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B. Jürgen · T. Schweder · M. Hecker

The stability of mRNA from the *gsiB* gene of *Bacillus subtilis* is dependent on the presence of a strong ribosome binding site

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Abstract In *Bacillus subtilis* IS58 starved of glucose or exposed to heat shock, ethanol or salt stress, the σ^B-dependent general stress protein GsiB is accumulated to a higher level than other general stress proteins. This high-level accumulation of GsiB can at least partially be attributed to the remarkably long half-life (~20 min) of the *gsiB* mRNA. Analysis of different *gsiB-lacZ* fusions revealed that this stability is not determined by sequences at the 3′ end of the transcript but rather by sequences upstream of the translational start codon. Site-directed mutagenesis established that a strong ribosome binding site was crucial for the increased stability of the *gsiB* mRNA. A comparison of the sequences upstream of the translational start codons of three general stress genes, *gsiB*, *gspA* and *ctc*, revealed a direct correlation between mRNA stability and the strength of their translational signals.

Key words *Bacillus subtilis* · General stress proteins · *gsiB* · mRNA stability · Ribosome binding site

Introduction

High-resolution two-dimensional (2-D) protein gel electrophoresis of *Bacillus subtilis* revealed that more than 50 proteins are induced in a σ^B-dependent manner (Antelmann et al. 1997; Bernhardt et al. 1997). The σ^B-dependent general stress proteins are induced after the imposition of stresses such as heat shock, salt stress, ethanol stress or glucose starvation. Among the general stress proteins, GsiB (glucose starvation inducible) (Mueller et al. 1991), which codes for a small, hydrophilic protein, is synthesised at higher rates and accumulated to a higher level than other σ^B-dependent general stress proteins such as GspA or Ctc. This is particularly striking in *B. subtilis* strain IS58, which shows a pronounced σ^B-dependent general stress response (Bernhardt et al. 1997). Although the strength of different σ^B-dependent promoters may differ, it is unlikely that the strength of the *gsiB* promoter is the only factor that accounts for the observed levels of GsiB. Thus, the question has been raised as to the mechanism(s) responsible for the accumulation of GsiB in response to stress.

In this study we report that an optimal ribosome binding site (RBS) appears to be responsible for the remarkably long half-life (~20 min) of *gsiB* mRNA and that this contributes to the high induction ratios of GsiB under stress situations or glucose starvation. Finally, comparative analysis of the half-lives and the sequences of the mRNAs of the σ^B-dependent genes *gsiB*, *gspA* and *ctc* showed what appears to be a correlation between the strength of the translational signals, mRNA stability and the rate of protein synthesis from these genes.

Materials and methods

Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* was routinely grown in LB medium and used as host for cloning experiments. *B. subtilis* strains were cultivated under vigorous agitation at 37° C in a synthetic minimal medium as described by Stülke et al. (1993). Stationary phase was induced by glucose limitation (0.05% w/v).

Assay of β-galactosidase activity

Cultures were grown in minimal medium supplemented with 0.05% glucose. Samples were taken at times throughout the growth phases

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B. Jürgen · T. Schweder · M. Hecker (✉)
Institut für Mikrobiologie und Molekularbiologie,
Friedrich-Ludwig-Jahn-Straße 15,
Ernst-Moritz-Arndt-Universität Greifswald,
D-17487 Greifswald, Germany
E-mail: hecker@microbio7.biologie.uni-greifswald.de
Tel.: +49-3834-864200, Fax: +49-3834-864202

