The aprE leader is a determinant of extreme mRNA stability in Bacillus subtilis

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The Bacillus subtilis aprE gene encodes subtilisin, an extracellular proteolytic enzyme produced in stationary phase. The authors examined the stability of aprE mRNA and aprE leader–lacZ fusion mRNA. Both mRNAs were found to be unusually stable, with half-lives longer than 25 min, demonstrating that the aprE leader contains a determinant for extreme mRNA stability. The half-lives were the same in growing and stationary-phase cells. This contrasts with the findings of O. Resnekov et al. (1990) [Proc Natl Acad Sci U S A 87, 8355–8359], which suggested a growth-phase-dependent mechanism for decay of aprE mRNA. The discrepancy is explained by the techniques used. Substitution of two bases or deletion of 25 nucleotides in the aprE leader led to a major difference in its predicted secondary structure and resulted in a fivefold reduction of the half-life of aprE mRNA. The authors also determined the half-life of amyE mRNA, which encodes α-amylase, another stationary-phase, excreted enzyme and found it to be around 5 min. This shows that extreme stability is not a general property of stationary-phase mRNAs encoding excreted enzymes.

Keywords: mRNA secondary structure, subtilisin, amyE, stationary phase

INTRODUCTION

The steady-state amount of mRNA in a cell is a function of its rate of synthesis and its rate of decay. The half-life of bulk mRNA in growing cells of Escherichia coli is about 3–5 min, but that of specific mRNA species can vary between less than 1 min and more than 30 min (Belasco, 1993). Furthermore, the half-lives of some mRNAs vary depending on growth stage and growth conditions (Paesold & Krause, 1999; Vytvytska et al., 1998). Thus, there exist specific mechanisms for the control of decay of various mRNA species. These mechanisms involve specific RNA–protein interactions and alternative mRNA secondary structures (Liu & Romeo, 1997; Vytvytska et al., 1998; Yamanaka et al., 1999).

Most of our ideas about bacterial mRNA degradation are based on experiments performed with E. coli. The 5’ untranslated leader of an mRNA often contains the major stability determinant(s) (Bechhofer, 1993). The rate-limiting step in mRNA degradation is thought to be an endonucleolytic cut in the 5’ part of the molecule, which ‘opens’ it for further endonucleolytic attacks towards the 3’ end. The resulting fragments are rapidly degraded by 3’ to 5’ exoribonucleases (Spickler & Mackie, 2000). RNase E is the major endoribonuclease executing the rate-limiting attack (Cohen & McDowall, 1997). The general model for mRNA degradation probably applies to other bacteria but with important modifications in details. For instance, no RNase E homologue has been found in Bacillus subtilis (Kunst et al., 1997) and different patterns of degradation have been found for the same mRNA species in B. subtilis and E. coli (Persson et al., 2000).

When B. subtilis enters stationary phase, several new genetic programmes are switched on for sporulation, competence development (Lazazzera et al., 1999) or production of extracellular enzymes (Ferrari et al., 1993). The aprE gene encodes subtilisin, an extracellular proteolytic enzyme produced by stationary-phase cells (Ferrari et al., 1988). Resnekov et al. (1990) measured the decay of aprE mRNA and found it to be extremely stable in stationary-phase cells, with a half-life of at least 25 min. This is not a general property of stationary-phase mRNA (Melin et al., 1989; Resnekov et al., 1992). Interestingly, when the bacteria were diluted into fresh medium and allowed to resume growth, the stability of aprE mRNA seemed to decrease four- to fivefold. These
findings indicated the presence of a specific control mechanism for decay of aprE mRNA related to growth phase and perhaps operating also on mRNA for other extracellular enzymes whose synthesis is induced in stationary-phase cells.

In the present work we re-examined the results of Resnekov et al. (1990) with the primary aim of identifying a possible control mechanism for growth-stage-dependent differential rate of decay of aprE mRNA. However, we found that aprE mRNA has the same extreme stability in both stationary-phase and growing cells. The apparent growth-stage-dependent stability of aprE mRNA can be fully explained by the techniques used by Resnekov et al. (1990). Our experiments also demonstrate that the determinant(s) for the extreme stability of aprE mRNA is contained within the leader.

