also been found by Northern (RNA) blot analysis (data not shown). These results indicate that translation of the entire *lacZ* coding region is not required to maintain the chemical stability of *lacZ* mRNA.

DISCUSSION

The frequency of translation initiation is thought to play a significant role in determining mRNA stability (41). The present study shows that efficient ribosomal binding but not translation is necessary for *lacZ* mRNA stability; in the presence of an inefficient initiation codon or a 5′ stop codon, mRNA stability is maintained by ribosomal binding via the SD.

Significance of SD and initiation codon changes. An adaptation of the Gualerzi model of the translation initiation

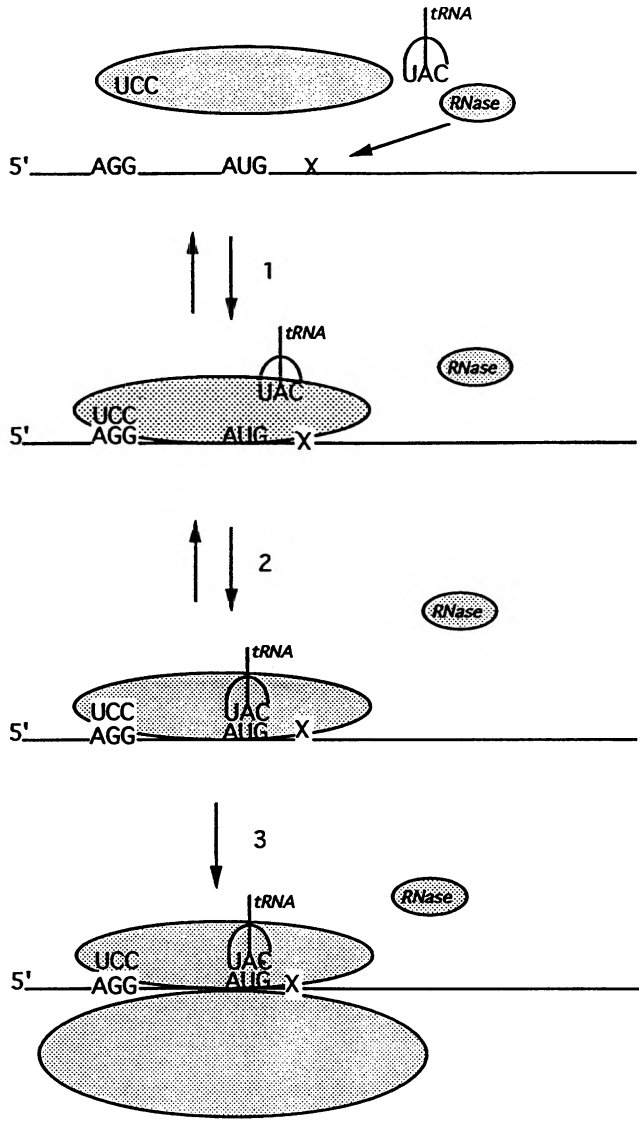

FIG. 7. Model of transcript stability via ribosomal protection. An adaptation of the Gualerzi model of the translation initiation process is shown to describe steps at which mRNA stability could be affected. Step 1 is 30S ribosomal subunit binding to the SD. Ribosomal binding efficiency determines protection of the transcript from RNases. A putative 5′ RNase site is indicated by an X. The rate of step 2, during which fMet-tRNA anticodon interaction with the initiation codon occurs, does not affect transcript stability. Step 3, during which the 50S subunit joins the preinitiation complex is also shown.

process is described to elucidate the steps at which mRNA stability could be affected (Fig. 7) (15, 16). This model is partly based on the observation that, in vitro, 30S subunit binding to the mRNA can occur independently of fMet-tRNA binding (16, 17, 35). The model states that fMet-tRNA and mRNA are assembled in a nonobligatory order onto the 30S ribosomal subunit (with bound initiation factors and GTP) into a ternary preinitiation complex. At this point the mRNA and the fMet-tRNA are not interacting. A transition forms the 30S initiation complex, during which the anticodon of fMet-tRNA base pairs with the initiation codon. In this model, ribosomal binding can occur independently of translation initiation.

Altered *lacZ* mRNA half-lives due to SD mutations suggest that the binding of the 30S ribosomal subunit to the mRNA via the SD interaction is important for mRNA stability, possibly because of ribosomal protection of an RNase site (Fig. 7). In the initiation codon mutants, a decrease in tRNA-anticodon interaction with the initiation codon did not affect mRNA stability, probably because 30S subunit binding to the transcript, and ribosomal protection of a putative 5′ RNase site, does not require fMet-tRNA (Fig. 7). Furthermore, the initiation codon itself does not appear to be part of an RNase site because the decay of a *lacZ* transcript containing both an inefficient SD and an altered initiation codon was rapid. Results from other systems also show that ribosomal protection of the coding region is not always necessary for mRNA stabilization. Translation of the coding region of *ermC* is not required for inducible stabilization of the transcript by erythromycin (2, 18). Likewise, a translational stop at codon 56 of the β-lactamase gene does not destabilize the transcript (27). Therefore, we propose that ribosomal binding via the SD may be protecting an RNase site near the 5′ end of the *lacZ* transcript.