Table 1 Bacterial strains and plasmids

Strain	Genotype or description	Source or reference
Escherichia coli		
DH5α	ϕ80d*lacZ*ΔM15, *endA*1, *recA*1, *hsdR*17, *supE*44, *thi*-1	Hanahan (1983)
Bacillus subtilis		
IS58	*trpC*2, *lys*-3	Smith et al. (1980)
BGB2	IS58*amyE*::pDH*gsiB*2, *cat*	This study
BGB4	IS58*amyE*::pDH*gsiB*4, *cat*	This study
BGB7	IS58*amyE*::pA*CgsiB*ATG, *kan*	This study
BGB15	IS58*amyE*::pDH*gsiB*SDSM1, *cat*	This study
BSA46	SPβ::*ctc-lacZ*	Benson and Haldenwang (1992)
Plasmids		
pBluscriptKS$^-$	Cloning vector *E. coli*, *bla*	Stratagene
pKS$^-$*gsiB*1	pBluescriptKS$^-$ + *gsiB*1	This study
pKS$^-$*gsiB*SDSM1	pKS$^-$ + *gsiB*SDSM1	This study
pDH32M	Integration vector for *B. subtilis*, containing *E. coli ori*, *bla*, *cat*	Kraus et al. (1994)
pAC7	Integration vector for *B. subtilis*, containing *E. coli ori*, *bla*, *kan*	Weinrauch et al. (1991)
pDH*gsiB*2	pDH32M + *gsiB*2	This study
pDH*gsiB*4	pDH32M + *gsiB*4	This study
pDH*gsiB*SDSM1	pDH32M + *gsiB*SDSM1	This study
pAC7*gsiB*ATG	pAC7 + *gsiB*ATG	This study
pBGT10	pDG148 + *lacZ* of pHV501	This study
pDG148	*B. substilis ori*, *E. coli ori*, P_{spac}, *lacI*, *bla*, *kan*, *phleo*	Stragier (1988)
pHV501	Integration vector for *B. subtilis*, containing *E. coli ori*, P_{spac}, *lacZ*, *lacI*, *bla*, *ermBD*	Vagner et al. (1995)

and stored at –20° C until the enzyme assay was carried out as described previously by Miller (1972).

mRNA analysis

Rifampicin (100 μg/ml final concentration) was added during exponential growth at an OD_{550nm} of 0.4 or 30 min after entry into stationary growth phase caused by glucose limitation. The antibiotics puromycin (20 μg/ml final concentration) or chloramphenicol (5 μg/ml final concentration) were added 1 min after the addition of rifampicin. The samples were taken just before the addition of the antibiotics and 0.5, 5, 10, 20, 25, 30, 40 and 50 min thereafter. Total RNA of the *B. subtilis* strains was isolated from exponentially growing or starved cells as described previously (Majumdar et al. 1991; Völker et al. 1994).

Northern blotting experiments were performed using 1, 2, 5 or 10 μg of total RNA as described by Wetzstein et al. (1992). In slot-blot experiments serial dilutions of total RNA were transferred directly onto positively charged nylon membranes. The RNA samples were hybridised with digoxigenin-labelled RNA probes as recommended by the manufacturer (Boehringer Mannheim).

The chemiluminograms were quantified with a personal densitometer from Molecular Dynamics. Induction ratios were calculated by setting the value of the control to 1. Hybridisation specific for *gsiB* mRNA was conducted with a digoxigenin-labelled RNA probe synthesised in vitro with T7 RNA polymerase from linearised plasmid pKS$^-$*gsiB*1.

Analysis of the mRNA secondary structure was performed according to the computer program MFOLD from Michael Zuker (http://www.ibc.wustl.edu/~zuker/rna/form1.cgi).

General methods

Cloning procedures were performed as described by Sambrook et al. (1989). Transformation of naturally competent *B. subtilis* cells was carried out by the two-step protocol of Hoch (1991).

The integration vector pDH32M (Kraus et al. 1994), which contains a promoterless *lacZ* gene with the translational signals of the *spo0V* gene, was used to construct chromosomal *lacZ* transcriptional fusions.

The different *gsiB* fragments were amplified from the plasmid pKS$^-$*gsiB*1 using forward primers that contained a *Sma*I restriction site and reverse primers that had a *Bam*HI restriction site at their ends. The appropriate vector was digested with *Eco*RI. After generation of blunt ends, the vector was digested with *Bam*HI. The *gsiB* fragments were cloned as blunt-ended *Bam*HI fragments.

The integration vector pAC7 (Weinrauch et al. 1991) was used for the construction of the *gsiB-lacZ* translational fusions. This vector contains a promoterless *lacZ* gene, but in contrast to pDH32M this gene is not preceded by an RBS. The vector pAC7 was digested with *Sma*I and *Bam*HI and ligated with the *Sma*I/*Bam*HI-digested *gsiB* fragment.

The plasmids were transformed into *E. coli* and transformants were selected on ampicillin-containing LB plates (100 μg/ml final concentration). The plasmids carrying the *gsiB-lacZ* fusions were isolated from *E. coli*, linearised with *Sca*I and then transformed into *B. subtilis* IS58. The transformants were selected on plates supplemented with the appropriate antibiotics. The chromosomal integration of the *gsiB-lacZ* fusions was verified by screening for an α-amylase-negative phenotype.

