The *Escherichia coli* antiterminator protein BglG stabilizes the 5′ region of the *bgl* mRNA

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The *b*-glucoside utilization (*bgl*) genes of *Escherichia coli* are positively regulated by the product of the *bglG* gene, which functions as an antiterminator by binding to specific sequences present within the *bgl* mRNA. BglG is inactivated by phosphorylation in the absence of *b*-glucosides by BglF, the *bgl*-specific component of the phosphotransferase system (PTS). Here, we present evidence for an additional function for BglG, namely the stabilization of the 5′ end of the *bgl* mRNA. Half-life measurements of the promoter-proximal region of the *bgl* mRNA indicate a five fold enhancement of stability in the presence of active (unphosphorylated) BglG. This enhancement is lost when the binding of BglG to mRNA is prevented by deletion of the binding site. Interestingly, stabilization by BglG does not extend to downstream sequences. The enhanced stability of the upstream sequences suggest that BglG remains bound to its target on the mRNA even after the downstream sequences have been degraded. Implications of these observations for the mechanism of positive regulation of the operon by BglG are discussed.

1. Introduction

Gene expression can be regulated at four independent levels: transcription of DNA into RNA, stability of RNA, translation of the transcript into protein, and stability of the protein product. Degradation of mRNA has been recognized as an important means of post-transcriptional regulation in prokaryotes (reviewed by Regnier and Arriano 2000). The stability of single transcripts or even entire pools of mRNA can be regulated by challenges such as antibiotics, nutritional stresses, transitions of growth stages, and bacterial growth rate (Georgellis *et al* 1993; Goldenberg *et al* 1996; Nilsson *et al* 1984; Resnekov *et al* 1990). The half-life of the bulk mRNA in *Escherichia coli* is 2·4 min at 37°C. However the stability of individual transcripts can vary by more than a factor of 50; transcript half-lives can be as short as a fraction of a minute or as long as half an hour. Further, variation in stabilities of different regions of a polycistronic mRNA can enable differential expression of genes.

Degradation of mRNA in bacteria does not follow a single pathway and the directionality can vary in different transcripts (5′ to 3′ or 3′ to 5′). Messenger RNA turnover is mediated by a combination of endo- and exonucleases. Studies in *E. coli* have shown that degradation commences with an initial endonucleolytic attack at the 5′ or 3′ end of the transcript, which opens up the molecule for subsequent internal cleavages (Regnier and Arriano 2000). The transcript is then degraded to mononucleotides by the concerted action of other ribonucleases. The 5′ and 3′ untranslated regions of bacterial transcripts can act as stability determinants in the decay process (Bechhofer and Dubnau 1987; Jain and Belasco 1995; Melin *et al* 1990). The promoter-proximal sequences of many transcripts, being rich in secondary structures, act as barriers for processive exonucleolytic degradation. Thus, an initial cleavage targeting these non-coding regions is critical for subsequent turnover (Belasco *et al* 1986; Lundberg *et al* 1990; Melefors and von Gabain 1988). Similarly, stable hairpins located at the 3′ ends of many mRNAs such as

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Abbreviations used: PTS, Phosphotransferase system; RAT, ribonucleic anti-terminator; RNP, RNA polymerase.

rho-independent terminators, protect the upstream message from degradation by 3′–5′ exonucleases (Schmeissner *et al* 1984).

The importance of terminal secondary structures in regulating mRNA stability has been proven unequivocally. Recently, a number of proteins (other than RNases) have been assigned important roles in stabilizing or destabilizing bacterial transcripts on account of their RNA binding properties. Mutations that cause an early arrest of translation or those that modify translation frequency, lower mRNA stability, suggesting that transcripts turnover more rapidly when the ribosome density decreases. Binding of ribosomes to the 5′ end of transcripts protect them from the initial degradation events (Rapaport and Mackie 1994; Jurgen *et al* 1998). The general termination factor, Rho, and the pBR322 copy number protein, Rop, have been shown to have roles in transcript turnover (Sozhamannan and Stitt 1997). In another instance, Hfq (host factor I) which binds to the *ompA* mRNA in a growth dependent manner, regulates its stability (Vytvytska *et al* 1998). Modification of the activity of such RNA-binding proteins, by signals from the external environment, can help modulate gene expression by alteration of mRNA stability, constituting a possible mechanism for signal transduction.

The *b*-glucoside utilization (*bgl*) operon of *E. coli*, is positively regulated by the product of *bglG*, the first gene of the operon, which functions as an anti-terminator at two rho-independent terminators flanking it (Mahadevan and Wright 1987; Schnetz and Rak 1988). The first terminator is located within the leader region preceding the *bglG* gene and spans the sequence from + 64 to + 105. The second terminator is located downstream of *bglG*, within the *bglG-bglF* intercistronic region. BglG is a sequence specific RNA-binding protein that interacts with a 32-nucleotide target sequence overlapping the terminator (Houman *et al* 1990) known as the ribonucleic anti-terminator (RAT) (Aymerich and Steinmetz 1992). The activity of BglG is altered by phosphorylation in response to the presence or absence of *b*-glucosides (Amster-Choder *et al* 1989; Amster-Choder and Wright 1990, 1992; Schnetz and Rak 1990) and glucose (Gorke and Rak 1999; Gulati and Mahadevan 2000). The product of the second gene of the operon, BglF, mediates phosphorylation of BglG in the absence of *b*-glucosides, leading to its inactivation. BglG exists in the active (unphosphorylated) form upon induction by *b*-glucosides and is constitutively activated in strains that carry deletions or mutations of the *bglF* gene. The BglG-BglF pair thus constitutes a new class of signal transducers. A number of homologues of BglG have been identified in other bacteria: SacY, SacT and LicT in *Bacillus subtilis*, BglR in *Lactococcus lactis* and ArbG in *Erwinia crysanthemi* (Mahadevan 1997; Rutberg 1997 for a review). These proteins are now classified as the BglG/SacY family of anti-terminators, a group increasing rapidly in number.

