

Different Processing of an mRNA Species in *Bacillus subtilis* and *Escherichia coli*

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Received 30 July 1999/Accepted 18 October 1999

Expression of the *Bacillus subtilis* *glpD* gene, which encodes glycerol-3-phosphate (G3P) dehydrogenase, is controlled by termination or antitermination of transcription. The untranslated leader sequence of *glpD* contains an inverted repeat that gives rise to a transcription terminator. In the presence of G3P, the antiterminator protein GlpP binds to *glpD* leader mRNA and promotes readthrough of the terminator. Certain mutations in the inverted repeat of the *glpD* leader result in GlpP-independent, temperature-sensitive (TS) expression of *glpD*. The TS phenotype is due to temperature-dependent degradation of the *glpD* mRNA. In the presence of GlpP, the *glpD* mRNA is stabilized. *glpD* leader-*lacZ* fusions were integrated into the chromosomes of *B. subtilis* and *Escherichia coli*. Determination of steady-state levels of fusion mRNA in *B. subtilis* showed that the stability of the fusion mRNA is determined by the *glpD* leader part. Comparison of steady-state levels and half-lives of *glpD* leader-*lacZ* fusion mRNA in *B. subtilis* and *E. coli* revealed significant differences. A *glpD* leader-*lacZ* fusion transcript that was unstable in *B. subtilis* was considerably more stable in *E. coli*. GlpP, which stabilizes the transcript in *B. subtilis*, did not affect its stability in *E. coli*. Primer extension analysis showed that the *glpD* leader-*lacZ* fusion transcript is processed differently in *B. subtilis* and in *E. coli*. The dominating cleavage site in *E. coli* was barely detectable in *B. subtilis*. This site was shown to be a target of *E. coli* RNase III.

The steady-state level of mRNA in a cell is a function of the rate of mRNA synthesis and the rate of its decay. For bacteria, there is a wealth of information on the regulation of mRNA synthesis (see, e.g., reference 27), while much less is known about mechanisms of mRNA decay (5, 6, 35).

Most of our knowledge about bacterial mRNA decay is based on studies of *Escherichia coli* (26, 34). In a simple model, an initial endoribonucleolytic attack at the 5' end of an mRNA opens up the molecule for internal downstream cleavages and the fragments generated are subsequently degraded by exoribonucleases (12). The initial cleavage is performed by one of two endoribonucleases, RNase E, encoded by the *rne* gene (7), or RNase III, encoded by the *rnc* gene (3, 8). The endonucleolytic activity of RNase E is localized to the N-terminal half of the protein, which, unlike the C-terminal half, is essential for *E. coli* viability (29, 31). RNase III is primarily involved in maturation of stable RNA but also in degradation of some mRNA species. The hydrolytic exoribonuclease RNase II and the phosphorolytic exoribonuclease polynucleotide phosphorylase (PNPase) are important for the final (3'-to-5') degradation of an mRNA to mono- and oligonucleotides. For a few *E. coli* mRNA species, binding of specific proteins has been found to have a decisive influence on mRNA half-life (28, 43).

Much less is known about mRNA degradation in *Bacillus subtilis*. In several bacterial species, but not *B. subtilis*, sequence homologues to the N-terminal part of RNase E have been found (24, 25). *E. coli* RNase III has a homologue in *B. subtilis* called Bs-RNase III which has been shown to cleave rRNA in an *E. coli* Rnc mutant (45). However, *E. coli* RNase III cannot cleave a *B. subtilis* phage SP82 mRNA species at a

site which is cleaved by Bs-RNase III (33). RNase III may be an essential enzyme in *B. subtilis* (36), but it is not in *E. coli* (3). PNPase accounts for more than 90% of the exoribonuclease activity in *B. subtilis* cell extracts. It is unclear if the cells also contain an enzyme related to RNase II (10). However, the gene for PNPase can be deleted in *B. subtilis* with little effect on overall cell physiology or the half-life of bulk mRNA (44).

Transcription of the *B. subtilis* *glpD* gene (and other *glp* genes) is controlled by termination or antitermination of transcription at an inverted repeat in the 5' untranslated leader of *glpD* mRNA (21, 22, 38). We have isolated a number of mutants carrying mutations in the *glpD* leader which allow increased transcription through the inverted-repeat region. These mutants have enhanced levels of the *glpD* gene product and grow on glycerol as a sole carbon and energy source in the absence of an activated form of the antiterminator protein GlpP. Some of the corresponding mutants are temperature sensitive (TS) for growth on glycerol. This phenotype has been shown to be due to an increased, temperature-dependent rate of degradation of *glpD* mRNA. The TS phenotype is suppressed by the GlpP protein in the presence of glycerol-3-phosphate (G3P), which is the inducer of the *glp* regulon (17). It is possible that the wild-type *glpD* transcript is also TS in the absence of GlpP and G3P. However, we have not yet succeeded in producing sufficient amounts of wild-type *glpD* transcript under noninducing conditions to test this possibility. With this caveat in mind, we will refer to the class of *glpD* leader mutations described above as TS.