A stop codon increases *lacZ* transcript stability. A translational stop at the 10th codon of *lacZ* increased mRNA stability, although the downstream coding region of the transcript is unprotected by ribosomes. Stop codons in a *MATα1-PGK1* fusion transcript in *Saccharomyces cerevisiae* also increased mRNA stability (28). One explanation for our result is that a translational stop causes the ribosome to pause and increases ribosomal protection from endoribonucleases at the 5′ end of the transcript. Ribosomes have been shown to pause on bovine preprolactin mRNA during translation initiation and termination (40). Our results show that translation of the first 10 codons of *lacZ* is sufficient to protect this transcript from rapid degradation. This suggests that rate-limiting cleavage of *lacZ* transcripts may occur at the 5′ end of the mRNA.

Conclusions. Mutations in the translation initiation region of *lamB-lacZ* gene fusions suggest that mRNA stability is related to translation initiation frequency (41). However, in the *ermC* transcript, translation of the coding region is unnecessary for inducible transcript stability (2, 18). In the present study, SD-mediated ribosomal binding during translation initiation was important for *lacZ* mRNA stability, but decreased translation initiation frequency due to inefficient initiation codons did not affect mRNA stability. Translation of the coding region of *lacZ*, downstream of codon 10, was also unnecessary to maintain transcript stability. Therefore, only 30S subunit binding, not translation of the entire coding region, may be necessary to maintain *lacZ* mRNA functional stability.

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REFERENCES

1. **Baumeister, R., P. Flache, O. Melefors, A. von Gabain, and W. Hillen.** 1991. mRNA is associated with a low efficiency of translation and a short half-life in *Escherichia coli*. Nucleic Acids Res. **19**:4596–4600.
2. **Bechhofer, D. H., and D. Dubnau.** 1987. Induced mRNA stability in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **84**:498–502.
3. **Belasco, J. G., G. Nilsson, A. von Gabain, and S. N. Cohen.** 1986. The stability of gene transcripts is dependent on determinants localized to specific mRNA segments. Cell **46**:245–251.
4. **Calogero, R. A., C. L. Pon, M. A. Canonaco, and C. O. Gualerzi.** 1988. Selection of the mRNA translation initiation region by

Escherichia coli ribosomes. Proc. Natl. Acad. Sci. USA **85**:6427–6431.