The role of most of the proteins belonging to the BglG/SacY family as anti-terminators has been established conclusively. The possibility that they have additional regulatory roles on account of their RNA binding ability is relatively unexplored. In this report, we show that BglG, in addition to functioning as an antiterminator, also enhances the stability of the 5′ region of the *bgl* mRNA. Since BglG is the prototype of the BglG/SacY group of anti-terminators, information generated using this system is significant in terms of investigating similar roles for the other members of the family.

2. Materials and methods

2.1 *Bacterial strains and growth conditions*

The bacterial strains used in this study are listed in table 1. All strains are *E. coli* K-12 derivatives. Bacteria were grown and maintained in LB liquid/agar medium. Transformants carrying different plasmid constructs were maintained with appropriate antibiotic selection. For RNA isolation, cells were grown in minimal medium supplemented with 0·4% succinate as carbon source.

2.2 *Plasmid constructs*

A list of the plasmid constructs used in this study is given in table 1. All plasmids are pBR322 derivatives. The plasmid pAG-G, harbouring the complete *bglG* gene and the regulatory region, *bglR*, was constructed by cloning a 1·8 kb *Ssp1* fragment from pMN22AE that carries an activated copy of the *bgl* operon, at the *Sca1* site of pBR322. The plasmid pΔRAT is a pBR322 derivative that also carries an intact *bglG* but lacks the BglG-binding site. It was constructed in two steps by amplifying the *bgl* sequences flanking the RAT sequence by PCR and subsequent ligation of the fragments. The resulting construct, pΔRAT carries a deletion of 44 nucleotides (from + 44 to + 87) spanning the boxA and boxB sequences for BglG-binding, but harbours the intact *bglG* gene. The plasmid pA8 that harbours an insertionally activated regulatory locus, the intact terminator (+ 64 to + 105) and a part of *bglG* coding for the N-terminal 66 amino acids, was constructed by PCR amplification of the *bglR-G* region (from – 250 to + 329) using the parent plasmid pMN22AE. The plasmid pANS5-16 is similar to pA8, but carries a single nucleotide deletion at position + 100 and a C to A transversion at + 25. Transcriptional studies have indicated that the transversion has no effect on the steady state levels of the *bgl* mRNA (Kharat and Mahadevan 2000).

2.3 *RNA isolation and analysis of transcripts*

Bacterial strains and transformants carrying appropriate *bgl* alleles were grown in M9 minimal succinate medium. RNA isolation and *in vivo* transcription analysis were carried out using an S1 nuclease protection assay as described previously (Singh *et al* 1995; Mukerji and Mahadevan 1997). Forty µg of total RNA was hybridized with a large excess of 5′ end-labelled oligonucleotide complementary to the region + 1 to + 42 (SM3) or + 121 to + 161 (SM 33) of the *bgl* transcript. After hybridization and digestion with S1 nuclease, the products were separated by electrophoresis on a 12% acrylamide-urea gel and the bands were visualized by autoradiography. The autoradiographs, after appropriate exposure for the bands to be in a linear range, were scanned on a densitometric scanner. The reproducibility of the results obtained was ensured by carrying out each experiment at least twice.

2.4 *mRNA half-life measurements*

Decay rates of mRNA were examined to determine the half-life of the *bgl* transcript under various conditions. JF201 transformed with the appropriate plasmid construct was grown to mid-log phase and transcription was arrested by addition of rifampicin at a final concentration of 200 µg/ml. Aliquots of the culture were collected at various time points (in most cases, at intervals of 2 min after addition of rifampicin). Total RNA was isolated and 5 to 20 µg of total RNA was subsequently subjected to S1 protection assays as described above.

To determine the half-life of the *bgl* transcript, the autoradiograms were densitometrically scanned. The percentage *bgl* mRNA remaining at each time point was calculated taking the signal obtained at 0 min as 100%. To obtain the mRNA decay profile, a semi-logarithmic graph of the percentage mRNA remaining versus time was plotted. The time at which 50% of the mRNA is already degraded was calculated from the decay plot to determine the half-life for each experiment. Although in most of the cases, a time course of 0 to 8 min was followed, some experiments were carried out with shorter or longer time scales to generate appropriate data points for calculation of the mRNA half-lives. Each experiment was carried out at least twice and the average half-life calculated.

3. Results

3.1 bglF *mutants show an enhancement in RNA levels in the promoter proximal region*

BglF, encoded by the second gene of the *bgl* operon, is the *b*-glucoside specific transport protein, belonging to the phosphotransferase system (PTS) family of transporters. It acts as a negative regulator of the operon by inactivating the anti-terminator protein, BglG, (Mahadevan *et al* 1987) by phosphorylation (Amster-Choder *et al* 1989; Amster-Choder and Wright 1990, 1992; Schnetz and Rak 1990). The strain MA200-1 carries a mutation in *bglF* and therefore, *bglG* is constitutively active (Mahadevan *et al* 1987). Transcription analysis using the oligonucleotide probe SM3, spanning the region from + 1 to + 42 of the *bgl* transcript, showed an enhancement in the steady-state levels of the promoter-proximal mRNA in MA200-1, when compared with its parent strain MA200

Table 1. Bacterial strains and plasmids.