In the present work we have studied the decay of *B. subtilis* wild-type and TS *glpD* leader transcripts in *B. subtilis* and *E. coli*. Fusions were constructed between *B. subtilis* wild-type and TS *glpD* leaders and the *E. coli* *lacZ* gene, and the fusions were integrated into the chromosomes of *B. subtilis* and *E. coli*. In *B. subtilis* we have found that the stability of the fusion transcript is determined by the *glpD* leader sequence, i.e., in the absence of GlpP, a TS leader causes rapid and temperature-dependent degradation of the fusion transcript. In *E. coli* the TS fusion transcript is much more stable and decays at the same rate as

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Characteristics	Source or reference
<i>B. subtilis</i>		
BR95	<i>trpC2 pheA1 ilvC1</i>	Our collection
LUR252	<i>trpC2 ilvC1 glpP12 glpD52</i> (insertion of an extra GC in the <i>glpD</i> leader)	17
LUZ9595	BR95 with insertion of a wild-type <i>glpD</i> leader- <i>lacZ</i> fusion into <i>amyE</i> ; Km ^r	18
LUZ1212	LUR252 with insertion of a LUR252 <i>glpD</i> leader- <i>lacZ</i> fusion into <i>amyE</i> ; Km ^r	This work
<i>E. coli</i>		
MC4100	F ⁻ <i>araD139 Δ(argF-lac)U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR</i>	14
MC4100D1	MC4100 [λφ(wild-type <i>glpD</i> leader- <i>lacZ</i> fusion)]	16
MC4100D2	MC4100 [λφ(LUR252 <i>glpD</i> leader- <i>lacZ</i> fusion)]	This work
BL321	F ⁻ <i>thi-1 argH1 gal-6 lacY1 mtl-2 xyl-7 malA1 ara-13 str-9 tonA2 lambda^d supE44 mc-105</i>	40
BL322	As BL321 but <i>mc</i> ⁺	40
Plasmids		
pHP13	Cm ^r Em ^r	19
pPHis1	Derivative of pHP13 carrying a <i>glpP</i> gene coding for GlpP with six extra carboxy-terminal histidine residues	18
pMD433	<i>ΔamyE::lacZ</i> ; Km ^r Ap ^r	9
pLUM1041	Derivative of pMD433; <i>ΔamyE::wild-type glpD</i> leader- <i>lacZ</i> fusion	18
pLUM1043	Derivative of pMD433; <i>ΔamyE::LUR252 glpD</i> leader- <i>lacZ</i> fusion	This work

the wild-type fusion transcript. Additionally, GlpP does not influence the decay of the fusion transcripts in *E. coli* although it does function as a specific antiterminator protein in the species (16). Finally, we show that the cleavage patterns at the 5' ends of the fusion transcripts are distinctly different in *E. coli* and *B. subtilis*. The most striking difference is that the major cleavage product in *E. coli* is barely detectable in *B. subtilis*. This cleavage product is missing in an *E. coli* Rnc mutant.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this work are listed in Table 1.

Growth of bacteria for extraction of RNA. *B. subtilis* was grown in minimal salts (1) with 0.5% casein hydrolysate and required amino acids (40 mg liter⁻¹) with shaking at 200 rpm. The bacteria were grown at different temperatures to an optical density at 600 nm (OD₆₀₀) of 0.5. The cultures were then induced with glycerol (1.5 g liter⁻¹) for 15 min. *E. coli* was grown in Luria broth containing 40 mM G3P with shaking at 200 rpm. The bacteria were grown at different temperatures to an OD₆₀₀ of 0.5.

Samples were taken for RNA extraction, or the cells were incubated with rifampin (*B. subtilis*, 100 mg liter⁻¹; *E. coli*, 500 mg liter⁻¹) and nalidixic acid (*E. coli*, 20 mg liter⁻¹) for various times before samples were taken. The 0-min samples were taken 2 min after addition of the antibiotics.

Construction of strains. *B. subtilis* LUZ1212 was obtained by transforming *B. subtilis* LUR252 with pLUM1043 and isolating a kanamycin-resistant, amylase-negative transformant according to the protocol for the isolation of *B. subtilis*

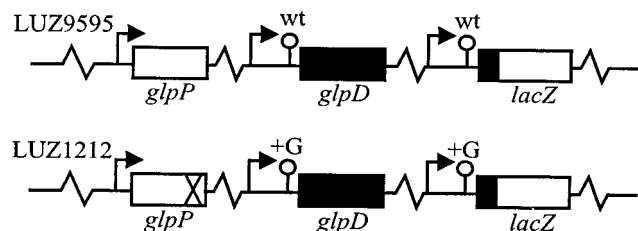


FIG. 1. Schematic representation of *B. subtilis* LUZ9595 and LUZ1212. The *glpD* promoter and leader from BR95 and LUR252 were amplified by PCR and cloned in frame with *lacZ* in pMD433. The *glpD* leader-*lacZ* fusions were integrated into the chromosome at the *amyE* locus in the cognate strain. \blacktriangleright , promoter; \circ , *glpD* leader with inverted repeat; X, *glpP12* mutation; wt, wild type; +G, insertion of an extra GC pair in the inverted repeat; \blacktriangleleft , intervening chromosomal DNA.