5. **Cannistraro, V. J., and D. Kennell.** 1979. *Escherichia coli lac* operator mRNA affects translation of β-galactosidase mRNA. Nature (London) **277**:407–409.
6. **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.
7. **Chevrier-Miller, M., N. Jaques, O. Raibaud, and M. Dreyfus.** 1990. Transcription of single-copy hybrid *lacZ* genes by T7 RNA polymerase in *Escherichia coli*: mRNA synthesis and degradation can be uncoupled from translation. Nucleic Acids Res. **18**:5787–5792.
8. **Cremer, K. J., L. Silengo, and D. Schlessinger.** 1974. Polypeptide formation and polyribosomes in *Escherichia coli* treated with chloramphenicol. J. Bacteriol. **118**:582–589.
9. **De Smit, M. H., and J. van Duin.** 1990. Secondary structure of the ribosome binding site determines translational efficiency: a quantitative analysis. Proc. Natl. Acad. Sci. USA **87**:7668–7672.
10. **Flache, P., R. Baumeister, and W. Hillen.** 1992. The Tn*10*-encoded tetracyline resistance mRNA contains a translational silencer in the 5′ nontranslated region. J. Bacteriol. **174**:2478–2484.
11. **Fry, M., M. Israeli-Reches, and M. Artman.** 1972. Stabilization and breakdown of *Escherichia coli* messenger ribonucleic acid in the presence of chloramphenicol. Biochemistry **11**:3054–3059.
12. **Gorski, K., J. M. Roch, P. Prentki, and H. M. Krisch.** 1985. The stability of bacteriophage *T4 gene 32* mRNA: a 5′ leader sequence that can stabilize mRNA transcripts. Cell **43**:461–469.
13. **Gren, E. J.** 1984. Recognition of messenger RNA during translation initiation in *Escherichia coli*. Biochimie **66**:1–29.
14. **Gross, G., C. Mielke, I. Hollatz, H. Blocker, and R. Frank.** 1990. RNA primary sequence or secondary structure in the translation initiation region controls expression of two variant *interferon*-β genes in *Escherichia coli*. J. Biol. Chem. **265**:17627–17636.
15. **Gualerzi, C. O., and C. L. Pon.** 1990. Initiation of mRNA translation in prokaryotes. Biochemistry **29**:5881–5889.
16. **Gualerzi, C. O., G. Risuleo, and C. Pon.** 1977. Initial rate kinetic analysis of the mechanism of initiation complex formation and the role of initiation factor IF-3. Biochemistry **16**:1684–1689.
17. **Hartz, D., D. S. McPheeters, R. Traut, and L. Gold.** 1988. Extension inhibition analysis of translation initiation complexes. Methods Enzymol. **164**:419–425.
18. **Hue, K. K., and D. H. Bechhofer.** 1991. Effect of *ermC* leader region mutations on induced mRNA stability. J. Bacteriol. **173**:3732–3740.
19. **Hui, A., and H. A. De Boer.** 1987. Specialized ribosome system: preferential translation of a single mRNA species by a subpopulation of mutated ribosomes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:4762–4766.
20. **Jacob, W. F., F. M. Santer, and A. E. Dahlberg.** 1987. A single base change in the Shine-Dalgarno region of 16S rRNA of *Escherichia coli* affects translation of many proteins. Proc. Natl. Acad. Sci. USA **84**:4757–4761.
21. **Kepes, A.** 1967. Sequential transcription and translation in the lactose operon of *Escherichia coli*. Biochim. Biophys. Acta **138**:107–123.
22. **Mayford, M., and B. Weisblum.** 1989. Conformational alterations in the *ermC* transcript in vivo during induction. EMBO J. **8**:4307–4314.
23. **Melefors, O., and A. von Gabain.** 1988. Site-specific endonucleolytic cleavages and the regulation of stability of *E. coli ompA* mRNA. Cell **52**:893–901.
24. **Morse, D. E., R. Mosteller, R. F. Baker, and C. Yanofsky.** 1969. Degradation of *tryptophan* messenger. Nature (London) **223**:37–43.
25. **Morse, D. E., and C. Yanofsky.** 1969. Polarity and the degradation of mRNA. Nature (London) **224**:329–331.
26. **Narayanan, C. S., and D. Dubnau.** 1987. Demonstration of erythromycin-dependent stalling of ribosomes on the *ermC* leader transcript. J. Biol. Chem. **262**:1766–1771.
27. **Nilsson, G., J. Belasco, S. N. Cohen, and A. von Gabain.** 1987. Effect of premature termination of translation on mRNA stability depends on the site of ribosome release. Proc. Natl. Acad. Sci. USA **84**:4890–4895.
28. **Parker, R., and A. Jacobson.** 1990. Translation and a 42-nucleotide segment within the coding region of the mRNA encoded by the Mat 1 gene are involved in promoting rapid mRNA decay in yeast. Proc. Natl. Acad. Sci. USA **87**:2780–2784.
29. **Petersen, C.** 1987. The functional stability of the *lacZ* transcript is sensitive towards sequence alterations immediately downstream of the ribosome binding site. Mol. Gen. Genet. **209**:179–187.
30. **Petersen, C.** 1991. Multiple determinants of functional mRNA stability: sequence alterations at either end of the *lacZ* gene affect the rate of mRNA inactivation. J. Bacteriol. **173**:2167–2172.
31. **Scherer, G. F. E., M. D. Walkinshaw, S. Arnott, and D. J. Morre.** 1980. The ribosome binding sites recognized by *E. coli* ribosomes have regions with signal character in both the leader and protein coding segments. Nucleic Acids Res. **8**:3895–3907.
32. **Schneider, E., M. Blundell, and D. Kennell.** 1978. Translation and mRNA decay. Mol. Gen. Genet. **160**:121–129.
33. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71**:1342–1346.
34. **Stormo, G. D., T. D. Schneider, L. Gold, and A. Ehrenfeucht.** 1982. Use of the 'perceptron' algorithm to distinguish translational initiation sites in *E. coli*. Nucleic Acids Res. **10**:2998–3011.
35. **Van Duin, J., G. P. Overbeek, and C. Backendorf.** 1980. Functional recognition of phage RNA by 30-S ribosomal subunits in the absence of initiator tRNA. Eur. J. Biochem. **110**:593–597.
36. **Varmus, H. E., R. L. Perlman, and I. Pastan.** 1971. Regulation of *lac* transcription in antibiotic-treated *E. coli*. Nature (London) New Biol. **230**:41–44.
37. **Wagner, L. A.** 1994. An investigation of 5′ messenger RNA stability determinants in *lacZ*. Ph.D. thesis. University of Utah, Salt Lake City.
38. **Wagner, L. A., R. B. Weiss, R. Driscoll, D. S. Dunn, and R. F. Gesteland.** 1990. Transcriptional slippage occurs during elongation at runs of adenine or thymine in *Escherichia coli*. Nucleic Acids Res. **18**:3529–3535.
39. **Weiss, R. B., W. M. Huang, and D. M. Dunn.** 1990. A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage *T4 gene 60*. Cell **62**:117–126.
40. **Wolin, S. L., and P. Walter.** 1988. Ribosome pausing and stacking during translation of a eukaryotic mRNA. EMBO J. **7**:3559–3569.
41. **Yarchuk, O., N. Jacques, J. Guillerez, and M. Dreyfus.** 1992. Interdependence of translation, transcription and mRNA degradation in the *lacZ* gene. J. Mol. Biol. **226**:581–596.