To construct plasmid pBGT10, plasmid pDG148 (Stragier et al. 1988) was digested with *Sal*I followed by treatment with the Klenow enzyme, to generate blunt ends, and digestion with *Hin*dIII. This plasmid was ligated with the *Hin*dIII/*Dra*I-digested *lacZ* fragment of plasmid pHV501 (Vagner et al. 1995).

Site-directed mutagenesis of the *gsiB* RBS

The exchange of three bases in the *gsiB* RBS was performed as described by Barik (1993) using the forward primer gsiB1forSma 5′-GGGCCCGGGACCGTACATATAAGAATGTCG, the reverse primer G7revBam 5′-GGGGGATCCAGCCAATTTCT-TGGTAGA and the degenerate primer gsiBM1 5′-TTCCTG-

CGTGAATTGGTGTT. The three bases in the *gsiB* RBS that were exchanged are shown in Fig. 2 (strain BGB15).

The first polymerase chain reaction (PCR) was performed to generate the megaprimer, which contains the mutated RBS, with the primers gsiB1forSma and gsiBM1. The product resulting from the first PCR was purified with the Promega Wizard DNA Purification Kit and used as a forward primer in the second PCR with the reverse primer G7revBam in order to generate the *gsiB* sequence containing the mutated RBS. To avoid secondary structures in the megaprimer in the second PCR, 5% glycerol was added.

The *gsiB* fragment with the mutated RBS was cloned into the vector pDH32M, yielding the plasmid pDH*gsiB*SDSM1. The *gsiB-lacZ* fusion strain BGB15 differs from the *gsiB-lacZ* fusion strain BGB2 only in the mutated *gsiB* RBS. The *Taq* cycle-sequencing kit of Promega was used to verify the sequence of the mutated *gsiB-lacZ* fusion.

Computer analysis of sequence data

DNA sequence analysis was performed using the Genetics Computer Group sequence software package.

Results

Stability of *gsiB* mRNA

The general stress protein GsiB is accumulated after stress to a greater extent than most other general stress proteins (Völker et al. 1994). In addition to the strength of its σ^B-dependent promoter, stabilisation of the *gsiB* mRNA might be another factor facilitating this high rate of synthesis. We investigated the stability of the *gsiB* mRNA by Northern blotting experiments and found a remarkably high *gsiB* mRNA half-life of about 20 min after entry into stationary phase due to glucose exhaustion (Fig. 1). High *gsiB* mRNA stability was also observed during exponential growth phase (Fig. 1) and in cells exposed to stresses such as heat shock or salt stress (data not shown).

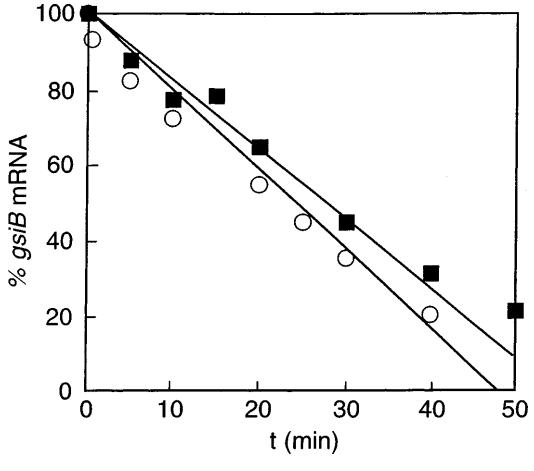


Fig. 1 Stability of *gsiB* mRNA during exponential growth phase (■) and under glucose starvation (○). Northern blot analysis was performed by using a digoxigenin-labelled RNA probe specific for *gsiB*. mRNA was quantified with the personal densitometer from Molecular Dynamics (see Materials and methods). These data represent the average of 2–3 different Northern blotting experiments

In order to determine whether a region at the 3′ end of the *gsiB* mRNA is responsible for the stability of this mRNA, *gsiB-lacZ* transcriptional fusions with differing lengths of the *gsiB* transcript downstream of the translational start codon were constructed and inserted into the chromosome (Fig. 2). The *gsiB-lacZ* fusion strain BGB2 lacks only the transcriptional terminator and contains 704 bp of the *gsiB* mRNA sequence. The *gsiB-lacZ* fusion strain BGB4 contains 470 bp of the *gsiB* sequence. The stability of these *gsiB-lacZ* mRNAs did not significantly differ from the stability of wild-type *gsiB* mRNA (Fig. 3). In order to test whether the sequences immediately downstream of the ATG codon of *gsiB* contribute to the stability of the mRNA, a translational fusion between the ATG translational start codon and *lacZ* (BGB7) was constructed. Even this *gsiB-lacZ* mRNA displayed the same stability as wild-type *gsiB* mRNA (Fig. 3C).