LUZ9595 (18). Plasmid pLUM1043 was constructed in the same way as pLUM1041 with chromosomal DNA from LUR252 as a template for PCR (18).

E. coli MC4100D2 was constructed in analogy with *E. coli* MC4100D1 as described by Glatz et al. (16), with chromosomal DNA from *B. subtilis* LUR252 as a template for PCR.

DNA and RNA techniques. PCR and DNA cloning techniques were applied according to standard protocols (39). Total RNA from *B. subtilis* was extracted as described by Resnekov et al. (37). Total RNA from *E. coli* was extracted as described by Emory et al. (13). Electrophoresis of RNA for Northern blots was done as described by Thomas (42), and the RNA was then blotted onto Hybond-N filters (Amersham). A single-stranded (ss) DNA probe for Northern blots was generated by ssPCR with primer GlpDBamII (18), cold d(A, G, T)TP, and [α -³²P]dCTP (Amersham). To generate the template for the ssPCR, a fragment was amplified by PCR from *B. subtilis* LUR252 with primers GlpDBamI (18) and GlpDBamII. The PCR fragment was cleaved with *Ava*II, and a 215-bp fragment containing part of the *glpD* leader together with the first 33 codons of *glpD* was isolated for use as a template. After hybridization, the radioactivities of the bands were quantitated with a PhosphorImager (Molecular Dynamics). Primer extension analysis was performed according to the method of Ayer and Dynan (2). The primer used was complementary to positions +111 to +130 of the *glpD* leader (5'-ATTGATGATTCATCATTACG-3').

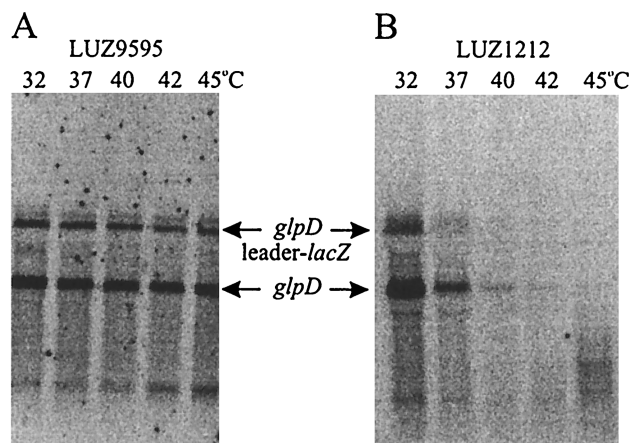


FIG. 2. Northern blots showing steady-state levels of *glpD* and *glpD* leader-*lacZ* mRNA in *B. subtilis* LUZ9595 (wild-type *glpD* leader) (A) and *B. subtilis* LUZ1212 (mutant *glpD* leader) (B). Total RNA was extracted from cells grown and induced at the temperatures indicated. The lanes contained 5 μ g of LUZ9595 RNA and 20 μ g of LUZ1212 RNA.

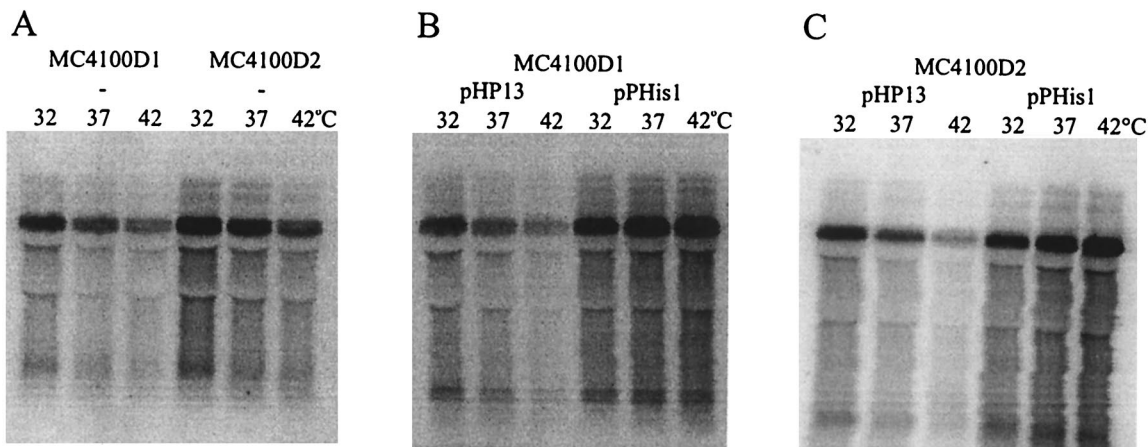


FIG. 3. Northern blots showing steady-state levels of *glpD* leader-*lacZ* mRNA in *E. coli* MC4100D1 (wild-type *glpD* leader) and MC4100D2 (mutant *glpD* leader) carrying no plasmid (-), pHP13, or pPHis1. Total RNA was extracted from cells grown at the temperatures indicated. The lanes contained 20 μ g of RNA, except the MC4100D1 plus pPHis lanes, which contained 10 μ g of RNA.