Strain	Description	Source/ Reference	Plasmid	Genotype and relevant description	Source/ Reference
JF201	F Δ*lac*X74 Δ(*bgl-pho*) 201 *ara thi gyrA*	Reynolds *et al* 1986	pMN22AE	*bglR::IS1 bglG*$^+$ *bglF*$^+$ *bglB*$^+$ Ap^R	Singh *et al* 1995
MA200	F$^-$Δ*lac*X174 *thi bglR1*(*bglR*::IS*1*) *srl*::Tn*10 recA56 l bgl R7 bglG¢ lacZ lacY f* (*bgl-lac)*	Mahadevan *et al* 1987	pAG-G	*bglR::IS1 bglG*$^+$ Tet^R	This work
MA200-1	MA200, *bglF201*	Mahadevan *et al* 1987	pA8	*bglR::IS1 bglG¢* Ap^R	Moorthy and S Mahadevan (unpublished)
AE328	Δ*lacX74 thi bglR11* (*bglR*::IS1), *tna*::Tn10 (Bgl$^+$)	A Wright	pMN25	*bglR bglG*$^+$ *bglF¢* Ap^R	Mahadevan *et al* 1987
R4	AE328 *bglF*	Yakkundi *et al* 1998	pΔRAT	*bglR::IS1 bglG*$^+$ Tet^R (lacks the BglG binding sequence)	This work
			pANS5-16	*bglR::IS1 bglG¢* Ap^R (harbours deletion of a G residue at + 100)	Kharat and Mahadevan 2000

(figure 1a). Similar results were observed in the strain R4, another spontaneous mutant carrying an inactive *bglF*. Compared to its parent strain AE328, steady-state levels of the promoter-proximal transcript were enhanced three fold (figure 1b). The two strain sets are isogeneic and therefore, the *bgl* transcripts compared are identical except for the presence or absence of BglF. Since BglF mediates regulation by modifying the activity of the anti-terminator, BglG, it was important to examine the role of the anti-terminator in the enhancement of the promoter-proximal transcript. The plasmid pAG-G, carrying an insertionally activated promoter, codes for BglG but lacks the *bglF* gene. Therefore, the antiterminator escapes negative regulation and is always in an active (unphosphorylated) form. In the absence of an active BglF, a three-fold enhancement in the steady-state levels of *bgl* mRNA was observed compared to the uninduced levels seen in the case of the parent plasmid, pMN22AE, that carries both *bglG* and *bglF* (figure 1c). Further, when the construct pA8, which codes for a truncated, non-functional BglG, was used for transcription analysis, RNA levels obtained were comparable to those obtained with pMN22AE (figure 1c). These experiments indicate that the enhancement seen in the steady-state level of the promoter proximal region of the *bgl* transcript in *bglF* mutants can be correlated to the presence of active (unphosphorylated) BglG in the cell.

3.2 *BglG increases the stability of the promoter-proximal region of the* bgl *transcript*

BglG interacts with its target sequence (RAT) in the 5′ untranslated region of the *bgl* transcript. The RAT sequence is a part of the transcript that overlaps with the rho-independent terminator. Binding of BglG to the sequence prevents premature termination of transcription. Thus, the presence of an active BglG leads to a higher read-through of transcription past the leader sequence. The results described in the previous section indicate that in the presence of BglG, increased steady-state levels of RNA are detected in a region upstream of the terminator as well. These observations cannot be explained by con-