Stability of *gsiB* mRNA after treatment with puromycin and chloramphenicol

Several studies on the stability of bacterial mRNA indicated that the binding of ribosomes to the mRNA might be a significant factor determining the mRNA stability (Nilsson et al. 1987; Baumeister et al. 1991). In order to determine the influence of elongating ribosomes on the stability of *gsiB* mRNA, Northern blotting experiments were performed using RNA samples isolated from glucose-starved cells after the addition of rifampicin and puromycin (Fig. 4A) or rifampicin and chloramphenicol (Fig. 4B). Puromycin causes the premature termination of translation and the detachment of the ribosomes from the mRNA (Singer and Berg 1991). In contrast to puromycin, chloramphenicol inhibits translational elongation but the ribosomes remain on the mRNA. Consequently, if the *gsiB* mRNA is stabilised by ribosomes engaged in elongation, the half-life should decrease after the addition of puromycin, but could increase after the addition of chloramphenicol. However, addition of puromycin or chloramphenicol after the treatment with rifampicin did not significantly alter the half-life of *gsiB* mRNA compared with the control.

Site-directed mutagenesis of the *gsiB* RBS

The RBS of *gsiB* has eight bases that match the anti-RBS of the 16S rRNA exactly and that might lead to very strong initiation of translation of *gsiB*. In order to determine whether this optimal RBS is important for the high stability of *gsiB* mRNA, we exchanged three bases of the *gsiB* RBS by site-directed mutagenesis. The mutations were integrated into the long transcriptional *gsiB-lacZ* fusion of strain BGB2. The translation of *lacZ* should not be influenced because it has its own RBS. However, compared with the control strain (BGB2), this