RESULTS

The *glpD* leader is a stability determinant of *glpD* leader-*lacZ* fusion mRNA. We have previously shown that the 5' untranslated leader of the *B. subtilis glpD* transcript affects its half-life (17). In order to determine whether interactions between the leader and other parts of the *glpD* transcript are important for stability, the following experiments were done. Gene fusions were made in which a DNA fragment of about 400 bp containing the 5' part of the *glpD* region, including the promoter, the leader sequence, and the first 33 codons, was coupled in frame to *E. coli lacZ*. Two fusions were made, one with the wild-type *glpD* leader sequence from *B. subtilis* BR95 and the other with the mutant *glpD* leader sequence from *B. subtilis* LUR252. The mutant leader sequence has an extra GC pair in the inverted repeat, i.e., the leader RNA has an extra G, which leads to increased constitutive (GlpP-independent) expression of the *glpD* gene. The *glpD* transcripts produced in the absence of GlpP are TS. The fusions were inserted in single copies into the *amyE* gene of *B. subtilis*, the wild-type *glpD* leader fusion was inserted into BR95, and the mutant *glpD* leader fusion was inserted into LUR252. A schematic description of the resulting strains, LUZ9595 and LUZ1212, is given in Fig. 1.

The steady-state levels of *glpD* mRNA and *glpD* leader-*lacZ* fusion mRNA in LUZ9595 and LUZ1212 were measured under inducing conditions and at different temperatures ranging from 32 to 45°C. The mRNA was analyzed in Northern blots with a probe specific for the *glpD* leader. As can be seen in Fig. 2, both a *glpD* and a *glpD* leader-*lacZ* fusion transcript are detected at all temperatures in induced LUZ9595, and the

amounts are similar at all temperatures. The smaller band, which increases in intensity with temperature, represents a truncated fusion transcript that also hybridizes with a *lacZ*-specific probe (data not shown). In LUZ1212, the steady-state levels of *glpD* and *glpD* leader-*lacZ* mRNA rapidly decrease with increasing growth temperature and transcripts are not

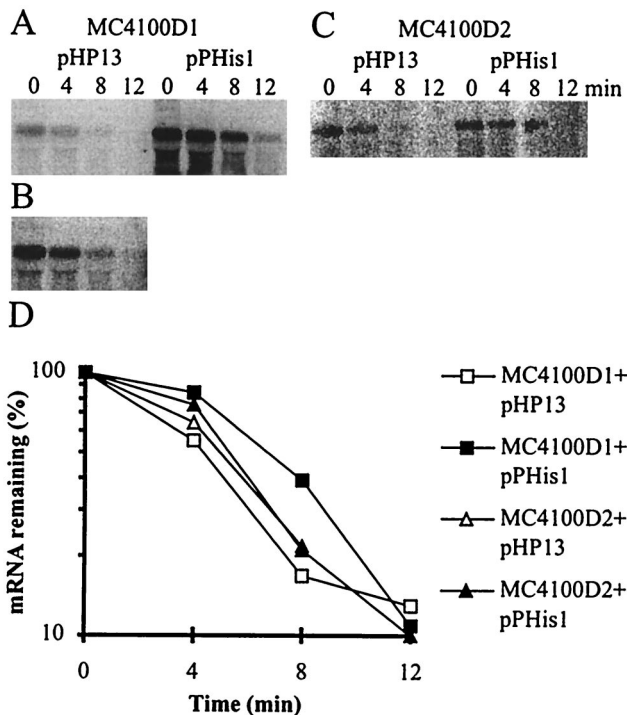


FIG. 4. Northern blots showing degradation of wild-type *glpD* leader-*lacZ* mRNA in *E. coli* MC4100D1 carrying pHP13 or pPHis1 (A), wild-type *glpD* leader-*lacZ* mRNA in *E. coli* MC4100D1 carrying pHP13 (overexposed film) (B), or mutant *glpD* leader-*lacZ* mRNA in *E. coli* MC4100D2 carrying pHP13 or pPHis1 (C). (D) Half-life plots. The cells were grown at 42°C, and total RNA was extracted at 0, 4, 8, and 12 min after the addition of rifampin and nalidixic acid. The lanes contained the following amounts of RNA: MC4100D1 plus pHP13, 20 μ g; MC4100D1 plus pPHis1, 10 μ g; MC4100D2 plus pHP13, 40 μ g; MC4100D2 plus pPHis1, 5 μ g.