**METHODS**

**Bacteria, plasmids and primers.** Bacteria and plasmids are listed in Table 1 and primers in Table 2.

**Growth of bacteria.** Bacteria were kept on TBAB plates. *E. coli* was grown in LB. *B. subtilis* was grown in NSMP (Fortnagel & Freese, 1968) at 37 °C on a rotary shaker at 200 r.p.m. Antibiotics were added to the following concentrations: chloramphenicol, 5 mg 1⁻¹; erythromycin,
Table 2. Primers

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ to 3’)</th>
</tr>
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<tbody>
<tr>
<td>aprEBam1</td>
<td>GTT GGA TCC AGT CTC TAC GGA AAT AGC GAG</td>
</tr>
<tr>
<td>aprEBam2</td>
<td>CAA GGA TCC GAT GCA CAA TTT TTT GCT TCTC</td>
</tr>
<tr>
<td>aprEconst</td>
<td>TTT GAT CTT TTT AAA TAA AGT AAT ACT ATG GTA TAA TGG</td>
</tr>
<tr>
<td></td>
<td>TTA CAC AGA ATA GTC TTT TAA GTA AGT CTA CTC TG</td>
</tr>
<tr>
<td>aprEconstS</td>
<td>TTT GAT CTT TTT AAA TAA AGT AAT ACT</td>
</tr>
<tr>
<td>aprEdel2S</td>
<td>GGT ATA ATG GCT ACG AGT GTA ACC TTAT ATA CC</td>
</tr>
<tr>
<td>aprEdel2Sinv</td>
<td>AAA ATT CAG AGT GTA ACC ATT ATA CC</td>
</tr>
<tr>
<td>aprEsubTT</td>
<td>AGT CTA CTC TTT ATT TTT TAA AAA GG</td>
</tr>
<tr>
<td>aprEsubTTinv</td>
<td>CCT TTT AAA AAA ATA AAG AGT AGA CT</td>
</tr>
<tr>
<td>amyE1</td>
<td>ATG TTT GCA AAA CGA TTC AAA ACC</td>
</tr>
<tr>
<td>amyESeq</td>
<td>CGA CGG TGC TGT AAG CTC ATT CGA</td>
</tr>
<tr>
<td>lacZ60</td>
<td>CGC CAG CTG GCG AAA GGG GG</td>
</tr>
<tr>
<td>amyEBam1</td>
<td>GAG GGA TCC GTT CAC AGT TTG GGG</td>
</tr>
<tr>
<td>amyEBam2</td>
<td>GGT TTT GGA TCC TTT TGC AAA CAT TC</td>
</tr>
<tr>
<td>amyE HindIII</td>
<td>GCG AAG CTT ATC CGT TCA CAG TTT CGG G</td>
</tr>
<tr>
<td>amyEconst</td>
<td>TTT GAT CTT TTT AAA TAA AGT AAT ACT ATG GTA TAA TGG</td>
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<td>GAA TAA AGC TTA AAG GTC ATT GTT GAC G</td>
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<tr>
<td>glpDBam1</td>
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</tr>
<tr>
<td>lacZ seq</td>
<td>GTT TTC CCA GTC ACG ACG TTG</td>
</tr>
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</table>

![Fig. 1. Schematic representation of the inserts of B. subtilis LUS1 and LUS2. The aprE promoter and leader and part of the aprE coding region from BR95 were amplified by PCR and cloned in-frame with lacZ in pMD432. The aprE promoter–aprE leader–lacZ fusion was integrated into the chromosome of BR95 at the amyE gene to give LUS1. In LUS2, the aprE promoter was substituted with the glpD promoter. +1 indicates the transcription start point. The thick lines below the constructs illustrate the probes used to detect aprE and lacZ mRNAs, respectively.](image)

0.5 mg l⁻¹; lincomycin, 12.5 mg l⁻¹; ampicillin, 50 mg l⁻¹; rifampicin, 100 mg l⁻¹.

Genetic techniques. B. subtilis was grown to competence as described by Arwert & Venema (1973). E. coli was made competent as described by Mandel & Higa (1970).