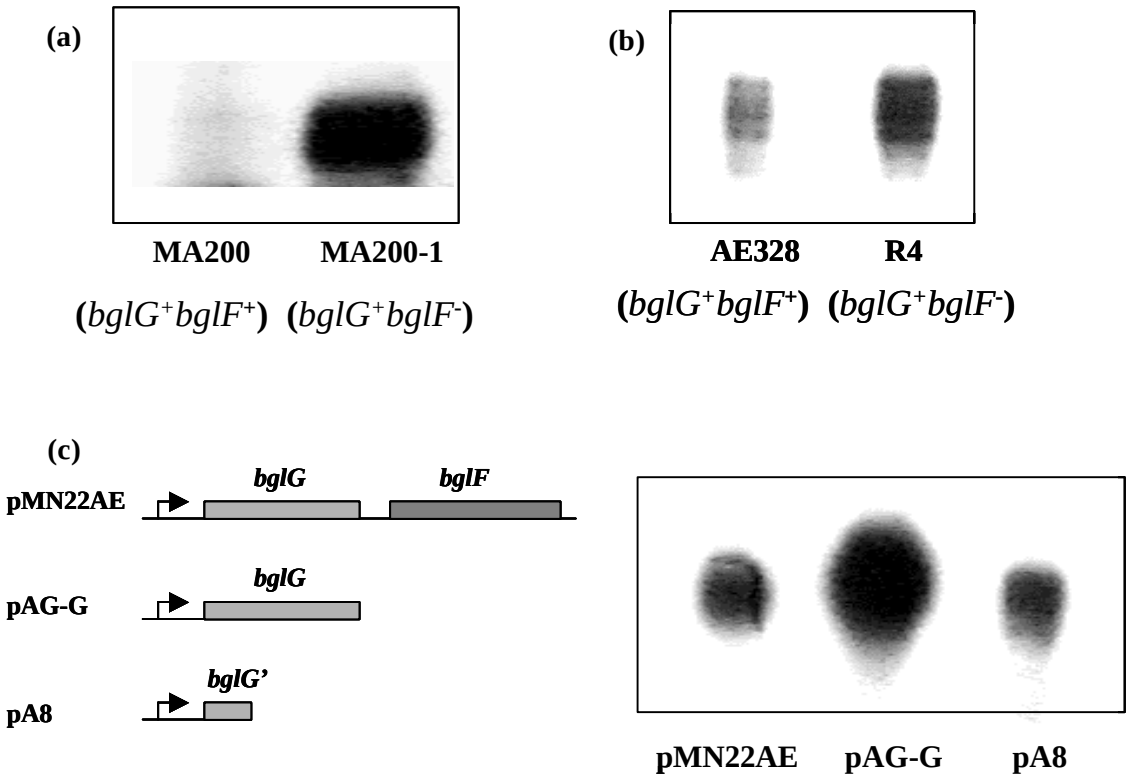

Figure 1. Enhancement in the steady-state RNA levels in the promoter-proximal region of the *bgl* transcript in *bglF* mutants. The results depict steady-state levels of the 5′ end of the *bgl* mRNA, detected by S1 nuclease protection assay using the oligonucleotide SM3, which hybridizes with the first 42 nucleotides of the transcript. (**a**, **b**) Transcription from the chromosomal *bgl* operon in wild-type (MA200 and AE328) and *bglF* (MA200-1 and R4) strains. The difference in the relative levels of *bgl* transcript seen in the two sets of strains is likely to be related to the nature of the *bglF* mutations present. (**c**) Results obtained with various plasmids (indicated on the left side) transformed in JF201, a strain carrying a deletion of the chromosomal *bgl* operon, detected by the same method as above.

sidering the role of BglG as an anti-terminator since the enhancement seen corresponds to the region upstream of the terminator. Therefore, some additional function of this protein in *bgl* expression needs to be invoked. BglG is incapable of binding to DNA (Houman *et al* 1990), which rules out the possibility that it acts as a transcription activator to increase the rate of transcription initiation. In view of these observations, one attractive possibility is that BglG enhances the steady-state mRNA levels in the promoter-proximal region by stabilizing the 5′ end of the *bgl* message as a secondary consequence of its binding to the RAT sequence. To examine this, the rate of mRNA decay was examined in the presence and absence of BglG, using two plasmid constructs: pMN22AE (which codes for both BglG and BglF) and pAG-G (harbouring only *bglG*). The plasmids were transformed in JF201 and transformants were grown in minimal succinate medium lacking the inducer. The levels of *bgl*-specific mRNA were examined at different time points after blocking initiation of transcription by addition of rifampicin, using the same oligonucleotide probe SM3 that hybridizes with the first 42 nucleotides of the mRNA. The decay profile represents the degradation of the 5′ segment of the *bgl* message (figure 2a). In the absence of the active form of BglG (i.e. under non-inducing conditions), the half-life of this segment was 1·7 min. In contrast, figure 2b shows that the average half-life of the same RNA segment in the presence of the constitutively active form of BglG was greater than 8 min. This five-fold enhancement in stability of the *bgl* message could be correlated with the presence of the active form of BglG. Transcription from the plasmid pA8, which encodes a non-functional anti-terminator, showed a decay pattern similar to pMN22AE (figure 2c). Based on these observations, it is conceivable that the role of BglG in positive regulation of the *bgl* operon is twofold: stabilization of the leader transcript upstream of the terminator and facilitation of transcription read-through past the terminator. Its action at these two distinct steps of RNA metabolism could coordinately bring about an enhanced expression of the *bgl* genes. To exclude the possibility that BglG causes a non-specific enhancement in stability of bacterial mRNAs, the decay rate of a segment of the *b*-lactamase message was analysed. The decay rates of the corresponding transcript in two plasmid constructs; pA8 and pMN25 (which encodes a functional BglG) was seen to be unaffected by the presence of BglG, indicating that it does not alter the decay rates of nonspecific messages in the cell (figure 3).

3.3 *BglG stabilizes the 5¢ segment of the* bgl *transcript by binding to its cognate site*

BglG binds to the newly transcribed message and its role in RNA stabilization may be related to its direct interaction with the RNA target. Alternatively, the protein might affect the decay process by modulating the activity of another, yet unknown factor, crucial for stabilizing the 5′ end of the message. To distinguish between these two possibilities, the plasmid pΔRAT, which harbours an intact *bglG* but lacks the target RAT sequence, was used for measuring mRNA decay. The 44 bp deletion in pΔRAT removes the entire BglG binding sequence and a part of the inverted repeat that forms the terminator (figure 4a). Transcription studies of downstream sequences indicated low levels of read-through, suggesting that the partial terminator is still active in the mutant (data not shown). When compared with its parent plasmid, pAG-G; mRNA from pΔRAT showed a significantly higher rate of decay of the 5′ end of the transcript (half-life of 0·7 min, figure 4b). The *bgl* sequences carried on the two plasmids are similar except for the BglG binding sequence. The drastic reduction in RNA half-life seen in the case of the latter can be attributed to the lack of BglG binding. Thus, the stabilization mediated by BglG is related to its direct interaction with its cognate binding sequence. The lower stability seen in the case of pΔRAT, in comparison to pMN22AE, is likely to be related to the fact that the latter can encode a low basal level of BglG that can bind to the RAT sequence present on the plasmid whereas no binding occurs in pΔRAT.