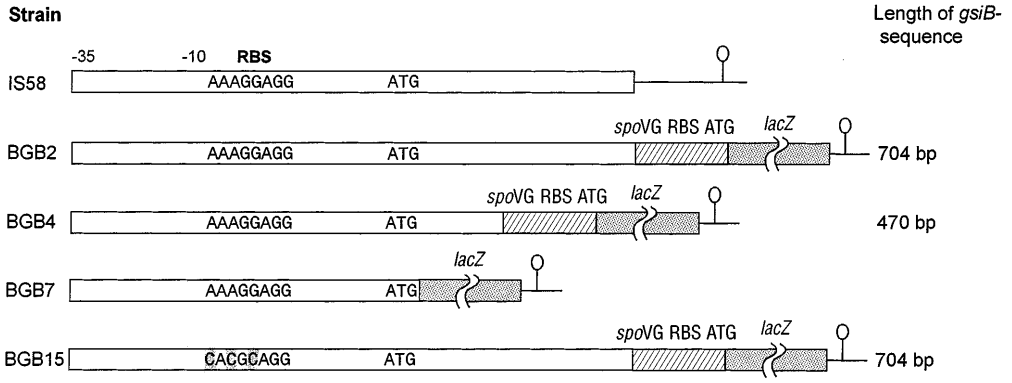


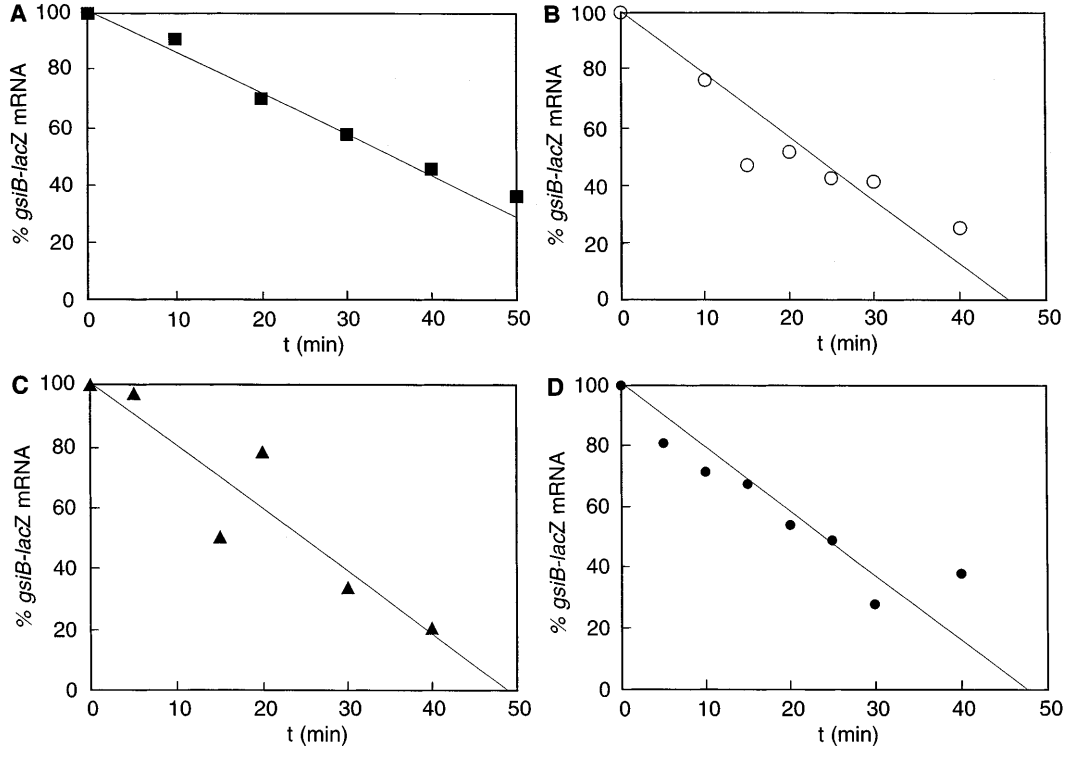
Fig. 2 Schematic representation of the different *gsiB-lacZ* fusion strains used in this study. (*RBS* ribosome binding site)

mutated strain BGB15 displayed a four- to fivefold reduction in β-galactosidase activity during both exponential growth and glucose starvation (Fig. 5). Northern blotting experiments using a *lacZ* probe revealed a dramatically reduced stability of the mRNA of the mutated *gsiB-lacZ* fusion of strain BGB15 (Fig. 6C), showing a half-life of only 5 min compared with 20 min for the control strain BGB2. These results indicate that the strong RBS of *gsiB* is important for stability of *gsiB-lacZ* mRNA. This conclusion was underlined by Northern blotting experiments using a *gsiB* probe in which the transcript of the mutated *gsiB-lacZ* fusion strain BGB15 was barely detectable (Fig. 6B) compared with the control BGB2 (Fig. 6A). The additional band, a transcript of about 1 kb in Fig. 6A, is presumably a degradation product of the *gsiB-lacZ* transcript. The signals at 2.4 kb and 1.4 kb also seem to be degradation products of the *lacZ* transcript that might be accumulated at the level of 23S rRNA and 16S rRNA (Leffers et al. 1989).

Fig. 3A–D Stability of the mRNA of the different *gsiB-lacZ* fusions. **A** BGB2 (■), **B** BGB4 (○), **C** BGB7 (▲) and **D** IS58 pBGT10 (●). Total mRNA was isolated from cells starved for glucose before and after addition of rifampicin. Northern blots were performed using a probe specific for *lacZ* and quantified as described in Materials and methods. The averaged data of 2–3 different experiments are shown

Stability of the mRNAs of other σ^B-dependent genes

Compared with GsiB, other σ^B-dependent proteins such as GspA and Ctc are synthesised at lower rates and accumulated to lower levels (Bernhardt et al. 1997). The rate of GsiB synthesis during glucose starvation, ana-



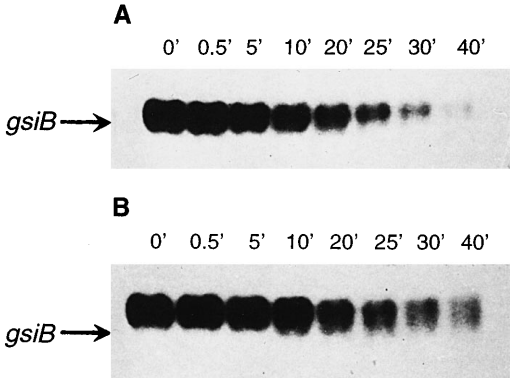


Fig. 4A, B Northern blot analysis showing stability of the *gsiB* mRNA of strain IS58 of *B. subtilis* during glucose starvation after the addition of **A** rifampicin and puromycin or **B** rifampicin and chloramphenicol (see Materials and methods)