Enzyme activities. β-Galactosidase activity was detected on TBAB plates containing 40 mg X-Gal l⁻¹. β-Galactosidase activity in liquid cultures was assayed according to Miller (1972) as described by Glatz et al. (1998). Amylase activity was detected on TBAB plates containing 1.5% starch. After incubation for 2 d, the plates were sprayed with an iodine solution (1 g iodine and 2 g potassium iodide in 300 ml distilled water). A clear halo is observed around amylase-positive colonies.

Construction of strains. A sequence containing the aprE promoter, leader sequence and the first 8 codons of the aprE gene, from −104 to +82 (Ferrari et al., 1988) was amplified from chromosomal DNA of B. subtilis BR95 with PCR using primers aprEBam1 and aprEBam2. The fragment was cleaved at both ends by restriction enzyme BamHI and ligated into the BamHI site of the B. subtilis integration plasmid pMD432. Competent E. coli XL-1 Blue was transformed with the ligate, with selection for ampicillin resistance. Transformants producing β-galactosidase were identified on X-Gal plates, and from one of these, pLUS1 was purified and the inserted fragment was sequenced. pLUS1 was then used to transform B. subtilis BR95, with selection for chloramphenicol resistance. Transformants producing β-galactosidase and lacking amylase activity were isolated. One of these was kept and the strain was named LUS1 (Fig. 1).

From plasmid pLUS1, the region containing the transcribed aprE part (+1 to +82) was amplified with PCR using primers aprEconst and aprEBam2. aprEconst substitutes the aprE promoter with the glpD promoter (−40 to −1) (Holmberg & Rutberg, 1992). After cleavage with BamHI, the amplified fragment was ligated to BamHI-cleaved pMD432. Competent E. coli XL-1 Blue was transformed with the ligate, with selection for ampicillin resistance. Transformants producing β-galactosidase were identified on X-Gal plates and from one of these, pLUS2 was purified and the inserted fragment was sequenced. pLUS2 was then used to transform B. subtilis BR95 and transformants were isolated as described above. The resultant strain was named LUS2 (Fig. 1).

Plasmid pLUS2 was used to construct two additional strains with modifications in the aprE leader sequence by running
PCRs with modified primers. A first PCR was run with primers aprEconstS and aprEdel25inv in one mix and aprEdel25 and aprEBam2 in another. aprEdel25inv and aprEdel25 are complementary to each other and introduce a deletion of 25 nt. The two fragments obtained were mixed and a second PCR was run with primers aprEconstS and aprEBam2. The fragment obtained was ligated to BamHI-cleaved pMD432 and E. coli XL-1 Blue was transformed with the ligate. Ampicillin-resistant transformants were selected and pLUS3 was isolated from one of these. pPLUS4 was obtained analogously but with the primers aprEdel25inv and aprEdel25 replaced with the primers aprEsubTTinv and aprEsubTT. These primers introduce T substitutions at +31 and +32. pPLUS3 and pPLUS4 were used to transform B. subtilis BR95, with selection for chloramphenicol resistance. Transformants were isolated and the resultant strains were named LUS3 and LUS4.

A sequence containing the amyE promoter, leader and part of the coding region, from −100 to +134 (Nicholson & Chamblish, 1986), was amplified by PCR with primers amyEbam1 and amyEbam2. The fragment was cleaved with BamHI and ligated to BamHI-cleaved pMD433. E. coli XL-1 Blue was transformed with the ligate, selecting for ampicillin resistance. Plasmid pPLUS5 was isolated from one of the transformants. From pPLUS5, the region containing the transcribed amyE part (+1 to +134) was amplified with PCR using primers amyEconst and amyEbam2. amyEconst substitutes the amyE promoter with the glpD promoter (−40 to −1) (Holmberg & Rutberg, 1992). After cleavage with BamHI, the amplified fragment was ligated to BamHI-cleaved pMD433. Competent E. coli XL-1 Blue was transformed with the ligate, selecting for ampicillin resistance. Transformants producing β-galactosidase were identified on X-Gal plates and from one of these, pPLUS6 was purified.

pPLUS5 and pPLUS6 were used as templates for PCRs with primer pairs amyEHindIII/lacZHindIII and amyEconst/lacZHindIII, respectively. After cleavage with HindIII, the fragments were ligated to HindIII-cleaved pDG1664. E. coli XL-1 Blue was transformed with the ligate, selecting for ampicillin resistance. pPLUS5b and pPLUS6b were isolated and used to transform B. subtilis BR95. Transformants resistant to erythromycin and lincomycin (MLS<sup>+</sup>) were selected and from each transformation a transformant that produced β-galactosidase and required threonine for growth was kept. The resultant strains were named LUS5 and LUS6, respectively.