3.4 *BglG does not stabilize the mRNA downstream of the terminator*

The 5′ untranslated sequences of a number of bacterial transcripts act as stability determinants by providing target sites for endonucleolytic attack. The experiments described above focus on the degradation rates of the first 42 nucleotides in the *bgl* message. In the absence of any information on decay kinetics of the *bgl* transcript or the knowledge of the ribonucleases involved, it is difficult to predict whether the RNA-stabilizing effect of BglG on the 5′ end will be reflected at downstream regions of the transcript as well. This question was difficult to address because of the obvious inability in obtaining sufficient RNA downstream of the terminator in the absence of BglG or under non-inducing conditions. To overcome this technical problem, mRNA decay experiments were carried out using pANS5-16, a plasmid identical to pA8 carrying a truncated *bglG*, except for a point mutation in the terminator that allows sufficient read-through of transcription. The decay profile of the 5′ segment of the *bgl* transcript showed that the point mutation in the terminator sequence did not have any significant effect on the half-life of the 5′ region of the RNA; mRNA half life was 1·2 min in the case of the mutant. The half-life of the transcript downstream of the terminator, measured using SM33, showed a decay rate of 0·9 min (figure 5a). To

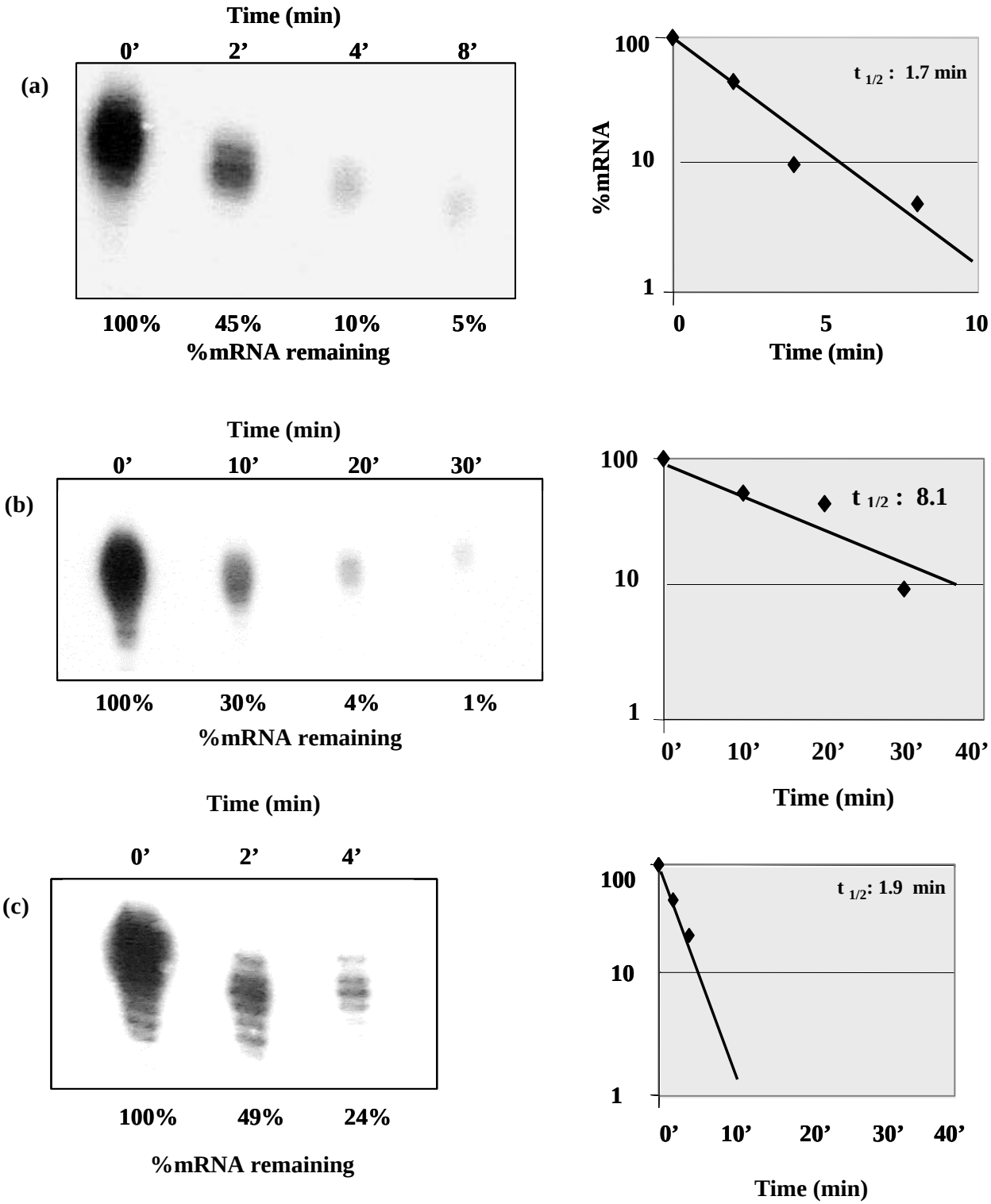

Figure 2. Decay profile of the promoter-proximal region of the *bgl* transcript in the presence and absence of BglG. (**a**) Degradation pattern of mRNA isolated from JF201 transformed with pMN22AE where the activity of BglG is negatively regulated by BglF. Cells were grown in minimal succinate medium and culture samples collected at different time points after addition of rifampicin. Following isolation of RNA, the first 42 nucleotides of the *bgl* transcript were quantitated by an S1 nuclease protection assay using the oligonucleotide SM3 as above. The percentage of mRNA remaining at each time point is indicated. (**b**) A similar mRNA degradation experiment carried out in the presence of active BglG using the plasmid pAG-G transformed in JF201. (**c**) Control study using the plasmid pA8 that carries a truncated (non-functional) *bglG*.

detect any possible effect of BglG on downstream transcription, a similar experiment was carried out in the presence of functional BglG using pAG-G. Transcription of downstream sequences in this case is constitutive because of the absence of the negative regulator BglF. The results of this experiment, shown in figure 5b, indicate that the anti-terminator did not have any effect on the decay kinetics of the downstream mRNA. The half-life of the downstream segment of the message was 0·9 min, which is identical to that obtained using pANS5-16. In