TABLE 2. Comparison of steady-state levels of wild-type and mutant *glpD* leader-*lacZ* mRNA in *E. coli* MC4100D1 and MC4100D2 at different temperatures^a

Plasmid	MC4100D1/MC4100D2 ratio		
	32°C	37°C	42°C
None	1	1.0	1.1
pHP13	1	1.0	1.1
pPHis1	1	1.1	0.8

^a Relative amounts of mRNA were calculated from Fig. 3.

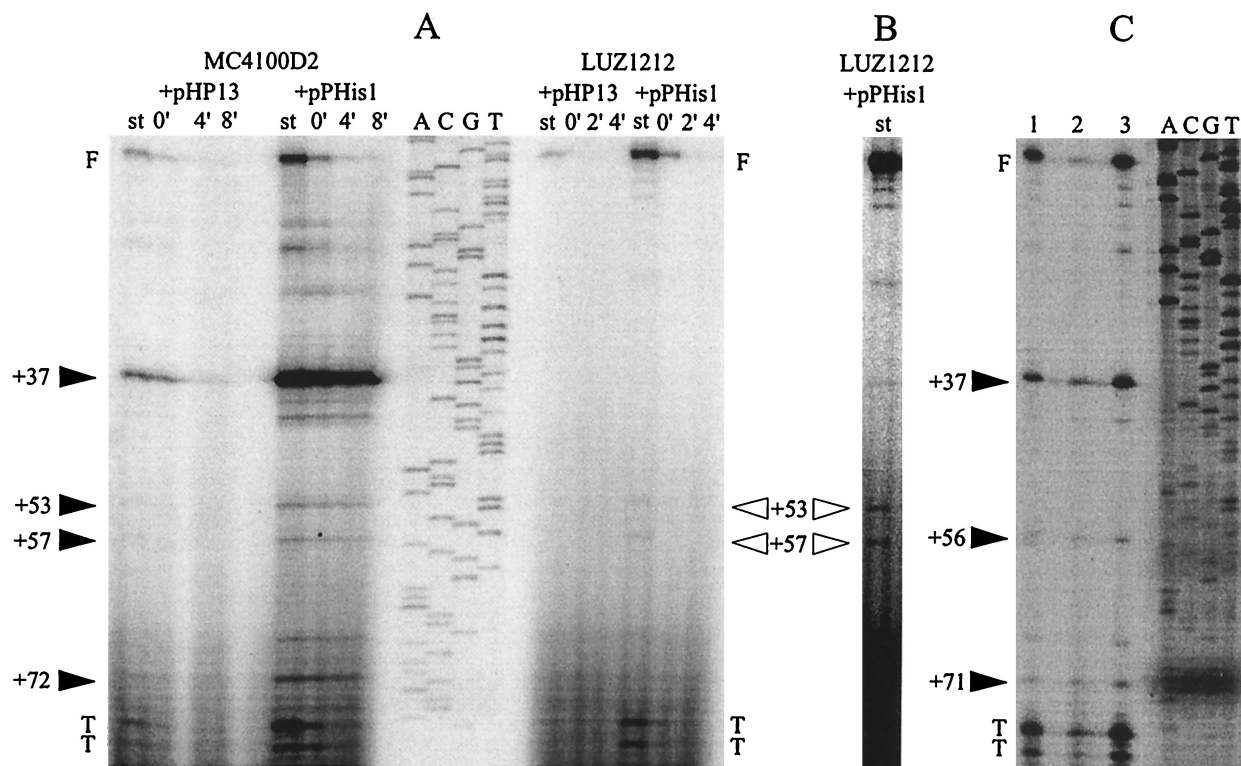


FIG. 5. (A) Primer extension analysis of 5' end points in *glpD* leader mRNA in *E. coli* MC4100D2 and *B. subtilis* LUZ1212 carrying pHP13 or pPHis1. *E. coli* was grown at 42°C, and *B. subtilis* was grown and induced at 45°C. Total RNA was extracted from samples taken immediately before the addition of rifampin (*B. subtilis*) or rifampin and nalidixic acid (*E. coli*) (st) and at various times thereafter. The 0-min (0') samples were taken 2 min after the addition of the antibiotics. The solid arrowheads indicate possible endonucleolytic cleavage sites in *E. coli*, and the open arrowheads indicate cleavage sites in *B. subtilis*. F indicates full-length transcripts, and T indicates fragments caused by primer extension termination at secondary structures in the base of the terminator. (B) A longer exposure of the lane containing steady-state RNA from LUZ1212 plus pPHis1. (C) Primer extension analysis of 5' end points in *glpD* leader mRNA in *E. coli*. Lane 1, MC4100 carrying pLUM1041 (contains a wild-type *glpD* leader-*lacZ* fusion); lane 2, MC4100D1 carrying pHP13; lane 3, MC4100D1 carrying pPHis1. Total RNA was extracted from cells grown at 42°C.

detectable above 40°C. After the membranes had been probed with the *glpD* probe, they were stripped and reprobbed with a DNA fragment specific for the *sdhC* gene (32). The steady-state levels of *sdhC* mRNA were essentially the same at all temperatures in both strains (data not shown). The β -galactosidase and G3P dehydrogenase (GlpD) activities of LUZ9595 and LUZ1212 measured under inducing conditions at 32 and 45°C correlated well with the corresponding mRNA levels (15). From these results we conclude that the *glpD* leader is a major stability determinant for both the *glpD* and the *glpD* leader-*lacZ* fusion transcripts in *B. subtilis*.