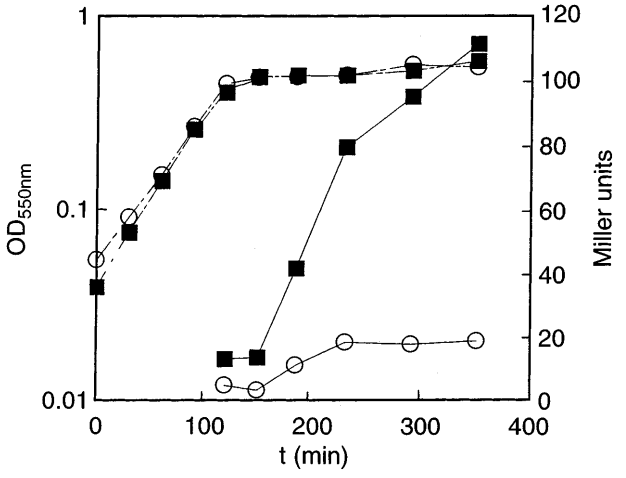


Fig. 5 Activity of β-galactosidase of the transcriptional *gsiB-lacZ* fusion strain BGB2 (■) and the mutated *gsiB-lacZ* fusion strain BGB15 (○). The growth curves are indicated by *dashed lines*. Stationary growth phase was induced by glucose limitation

lysed by analytical 2-D gel electrophoresis after labelling with L-[^{35}S]methionine in relation to the total protein on the gel and the methionine content of the appropriate proteins, is about five times higher (1.5%) than that of GspA and Ctc (0.2–0.3%) (Bernhardt et al. 1997, unpublished results).

It is unlikely that these differences can be explained solely by the different strengths of the promoters, but the strengths of their translational initiation signals are likely to be an additional factor. The most important factor for optimal translational efficiency seems to be a highly conserved RBS. The five bases GGAGG are crucial for efficient recognition by the ribosomes and the sequence around the RBS must be free of secondary structures (Vellanoweth and Rabinowitz 1992). A spacing of seven to nine nucleotides between the RBS and the start codon ATG appears to be optimal. In addition this spacer region should be free of cytosines and guanines because adenines and thymidines are more conducive to high-level expression. Following these rules for the strength of translational initiation signals of the σ^B-dependent stress genes, that of *gsiB* is the strongest followed by *gspA* and *ctc* (Fig. 7). The RBS of *gsiB* has eight bases complementary to the anti-RBS of the 16S rRNA. Furthermore *gsiB* has an adenine-rich spacer of eight nucleotides between the RBS and the translational start codon ATG. In contrast, the RBS of *gspA* differs in two of the eight nucleotides, the start codon of *gspA* is TTG and the spacer contains ten nucleotides, only five of which are adenines. The *ctc* RBS has six nucleotides complementary to the 16S rRNA and the spacer region contains only six bases instead of the optimal seven to nine. Only two of the spacer nucleotides are adenines. The calculated energy of the binding of the RBS of *gsiB*, *ctc* and *gspA* to the anti-RBS of the 16S rRNA, shown in Fig. 7, reveals that *gsiB* is likely to have the most efficient translational initiation signals followed by *gspA* and then *ctc*.

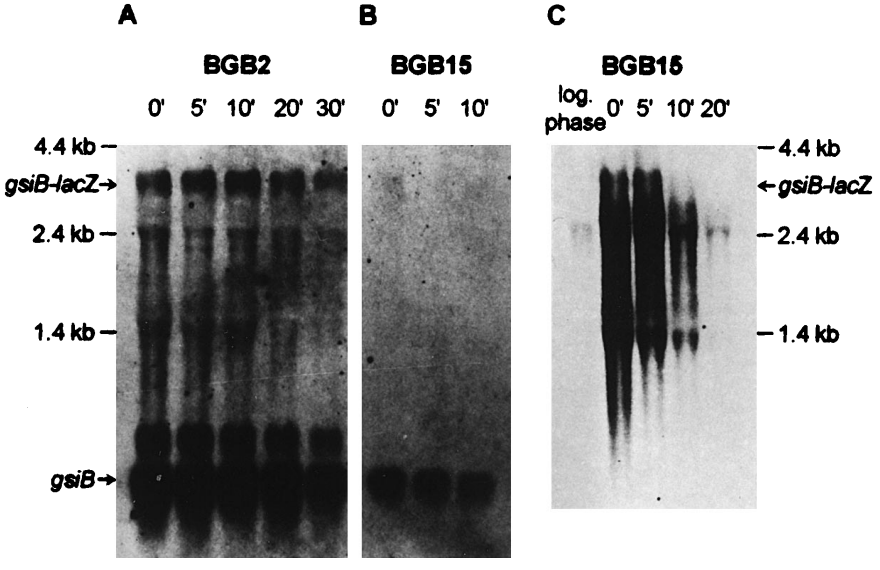


Fig. 6A–C Comparison by Northern blot analysis of the mRNA stability of the *gsiB-lacZ* transcript of strain BGB2 (**A**) and the mutated *gsiB-lacZ* transcript of strain BGB15 (**B**) using a probe specific for *gsiB*. In **C** the stability of the mutated *gsiB-lacZ* transcript is shown by using a probe specific for *lacZ*