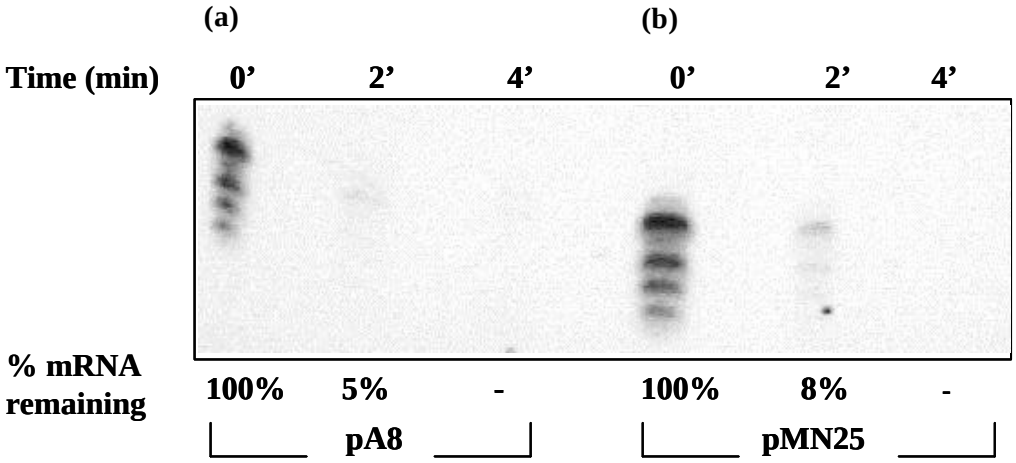

Figure 3. Analysis of the stabilities of the non-specific *b*-lactamase gene transcript, using an oligonucleotide probe complementary to the central region of the *bla* gene of the pBR322 vector. (**a, b**) RNA decay experiments in the presence and absence of BglG respectively.

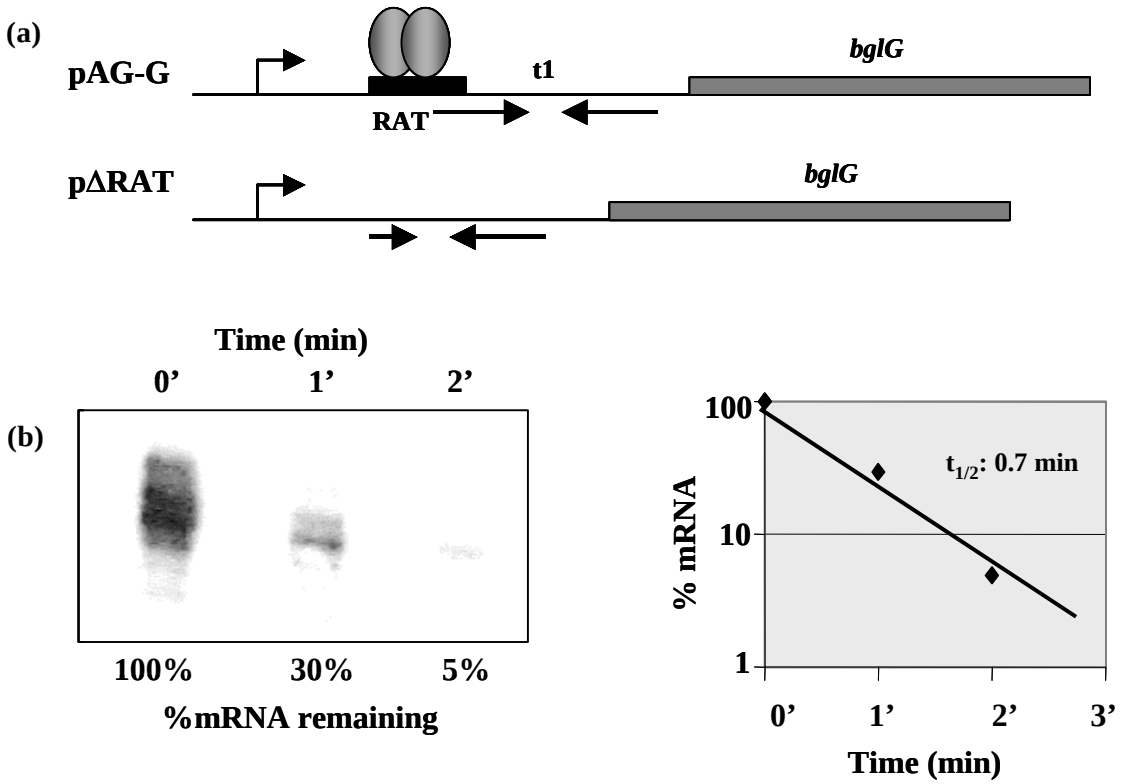

Figure 4. BglG does not stabilize the 5′ region of the *bgl* mRNA when the RAT target site is deleted. (**a**) Organization of the control plasmid pAG-G (*bglG*$^+$) and pΔRAT that carries a 44 bp deletion of the RAT sequence from + 44 to + 87. (**b**) Decay analysis of *bgl* mRNA from pΔRAT, using the probe SM3.

conclusion, the RNA-stabilizing effect of BglG is localized to the promoter-proximal region and is not reflected on the decay kinetics of the message downstream of the terminator.