A *B. subtilis glpD* leader transcript is more stable in *E. coli* than in *B. subtilis*. More is known about mRNA degradation in *E. coli* than in any other bacterium, and mutants affected in different components of the mRNA degradation machinery are available (26). We next wanted to take advantage of *E. coli* to further analyze the decay of *B. subtilis* wild-type and TS *glpD* leader-*lacZ* fusion transcripts. It should be emphasized that GlpP also promotes antitermination of transcription at the *glpD* leader in *E. coli* (16). The inverted repeat in the *glpD* leader sequence is, however, a less efficient stop signal in *E. coli* than in *B. subtilis*, as evidenced by a high background of expression of *glpD* leader-*lacZ* fusions in *E. coli*. This difference makes possible an analysis of wild-type *glpD* leader-*lacZ* fusion transcripts in *E. coli* in the absence of GlpP. In vitro runoff transcriptional analysis has shown that *E. coli* sigma-70 RNA polymerase passes through the inverted repeat unaided,

whereas no readthrough was detected with *B. subtilis* sigma-A RNA polymerase holoenzyme (15).

In a previous report (16), the wild-type *glpD* leader-*lacZ* fusion was integrated into the chromosome of *E. coli* to give strain MC4100D1, and similarly, the mutant *glpD* leader-*lacZ* fusion was now integrated to give strain MC4100D2. Plasmids pHP13 and pPHis1 were then introduced into MC4100D1 and MC4100D2. pHP13 is a *B. subtilis*-*E. coli* shuttle plasmid, and pPHis1 is a derivative which carries a gene coding for a His-tagged and biologically active derivative of GlpP (18). The relative steady-state levels of *glpD* leader-*lacZ* mRNA were measured in MC4100D1 and MC4100D2 grown at 32, 37, and 42°C and in the presence (pPHis1) or absence (pHP13) of GlpP. The resulting Northern blots are shown in Fig. 3. The relative steady-state level of the fusion transcript at 32°C was assigned an arbitrary value of 1 for each strain. The steady-state levels at the other temperatures were then calculated relative to the value at 32°C. By dividing the values for the MC4100D1 strains with the values for the MC4100D2 strains at each temperature, we obtained a comparative measure of the temperature stability of the two fusion transcripts (Table 2). These experiments demonstrate that there is no temperature-dependent difference between the steady-state amounts of the *glpD* leader-*lacZ* fusion transcripts in MC4100D1 and MC4100D2. The steady-state amounts of the transcripts increase in the presence of GlpP. Since it is shown below that GlpP does not increase the relative stability of the transcripts,

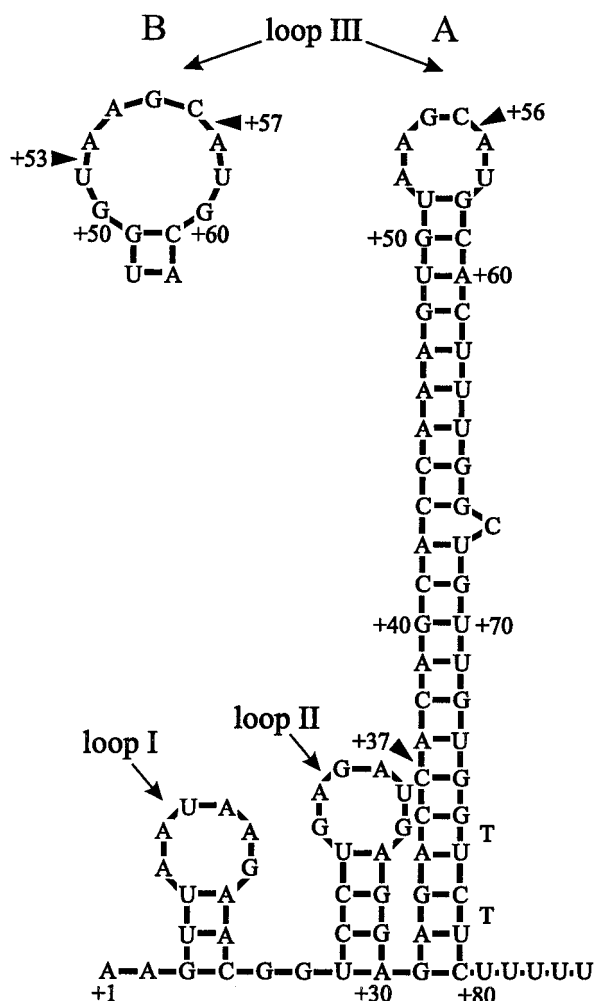


FIG. 6. (A) Computer-predicted folding of the first 84 nucleotides (46) of wild-type *glpD* leader mRNA. The arrowheads indicate the +37 and +56 cleavage sites in *E. coli*. T indicates sites of primer extension termination caused by secondary structures. (B) Predicted folding of loop III of mutant *glpD* leader mRNA. The arrowheads indicate the +53 and +57 cleavage sites, which are seen in both *B. subtilis* and *E. coli*.

this increase should be due to the antitermination effect of GlpP (16). For unknown reasons, this effect is more pronounced at higher temperatures.