**RNA techniques.** Total RNA was extracted from B. subtilis as described by Resnekov et al. (1990). For measuring mRNA half-lives, rifampicin (100 mg l<sup>−1</sup>) was added and samples were then removed at intervals for extraction of total RNA. Electrophoresis of RNA for Northern blots was done as described by Thomas (1980), and the RNA was then blotted onto Hybond-N filters (Amersham). Twenty micrograms of RNA was added to each well unless otherwise indicated. Single-stranded (ss) DNA probes were generated as described before (Persson et al., 2000) with the following primers: lacZ60 for lacZ constructs, aprEBam2 for aprE, and amyEseq for amyE. To generate templates for the ssPCR, specific fragments were amplified with PCR using the following primers and templates: glpDBam1 and lacZ60 with pLUS4, aprEsubTT and aprEBam2 with pLUS1, and amyE1 and amyEseq with chromosomal B. subtilis DNA. The glpDBam1–lacZ60 fragment was cleaved with BamHI and the lacZ part was isolated prior to the ssPCR. After hybridization, the radioactivity of the bands was quantified with a PhosphorImager (Molecular Dynamics). 23S rRNA and 16S rRNA were used as size markers. Primer extension analysis was performed according to the method of Ayer & Dynan (1988). The primer used was lacZseq.

**RESULTS**

**Construction and transcriptional control of aprE–lacZ fusions**

A fusion was made between the aprE promoter, leader and the first eight codons of aprE (−104 to +82) (Park et al., 1989) and the coding region of E. coli lacZ (Fig. 1). The fusion was inserted into the amyE locus of B. subtilis BR95. The resulting strain was named LUS1. A second fusion was made in which the aprE promoter region was substituted with the constitutive B. subtilis glpD promoter (−40 to −1) (Fig. 1). The fusion was inserted into the amyE locus in B. subtilis BR95 to give strain LUS2. The identity of the fusions was verified by DNA sequencing and the transcriptional start site (+1) was verified by primer extension experiments.

LUS1 and LUS2 were grown as described in Methods. At different times, samples were taken for measurement of OD<sub>600</sub> and β-galactosidase activity. In LUS1, β-galactosidase activity was detected only after the cells had entered stationary phase, whereas in LUS2, β-galactosidase was produced throughout exponential growth (Fig. 2). These results are in good agreement with the fact that a major control of aprE expression occurs at initiation of transcription through the AbrB/SpoOA switch (Olmos et al., 1996).

The aprE leader is a determinant of extreme mRNA stability

LUS1 was grown to t = 1.5, i.e. for 1.5 h past the entry into stationary phase, at which time rifampicin (100 mg l<sup>−1</sup>) was added to the culture. RNA was extracted at different times thereafter. The relative amounts of aprE and aprE–lacZ transcripts were de-
Stability of \emph{B. subtilis} aprE mRNA

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Northern blot analysis of (a) the native aprE transcript of LUS1; (b) the aprE promoter–aprE leader–lacZ transcript of LUS1; (c) the glpD promoter–aprE leader–lacZ transcript of LUS2. The bacteria were grown to \( t = 15 \) and RNA was extracted at 0 min and at 7.5, 15 and 30 min after the addition of rifampicin. The experiments were repeated at least three times with similar results.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Predicted secondary structures of the aprE leader in (a) LUS2, (b) LUS4, and (c) LUS3. In (a), the substitution of 2 nt in LUS4 is marked with arrows and the deleted 25 nt in LUS3 are bold-faced. The boxed nucleotides represent the RBS. The free energies of the secondary structures are for (a) \(-44.8\) kJ \((-37.7\) kJ for the first and \(-7.1\) kJ for the second stem–loop); (b) \(-65.3\) kJ; (c) \(-33.6\) kJ (http://mfold.wustl.edu/~folder/rna/form1.cgi).}
\end{figure}

termined in Northern blots using probes specific for aprE and lacZ. The half-lives of the two transcripts were similar and at least 25 min (Fig. 3a, b). These results indicate that the aprE leader determines the stability of both transcripts and that sequences downstream of +82 in the aprE gene have little effect on mRNA stability.