4. Discussion

RNA-protein interactions are found to occur in a large number of biological processes such as protein synthesis, pre-mRNA splicing and mRNA degradation. One important regulatory process in bacteria that involves such interactions is anti-termination of transcription. Regulation by controlling elongation of transcription has been identified in nine chromosomal operons/regulons in bacteria (Rutberg 1997). A majority of these proteins belong to the BglG/SacY family of anti-terminators. Members of this family are of similar size and show extensive sequence homology. Anti-terminator proteins bind to very specific sequences in the nascent transcripts called RAT and prevent formation of 'conditional terminators'. RAT targets of various anti-terminators differ only at a few positions and the nucleotides present at these positions act as specificity determinants (Aymerich and Steinmetz 1992). Genetic and biochemical analyses have led to the understanding that members of this family contain a novel RNA binding motif (Manival *et al* 1997). In spite of a wealth of information available on this subject, the role of anti-terminators in regulation of RNA stability has remained an attractive, yet unexplored possibility. Since the activity of most of these proteins can be regulated by external signals, if they could mediate stabilization of their corresponding mRNAs, it would add to the effectiveness of anti-termination as a means of controlling gene expression.

Interaction of the *bgl*-specific anti-terminator, BglG (*E. coli*), and SacT and SacY (*B. subtilis*) with their respective mRNA sequences has been experimentally demonstrated (Houman *et al* 1990; Arnaud *et al* 1996). Could they also stabilize the resultant transcripts? To address this question, one needs experimental conditions or mutants that allow the mRNA stabilizing effect of these proteins to be separated from their anti-termination activity. In the *glp* operon of *B. subtilis*, mutant studies allowed the elucidation of a dual role for the anti-terminator, GlpP (Glatz *et al* 1996). The isolation of mutations in the loop part of the corresponding terminator sequence allowed higher read-through of *glpD* mRNA but also led to destabilization of the message. Interestingly, introduction of multiple copies of GlpP in the cells stabilized the mRNA and this effect could be attributed to the RNA binding property of the protein. GlpP however does not harbour any homology to the BglG/SacY family of anti-terminators. The only suggestion that proteins belonging to this group could have additional role, came from the observation that the RAT site for SacY and SacT is at least 100 nucleotides from a terminator-like structure. This prompted the hypothesis that these proteins have an additional positive role in gene expression by stabilizing the RNAs to which they bind (Crutz and Steinmetz 1992).

To examine the role of one of the well-characterized anti-terminators, BglG, in mRNA stability, decay profiles of different segments of the *bgl* message were examined in the presence and absence of the anti-terminator. The degradation rates of the fragments upstream and downstream of the terminator were examined independently. BglG stabilizes the first 42 nucleotides of the transcript by more than four-fold. This is a reflection of the protection conferred by BglG binding because deletion of the RAT

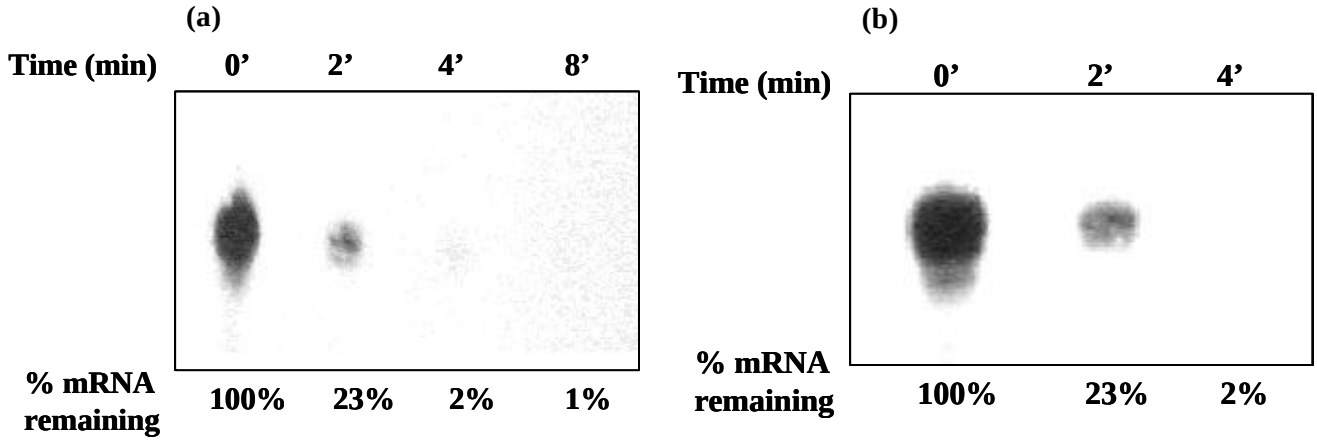

Figure 5. BglG does not stabilize mRNA downstream of the terminator. (**a**) A decay experiment carried out in the absence of BglG, with RNA isolated from JF201/pANS5-16 (terminator mutation at + 100). RNA downstream of the terminator was analysed using the probe SM33 (+ 141 to + 161). (**b**) Decay pattern of the downstream segment of *bgl* mRNA, in the presence of BglG. RNA was isolated from JF201/pAG-G (*bglG*$^{+}$) and analysed using SM33 as above.

sequence severely destabilizes the same segment of the transcript even in the presence of active BglG. Further support for this came from an independent study where a two to four-fold enhancement in steady-state levels of the 5′ region of the *bgl* mRNA was seen when cells were grown under inducing conditions (Schnetz and Rak 1988). However, stabilization of the promoter proximal mRNA is probably a reflection of localized protection brought about by BglG-mRNA binding since degradation of the transcript segment downstream of the terminator is not affected by the presence of the anti-terminator. Although the oligonucleotide probe used for quantifying the promoter proximal mRNA does not overlap with the RAT sequence, it is conceivable that BglG binding protects the 5′ end of the transcript, thus physically blocking cleavage sites on RNA from the attacking ribonucleases. Another possibility is that the alternative secondary structure that the 5′ end of the *bgl* RNA adopts in the presence of BglG, is resistant to endonucleases. A schematic representation of the pattern of decay of the *bgl* message, based on the results described here, is shown in figure 6.