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                -35              -10         +1                     RBS        Start ΔE
16S rRNA                                                            AAAGGAGG

gsiB     GTTTAAAAGAATTGTGAGC---GGGAATACAACAACCAACACCAATT----------AAAGGAGGAATTCAAA--ATG -9.3

gspA     GTTTATTTTTTTGAAAAA----GGGTATGTAACTTGT-ACAACAA-----------AAAGGGAGATGAATACCATTG -7.1

ctc      GTTTAAATCCTTATCGTTA---GGGTATTGTTTGTAATAGGACAACTAAAACGACA-AGAGGATGGTGAAT----ATG -6.4
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Fig. 7 Alignment of the RBS, the spacer between the RBS and the translational initiation codon, and the translational start codon of the σ^B-dependent genes *gsiB*, *gspA* and *ctc* and the anti-RBS of the 16S rRNA. The energy of the hypothetical binding of the 16S rRNA with the RBS of the three genes was determined by using the mRNA-folding program (MFOLD) of Zuker (see also Materials and methods)

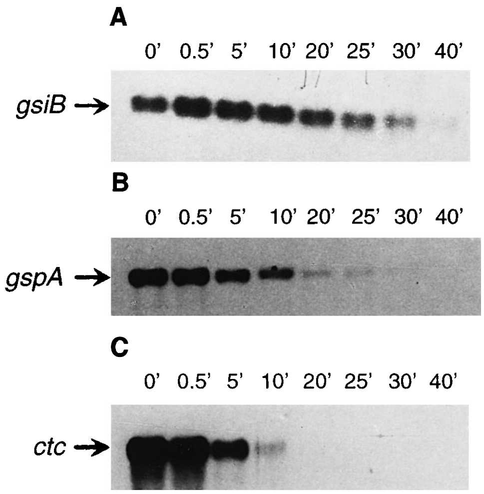

Fig. 8A–C Comparison of the mRNA half-lives of the σ^B-dependent genes **A** *gsiB*, **B** *gspA* and **C** *ctc* by Northern blot analysis using RNA probes specific for *gsiB*, *gspA* and *ctc*

Since a correlation was found between the stability of the *gsiB* mRNA and the strength of the RBS we wanted to determine whether such a correlation also holds true for *gspA* and *ctc*. In Northern blotting experiments with *gspA*- or *ctc*-specific probes, the half-lives of *gspA* and *ctc* mRNA from glucose-starved cells was determined to be 5–7 min and less than 5 min, respectively (Fig. 8).

Discussion

In this study we show that the mRNA of the σ^B-dependent general stress gene *gsiB* is remarkably stable. We conclude that its high degree of stability is one of the main reasons for the strong induction of the small hydrophilic GsiB protein in response to stress. The enhanced stability of the *gsiB* mRNA enables the cells to produce GsiB under different stress situations at higher concentrations than most other general stress proteins. This indicates an essential role of GsiB in the stress adaptation of *B. subtilis* (Maul et al. 1995).

Usually, a rapid turnover of mRNA allows the cell to respond quickly to changes in the environment. However, under certain circumstances, enhanced mRNA stability might become a crucial factor for regulating gene expression. Previous studies indicate that the decay of some mRNAs is controlled by environmental conditions. The half-lives of the *sdh* and *aprE* mRNAs of *B. subtilis*, for instance, are growth rate dependent (Melin et al. 1989; Resnekow et al. 1990). Georgellis et al. (1993) showed that the functional half-life of bulk mRNA was increased nearly fourfold in *E. coli* during slow anaerobic growth. Similar results were found by Albertson and Nyström (1994), who showed that the half-life of the mRNA pool increased in starving cells in comparison with exponential growing cells. Allmansberger (1996) observed that the half-life of the transcript of the β-xylanase gene (*xynA*) of *B. subtilis* seems to be controlled by stress or starvation. The *xynA* mRNA is relatively stable during exponential growth phase (8 min), but under various stress conditions, e.g. heat shock, ethanol or osmotic stress, the half-life drops to 2.5 min. However, in the case of the σ^B-dependent general stress genes examined in this study, we did not find any difference in their mRNA stability during exponential growth phase or during stress. This indicates that neither stress nor growth rate influences the half-life of their mRNAs.