The decay of the above two transcripts was also determined by primer extension analysis using a primer specific for the lacZ part of the aprE–lacZ transcript. The results of these experiments were essentially identical to those obtained with Northern blots (data not shown).

The decay of the aprE–lacZ transcript in stationary-phase cells was next measured in LUS2, in which the aprE promoter is replaced by the glpD promoter. Again, a half-life longer than 25 min was observed, indicating that the rate of degradation of the aprE transcript is not affected by sequences upstream of aprE +1 (Fig. 3c).

A truncated transcript of about 700 nt was seen in the Northern blots for strains LUS1 and LUS2 (Fig. 3b, c). This represents the 5' part of the full-length transcript and has been found also in other types of transcriptional-translational fusions with lacZ in \emph{B. subtilis} (Persson et al., 2000). The truncated transcript decayed at the same rate as the full-length transcript, which further supports the notion that the aprE leader is the major stability determinant also in the fusion transcript.

To examine how changes in the aprE leader transcript would affect mRNA stability, two modifications were introduced into the aprE leader of the lacZ fusion in LUS2. These were chosen so as to significantly alter the secondary structure of the leader (Fig. 4). Prediction of
secondary structures is very uncertain. Alternative structures are often suggested with similar free energies. One modification was a deletion of nucleotides +1 to +25, resulting in strain LUS3. In the second modification, a G and an A residue, at positions +31 and +32, respectively, were exchanged for two T residues, resulting in strain LUS4. According to computer-predicted folding (Zuker, 1989), the RBS of both LUS3 and LUS4 leader is contained within a strong stem–loop structure (Fig. 4b, c). In LUS3, the stem–loop is located at the very 5’ end and in LUS4, 22 unpaired bases precede the stem–loop.

LUS3 and LUS4 were grown to $t = 1.5$, at which time rifampicin was added to the cultures. RNA was extracted at different times and Northern blots were performed. The modifications in LUS3 and LUS4 destabilized the aprE–lacZ transcript, giving a half-life of 5–6 min (Fig. 5).

**The extreme stability of aprE mRNA does not change with growth stage**

Resnekov et al. (1990) reported that when a B. subtilis culture in early stationary phase was diluted into fresh medium, the steady-state level of aprE mRNA rapidly declined. This finding was interpreted as being due to a growth-stage-related destabilization of aprE mRNA. We have repeated and confirmed these results (Fig. 6). However, the relative rate of synthesis of rRNA rapidly increases following dilution. In Fig. 7 it is seen that the amount of rRNA increased about threefold within 30 min after a shift-up. This is in accordance with the classical observations made with E. coli following a growth shift-up (Bremer & Dennis, 1996). Thus, a rapid decline of aprE mRNA upon dilution into fresh medium could reflect a rapid dilution of this mRNA in the total RNA pool rather than accelerated degradation. To determine the half-life of aprE mRNA at various times after dilution is difficult because transcription from the aprE promoter is rapidly shut off. However, the half-life of the aprE–lacZ fusion transcript produced from the constitutive glpD promoter can readily be determined after dilution of stationary-phase cells into fresh medium as well as in exponentially growing cells. The results of such experiments using strain LUS2 show that even 30 min after dilution into fresh medium, when growth has well resumed, the aprE–lacZ mRNA is still ex-
The aprE gene is one of a number of B. subtilis genes that code for secreted proteins which are synthesized after growth has stopped and which are under (partly) common transcriptional control (Ferrari et al., 1993). Another well-studied gene in this category is amyE, which encodes α-amylase. The half-life of amyE mRNA was measured in B. subtilis in early stationary phase and determined to be about 5 min (Fig. 9), i.e. similar to that of bulk B. subtilis mRNA. To compare the properties of the fusion transcripts with those of the native amyE transcript, a fusion between the amyE promoter region, the amyE leader and lacZ was made analogously to the previously described fusion involving aprE. The fusion was inserted into the B. subtilis thrC gene to give strain LUS5. LUS5 was grown in NSMP and at different points, samples were taken for determination of β-galactosidase activity. The activity was very low during growth and increased fivefold as the cells entered stationary phase (data not shown). These results confirm that expression of the amyE–lacZ fusion is under the same temporal control as the amyE gene itself.