Binding of BglG to its cognate sequence cannot stabilize the entire *bgl* message. Degradation experiments suggest that, while the upstream region of the nascent mRNA is protected by BglG binding, mRNA downstream of the terminator is efficiently degraded. This observation suggests that BglG binding to its cognate sequence is relatively stable and lasts even after the downstream mRNA is degraded. What is the advantage of an anti-terminator protein staying bound to the RNA even after its role in ensuring processivity of transcription through the terminator is fulfilled? To ensure the effectiveness of a regulatory pathway, the level of an activator/repressor should be limiting. Most regulators are present in catalytic amounts in the cell, a small alteration resulting in efficient control of gene expression, while a more dramatic one leads to short-circuiting of the same signal transduction pathway. BglG exists as a soluble protein and can alternate between the two structural forms, an active dimer or an inactive monomer, depending on the inducing conditions. The level of BglG is under its own positive control, the *bglG* coding sequence being preceded by a terminator, relief from which requires the anti-terminator. As a result of this positive feedback loop, *bglG* mRNA levels in the cell can escalate dramatically upon induction. For a fast reversal of response when inducers are depleted, BglG has to be immediately inactivated by the membrane-bound BglF. The results described here suggest that yet another form of BglG exists in the cell. A stable binding of BglG dimers to small RNA fragments would amount to sequestration of the protein in a dimeric, yet inactive form, thereby providing a sink for the regulator. Thus at a given point of time the BglG protein can be distributed into an active, dimeric form; a monomeric, non-functional counterpart and an inactive, dimeric form. The latter form probably prevents the system from getting overloaded with the positive regulator and keeps it sensitive to changes in inducer concentrations. This is supported by the observation that over expression of the leader mRNA using a multicopy plasmid results in down-regulation of a reporter gene that is positively regulated by a single copy of the chromosomal *bglG* gene (Mahadevan and Wright 1987). In other words, multiple copies of the leader mRNA are able to titrate out active copies of BglG within the cell, indicating that the functional dimer of BglG is sequestered by binding to its target sequence. However, the tritration experiments described above do not address the strength of the BglG-RNA interaction or the effect of this

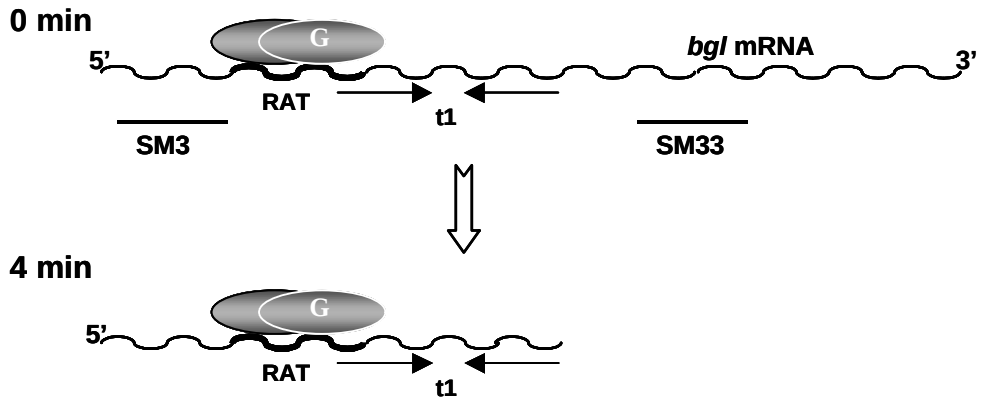

Figure 6. Degradation of *bgl* mRNA. The BglG dimer binds to its cognate RAT sequence as the nascent transcript emerges from the elongating complex and brings about anti-termination. The transcript is then subjected to degradation by RNAases but the promoter-proximal region remains protected. This protection can be attributed to BglG-binding since the anti-terminator stays bound to the 5′ segment of the mRNA even as the rest of the transcript decays (see text for details).

binding on mRNA stability. The results presented here offer the first direct *in vivo* analysis of the effect of BglG binding on mRNA stability. These results suggest that the two RAT sequences present in the *bgl* operon can act as effective sinks that prevent recycling of BglG, thus avoiding escalation of BglG concentration in the cell as a result of positive autoregulation of *bglG* upon induction.

In a recent report, BglG was shown to interact with the *b′* subunit of the *E. coli* RNA polymerase (RNP) (Nusbaum-Shochat and Amster-Choder 1999). In addition to facilitating the function of BglG at the terminator region, it was suggested that BglG might continue to be bound to the RNP and travel along with it as a part of the elongation complex. This was hypothesized to ensure the processivity of *bgl* transcription even after anti-termination at the rho-independent terminators has occurred. The results described here suggest that BglG remains bound to the RAT sequence with a relatively high half-life. The partitioning of BglG between the two functions will depend on its relative affinities to RNA and RNA polymerase.

In conclusion, this study focussing on the degradation of *bgl* mRNA and the involvement of BglG in the decay process, suggests a specific role for the 5′ stabilization in BglG-mediated positive regulation. Confirmation of these roles will enhance our understanding of the BglG-mediated signaling process. Further, similar studies for other members of the BglG/SacY family of anti-terminators will help reinforce the conclusions reported here.

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