The stability of mRNA molecules is frequently influenced by secondary structure (Wong and Chang 1986; Klug et al. 1987; Brawerman 1990). A typical example is the stabilisation of *E. coli ompA* mRNA by a secondary structure at its 5′ end (Gabain et al. 1983; Chen et al. 1991; Hansen et al. 1994). This secondary structure, which seems to be a target for GroEL binding (Georgellis et al. 1995), might protect the *ompA* mRNA against decay by ribonucleases. According to the MFOLD computer program of Zuker (see Materials and methods) there is no evidence for a secondary structure of the *gsiB* mRNA, except the putative transcriptional terminator at the 3′ end of *gsiB* (data not shown). This terminator does not seem to be involved in the enhanced stability of the *gsiB* mRNA.

The question arises as to whether fusing *lacZ* sequences on to *gsiB* in some way influences the stability of its mRNA. However, in none of the experiments in which *lacZ* has been used as a reporter gene has it been shown to influence the stability of the truncated target mRNAs. The different *gsiB*-*lacZ* fusions exhibited the same half-life as wild-type *gsiB* mRNA. Furthermore, the unstable mutated *gsiB* mRNA was not stabilised by fusion to the *lacZ* gene. In contrast, the fused *lacZ* mRNA was destabilised by the mutated *gsiB* sequence. Similar results were observed in the case of a tran-

scriptional *ctc-lacZ* fusion of strain BSA46 in which *lacZ* was as unstable as the wild-type *ctc* mRNA (data not shown). These data are consistent with the results of Belasco et al. (1986) who revealed that exchange of the terminator structure of an unstable mRNA by the terminator structure of a stable mRNA did not stabilise the unstable transcript if the stabilising elements were located at the 5′ end of the mRNA.

It has been assumed that elongating ribosomes protect mRNAs against nucleolytic attack (Petersen 1992). Bulk mRNA and specific transcripts from the *lac* and *trp* operons of *E. coli* were destabilised by puromycin; however, when ribosomes were stalled by chloramphenicol, stabilisation of these mRNAs was observed (Varmus et al. 1971; Craig 1972; Imamoto 1973; Pato et al. 1973). In the case of *gsiB*, treatment with puromycin or chloramphenicol did not significantly change the half-life of its mRNA compared with the control. These experiments might indicate that the *gsiB* coding sequence is free of ribonucleolytic target sites, but the experimental evidence is still lacking. In agreement with these data, the Northern blotting experiments with the different *gsiB-lacZ* fusions revealed that it is the sequence upstream of the translational start codon rather than the translated sequence that is crucial for the long half-life of the *gsiB* mRNA.

Flache et al. (1992) have shown that a sequence upstream of the *tetA* RBS influences the stability of the mRNA of *tetA-lacZ* translational fusions most probably by the efficiency of translational initiation. Guillerez et al. (1991) investigated the effects of different RBSs on β-galactosidase activity in *E. coli*. They constructed a series of *E. coli* strains in which the RBS of the chromosomal *lacZ* gene had been replaced by RBSs from other genes or artificial RBSs. The authors speculated that mRNA stability is tightly coupled to translational initiation. The general stress gene *gsiB* has optimal translational initiation signals, above all a strong RBS, and is likely to be efficiently translated. By use of site-directed mutagenesis, we show that the strength of the RBS correlates with the stability of *gsiB* mRNA. Our results suggest that the binding of the ribosomes to the *gsiB* RBS and thus the initiation of translation, is crucial for the high stability of *gsiB* mRNA. The initiating ribosomes could mask nuclease recognition sites near the *gsiB* RBS, thereby preventing the attack of the 5′ endoribonucleases. This interpretation is consistent with the assumption that the initiation of mRNA degradation mainly starts at the 5′ end of the mRNAs (DiMari and Bechhofer 1993). The idea that the strength of the translational signals might contribute to the high stability of *gsiB* mRNA is supported by our data on the half-lives of the *ctc* and *gspA* mRNAs. In summary, there is a correlation between the strength of the translational initiation signals of these three general stress genes and their mRNA stability; both are factors that determine the frequency of translational initiation. It seems that the stronger the translational signals, the higher the stability of the appropriate mRNA and finally the higher the translational rate.

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