To measure the half-life of the amyE–lacZ fusion transcript, we constructed LUS6, in which the tightly controlled amyE promoter is replaced by the constitutive glpD promoter. LUS6 was grown in NSMP to early stationary phase, at which time rifampicin was added to the culture. Total RNA was extracted at different times and the relative amounts of amyE–lacZ transcript were determined in Northern blots using a probe specific for lacZ. The half-life of the amyE–lacZ fusion transcript was similar to that of the native amyE transcript, i.e. about 5 min (Fig. 9). This demonstrates that the amyE leader is a major determinant of amyE mRNA stability and also shows the general validity of using fusion transcripts for comparisons of rates of mRNA decay.

**DISCUSSION**

B. subtilis aprE mRNA has been shown previously to be extremely stable in stationary-phase cells, with a half-life of at least 25 min (Resnekov et al., 1990); the present work confirms this finding. Furthermore, in that earlier paper, experiments were presented which were interpreted to show that in growing cells, the rate of degradation of aprE mRNA was increased four- to fivefold, indicating that the stability of this mRNA was regulated by growth stage. In growing cells, the aprE promoter is effectively shut off, which complicates measurements of the decay of the aprE transcript in a growth-shift experiment. To circumvent this problem, decay of aprE mRNA was measured as decay of the steady-state amount of the transcript at various times after a shift from stationary phase to growth.

In the present work we have shown that the determinant(s) for the extreme stability of aprE mRNA is located in the 5′ leader. A transcript of a transcriptional-translational fusion between the aprE leader and E. coli lacZ was found to have the same stability as the native aprE transcript. Furthermore, substituting the aprE promoter with the constitutive B. subtilis glpD promoter had no effect on the rate of decay of the fusion transcript. By using the glpD promoter one can directly measure the rate of decay of the aprE–lacZ fusion transcript (or the aprE transcript) in growing and stationary-phase cells.
The results of such measurements show that the half-life of the aprE-lacZ fusion transcript is at least 25 min under all conditions tested. Thus, contrary to previous suggestions (Resnekov et al., 1990) there is no growth-stage-related control of aprE mRNA stability. The increased rate of degradation of aprE mRNA on shift-up observed by Resnekov et al. (1990) is spurious. The shift-up is immediately followed by a selective increase in stable RNA whereas the aprE promoter is turned off. In experiments of Resnekov et al. (1990) this leads to an apparent increase in degradation rate of aprE mRNA which is simply due to a dilution effect.

There are few data available which compare the stability of a specific B. subtilis mRNA at different growth stages, but increased stability is not a general property of stationary-phase mRNA (Melin et al., 1989). In the present work we have also determined the half-life of amyE mRNA, which encodes another stationary-phase-specific B. subtilis exoenzyme, to be 5 min. This shows that extreme stability is not a general property of exoenzyme transcripts. Whatever the physiological reason for the exceptionally high stability of the aprE mRNA, it contributes to a high level of production of subtilisin in the ‘stationary-phase window’ where the aprE promoter is active.

Very few bacterial transcripts of extreme stability have been characterized, ermC and gsiB being two of the best-known examples in B. subtilis. The half-life of the ermC transcript increases about 20-fold upon exposure of the bacteria to erythromycin (Bechhofer & Zen, 1989). Binding of the drug to ribosomes translating a short ORF preceding the coding region for the ErmC protein stalls the ribosomes at this ORF. The stalling protects the whole ermC transcript from degradation, possibly by shielding a nuclease-sensitive site and/or affecting the secondary structure of the transcript. The gsiB transcript, which is produced from a SigB-dependent promoter and encodes a stress-related protein, has a half-life of at least 20 min (Jurgen et al., 1998). The gsiB gene has a very strong RBS with an optimal spacing to the AUG start codon. Mutations that weaken the gsiB RBS lead to a decrease in the half-life of the transcript, suggesting that a major factor in determining the gsiB mRNA half-life is the interaction between the RBS and 16S rRNA. However, RBS mutations also lead to some changes in the predicted secondary structure of the gsiB mRNA leader sequence, which complicates the interpretation of these experiments.