To confirm the above-mentioned results, the half-lives of the two fusion transcripts were measured at 42°C in the presence and absence of GlpP (Fig. 4). Linear regression analysis gave a half-life of 3 to 4 min in all cases. Importantly, the experiments show that the mutant *glpD* leader-*lacZ* fusion transcript decays much more slowly in *E. coli* than in *B. subtilis*, where a transcript from the mutant *glpD* leader has a half-life of about 1 min at 32°C and less than 20 s at 45°C (17). Furthermore, the presence of GlpP does not increase the stability of the transcript as it does in *B. subtilis*.

A *glpD* leader transcript is differently processed in *B. subtilis* and *E. coli*. The previous experiments showed that a *glpD* leader transcript which is TS in *B. subtilis* is much more stable in *E. coli*. The following experiments were done to investigate whether this reflects different processing of the 5' region of the transcript in the two bacteria. RNA was extracted from *B. subtilis* LUZ1212 and *E. coli* MC4100D2, both having in their

chromosomes the mutant *glpD* leader-*lacZ* fusion and carrying pPHP13 or pPHis1. *B. subtilis* was grown at 45°C and *E. coli* at 42°C. RNA samples were taken immediately before the addition of rifampin (*B. subtilis*) or rifampin and nalidixic acid (*E. coli*) and at various times thereafter. Primer extension products obtained with RNA from each sample were then characterized. The primer used is complementary to a region just downstream of the inverted repeat of the *glpD* leader. Very different patterns of primer extension products were obtained from the two bacteria (Fig. 5A and B). The most prominent band in *E. coli* (MC4100D2) is at position +37. This band is barely detectable in *B. subtilis* (LUZ1212 plus pPHis1). It was not possible to identify breakdown products from *B. subtilis* in the absence of GlpP (LUZ1212 plus pPHP13) due to the small amounts of fusion mRNA obtained. The bands obtained with *E. coli* have higher intensities in the presence of GlpP (MC4100D2 plus pPHis1), but otherwise the pattern is not different from that seen in the absence of GlpP (MC4100D2 plus pPHP13). Besides the +37 band, many less prominent bands are seen in *E. coli*, while only a few are seen in *B. subtilis*. Two bands, +53 and +57, are clearly seen in both bacteria. Figure 5C shows the results obtained with wild-type *glpD* leader-*lacZ* mRNA from *E. coli* MC4100D1 and MC4100 carrying pLUM1041, which contains a wild-type *glpD* leader-*lacZ* fusion. The patterns of primer extension products, including the +37 band, are similar to those of mutant *glpD* leader mRNA from *E. coli* MC4100D2. However, the +53 band is not obtained with wild-type *glpD* leader mRNA in *E. coli*. We will return to this in the

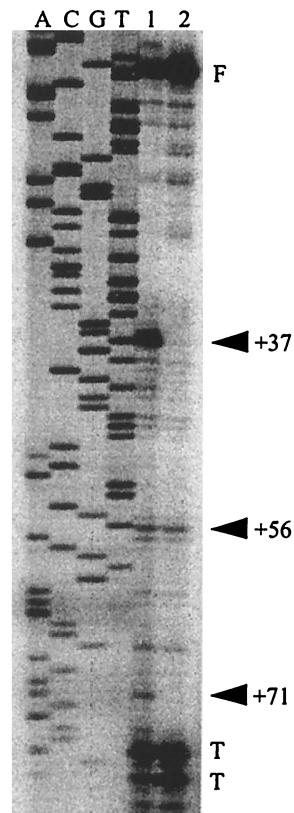


FIG. 7. Primer extension analysis of 5' end points in wild-type *glpD* leader mRNA in *E. coli* carrying pLUM1041 (containing a wild-type *glpD* leader-*lacZ* fusion). Lane 1, BL322 (wild type); lane 2, BL321 (RNase III deficient). Total RNA was extracted from cells grown at 42°C. The symbols are defined in the legend to Fig. 5.

discussion. It should be noted that the +56 and +71 bands in the wild-type *glpD* leader mRNA correspond to the +57 and +72 bands in the mutant *glpD* leader mRNA due to the extra G in the latter. In Fig. 5C, it is also seen that, similar to what was found with mutant *glpD* leader mRNA, the cleavage pattern of wild-type *glpD* leader mRNA in *E. coli* is not affected by GlpP. Figure 6 shows the predicted secondary structures of wild-type and mutant *glpD* leader mRNAs, with cleavage sites +37, +53, and +56-57 indicated.