The free energy of binding of the RBS and the 3′ end of 16S rRNA is very similar for gsiB, aprE and amyE (Tinoco et al., 1973) and yet the amyE transcript decays at least five times faster than those of the other two genes. It should be noted that the start codon is AUG for gsiB and amyE but GUG for aprE. Thus, a model suggesting the RBS–16S rRNA interaction to be a major determinant for mRNA stability is hardly of general validity. However, the interaction between 16S rRNA and the mRNA leader in forming the initiation complex is a function not only of base complementarity but also of the secondary structure of the leader (Yamanaka et al., 1999). The favoured predicted secondary structure of the wild-type aprE leader is a stem–loop at the very 5′ end with the RBS contained in a region with weak interactions (Fig. 4a). The computer prediction also suggests an alternative structure with similar free energy. However, the formation of this structure involves opening the already folded stem-loops shown in Fig. 4(a). That makes this alternative structure less likely. Changing two bases in the leader (G31 and A32 both changed to T) leads to a large structural change with a perfect stem–loop containing the RBS and preceded by 22 unpaired bases (Fig. 4b). This transcript has a half-life of about 5 min. The same stem–loop was obtained by deleting 25 nt in the leader (Fig. 4c). Also this modification led to a major destabilization of the transcript. Deleting a single-stranded 5′ end is known to stabilize some E. coli transcripts (Emory et al., 1992).

Clearly, the secondary structure of the aprE transcript is important for the extreme stability of aprE mRNA. This may simply depend on occlusion of RNase-sensitive sites, but other factors may also be involved, e.g. specific (temporary or permanent) binding of proteins to the transcript. This is a known mechanism for stabilizing or destabilizing transcripts. For example, B. subtilis glpD mRNA can be stabilized by the GlpP antiterminator protein (Glatz et al., 1996) and in E. coli, the ompA and glgC transcripts are destabilized by the Hfq and CsrA proteins, respectively (Vytvytska et al., 1998, Liu & Romeo, 1997). Finally, we wish to point out that there are striking sequence similarities between the sequence around the aprE RBS and that of a polypurine-rich sequence in bacteriophage SP82 which functions as a 5′ stabilizer in B. subtilis (Hue et al., 1995).

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REFERENCES


Ayer, D. E. & Dynan, W. S. (1988). Simian virus 40 major late promoter: a novel tripartite structure that includes intragenic sites, but other factors may also be involved, e.g. specific (temporary or permanent) binding of proteins to the transcript. This is a known mechanism for stabilizing or destabilizing transcripts. For example, B. subtilis glpD mRNA can be stabilized by the GlpP antiterminator protein (Glatz et al., 1996) and in E. coli, the ompA and glgC transcripts are destabilized by the Hfq and CsrA proteins, respectively (Vytvytska et al., 1998, Liu & Romeo, 1997). Finally, we wish to point out that there are striking sequence similarities between the sequence around the aprE RBS and that of a polypurine-rich sequence in bacteriophage SP82 which functions as a 5′ stabilizer in B. subtilis (Hue et al., 1995).

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REFERENCES


Ayer, D. E. & Dynan, W. S. (1988). Simian virus 40 major late promoter: a novel tripartite structure that includes intragenic sites, but other factors may also be involved, e.g. specific (temporary or permanent) binding of proteins to the transcript. This is a known mechanism for stabilizing or destabilizing transcripts. For example, B. subtilis glpD mRNA can be stabilized by the GlpP antiterminator protein (Glatz et al., 1996) and in E. coli, the ompA and glgC transcripts are destabilized by the Hfq and CsrA proteins, respectively (Vytvytska et al., 1998, Liu & Romeo, 1997). Finally, we wish to point out that there are striking sequence similarities between the sequence around the aprE RBS and that of a polypurine-rich sequence in bacteriophage SP82 which functions as a 5′ stabilizer in B. subtilis (Hue et al., 1995).

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Stability of B. subtilis aprE mRNA


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