In a control experiment, *glpD* leader mRNA produced in vitro was used as a template for primer extension. The major bands found were a full-length transcript and some shorter products representing a stop at the 3' end of the stem-loop (data not shown). Thus, we can conclude that degradation intermediates from the 5' end of the *glpD* leader-*lacZ* fusion transcript are very different in *B. subtilis* and in *E. coli*.

To examine the possibility that the +37 fragment in *E. coli* is produced from a promoter downstream of the *glpD* promoter, we made a deletion starting at the 5' end of the DNA fragment containing the *glpD* promoter and leader sequence and ending at position -6. The deletion caused the fusion transcript to disappear, indicating that no additional promoter is present downstream of the *glpD* promoter.

The +37 cleavage product is missing in an *E. coli* Rnc mutant. Next, we investigated whether either of the two major endoribonucleases of *E. coli* is responsible for cleaving at +37 in *glpD* leader mRNA. Plasmid pLUM1041 was introduced into an *E. coli* TS Rnc mutant and an *E. coli* Rnc mutant. The Rnc mutant was grown at 32°C and shifted to 45°C for 30 min; the Rnc mutant was grown at 37°C. Total RNA was extracted, and primer extension products were characterized. The result with the Rnc mutant is shown in Fig. 7, where it is seen that the +37 band is missing, which implies that this band is generated by the action of RNase III. A band of much lower intensity at +71 in the wild type is also missing in the mutant. The RNase E-deficient mutant gave the same pattern of primer extension products as the wild type (data not shown).

DISCUSSION

There exists considerable experimental evidence that the 5' end of an mRNA molecule is an important stability determinant in both *B. subtilis* and *E. coli* (4, 11, 13, 20, 30, 32, 41). However, the structures or conditions at the 5' end which influence mRNA stability may not always be the same in the two bacteria. For example, ribosome-binding sites, whether coupled to translation or not, can stabilize a *B. subtilis* transcript but not an *E. coli* transcript (23). The present experiments demonstrate that the *glpD* leader sequence determines the steady-state amounts of a TS *glpD* leader-*lacZ* fusion transcript in *B. subtilis*. Thus, the *B. subtilis glpD* leader contains the major stability determinant for the corresponding mRNA. The TS fusion transcript is about 10-fold more stable in *E. coli* than in *B. subtilis*, indicating different degradation pathways in the two bacteria.

Different processing of the *glpD* leader-*lacZ* fusion transcripts was reflected in the cleavage patterns obtained from the 5' ends of the transcripts. Most striking is the fact that the major cleavage product at +37 in *E. coli* was barely seen in *B. subtilis*. The +37 fragment was absent in an *E. coli* Rnc mutant, implying that it results from cleavage by RNase III. When the patterns of primer extension products are further compared, some additional points can be made. GlpP has no apparent effect on the patterns in *E. coli* (Fig. 5A and C). A comparison of the cleavage pattern of the mutant *glpD* leader (Fig. 5A) with that of the wild-type *glpD* leader in *E. coli* (Fig. 5C) shows

that the +56-57 band is present in both while the +53 band is missing in the latter. We recall that +53 and +57 bands in the mutant leader correspond to +52 and +56 bands in the wild-type leader. Also, in *B. subtilis*, the mutant leader gives rise to +53 and +57 bands (Fig. 5A and B) whereas only the +56 band is obtained with the wild-type leader (data not shown). We suggest that expansion of loop III due to the G insertion in the mutant leader (Fig. 6) increases the probability for endoribonuclease cleavage between U and A at +53 in the first part of the loop. Loop III thus seems to be a target for endoribonucleases of similar specificities in the two bacteria. It has been shown for a *B. subtilis* phage SP82 transcript that Bs-RNase III will cleave in a bulge containing the sequence CAUG (33). We note that the same sequence is found at the cleavage site +57 in the loop of *glpD* leader mRNA.

Our knowledge of mRNA turnover in *B. subtilis* and of RNases as well as other proteins involved is quite limited. The fact that *E. coli* is often taken as the paradigm for mRNA decay in bacteria mainly reflects a lack of data from other species. We therefore thought that a comparison of the decay of an mRNA in *B. subtilis* and *E. coli* should provide valuable information. Our data on the stability and processing of *glpD* leader-*lacZ* fusion transcripts point to important differences in the mechanisms of mRNA decay in the two bacteria. That such differences can exist should be taken into account in comparative studies of gene control in different bacteria. We find it particularly interesting that RNase III appears to generate the major cleavage product in *E. coli*, a product which can hardly be detected in *B. subtilis*. This raises questions about the roles and substrate specificities of RNase III and its homologue in *B. subtilis*.

ACKNOWLEDGMENTS

We thank Lars Rutberg for valuable discussions, Lars Hederstedt, Charles Kurland, and Lars Rutberg for critically reading the manuscript, and Bernt Eric Uhlin for sending the RNase-deficient *E. coli* mutants.

This project was supported by grants from the Swedish Medical Research Council and the Emil and Wera Cornell Foundation.

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