Polyadenylation of a functional mRNA controls gene expression in Escherichia coli

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ABSTRACT

Although usually implicated in the stabilization of mRNAs in eukaryotes, polyadenylation was initially shown to destabilize RNA in bacteria. All the data are consistent with polyadenylation being part of a quality control process targeting folded RNA fragments and non-functional RNA molecules to degradation. We report here an example in Escherichia coli, where polyadenylation directly controls the level of expression of a gene by modulating the stability of a functional transcript. Inactivation of poly(A)polymerase I causes over-expression of glucosamine-6-phosphate synthase (GlmS) and both the accumulation and stabilization of the glmS transcript. Moreover, we show that the glmS mRNA results from the processing of the glmU-glmS cotranscript by RNase E. Interestingly, the glmU-glmS cotranscript and the mRNA fragment encoding GlmU only slightly accumulated in the absence of poly(A)polymerase, suggesting that the endonucleolytically generated glmS mRNA harbouring a 5' monophosphate and a 3' stable hairpin is highly susceptible to poly(A)-dependent degradation.

INTRODUCTION

It is well established now that mRNA decay has an integral role in the control of gene expression. mRNAs can be degraded by a number of mechanisms that act independently, in parallel or sequentially. Several cis- and trans-acting factors including higher order RNA structures (1,2), RNA-binding proteins (3–5) and translating ribosomes (6,7) control the sensitivity of an mRNA molecule to degradation. Hairpin structures located at the 3' end of operons or in intercistronic regions protect mRNA molecules from digestion by 3'-5' exonucleases. To overcome these barriers, bacteria initiate RNA degradation by endoribonucleolytic cleavages mostly carried out by RNase E in E.coli. RNase III and RNase G have also been found to initiate RNA decay in certain cases (8,9). RNA fragments resulting from endonucleolytic cleavages can be either further degraded endonucleolytically or by the 3'-5' exonucleases, poly-nucleotide phosphorylase (PNPase), RNase II and RNase R [reviewed in (10)]. Oligo(A) tails can be added to RNA fragments resulting from endo- or exonucleolytic cleavages and promote their exonucleolytic degradation (11).

As opposed to their predominant role in eukaryotes, poly(A) tails act as RNA-stabilizing elements in bacteria (12). A stabilizing function for poly(A) tails has also been subsequently reported in mitochondria of higher plants (13), chloroplasts (14), and the nucleus of yeast and humans [reviewed in (15,16)]. The enzyme responsible for the majority of polyadenylation in E.coli, poly(A)polymerase (PAP I), is encoded by the pncB gene (17). The consensus idea emerging from numerous studies of pncB mutations is that the main function of polyadenylation is to facilitate degradation of short folded RNAs such as RNA fragments produced during the processing of longer RNA molecules (17–19) and of a non-functional mutated tRNA (20). This latter example suggested that poly(A)-dependent decay is involved in quality surveillance of bacterial RNA, as previously reported in yeast (21). A special function of polyadenylation is in the control of copy number of ColEI plasmids. The decay of the regulatory RNA I is initiated by RNase E cleavage five nucleotides from the 5' end, which activates very fast decay via the PAP I-PNPase pathway (22). There are also data indicating that PAP I may control the stability of primary transcripts (23,24).

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For instance, mutations that inactivate RNase E-dependent decay render mRNA more sensitive to poly(A)-dependent degradation (25–27). In addition, Aiso et al. showed that the pcnB mutation affected degradation of rnf mRNA in stationary phase, but this effect could be indirect as it required de novo mRNA synthesis (24). However, there has been no evidence so far that polyadenylation directly controls protein levels by modulating mRNA stability.

In an attempt to identify genes whose expression is affected by mRNA polyadenylation, we compared proteins of a wild-type and PAP I-deficient strains on 1D and 2D PAGE gels (2D-gel). There were several differences and, in particular, one polypeptide; glucosamine-6-phosphate synthase (GlmS) was significantly more abundant in the mutant strain. In this work, we demonstrate that the overexpression of GlmS is correlated with the accumulation and stabilization of the monocistronic glmS mRNA resulting from the processing of the glmU-glmS cotranscript.

**MATERIALS AND METHODS**

**Bacterial strains**

The name and origin of the strains used here are listed in Table 1. New strains were constructed by transduction of the ΔpcnB (KanR) and rng::CmR alleles of strains SK7988 and CA244 cafA, respectively, with bacteriophage P1. Cells were grown in a MOPS medium supplemented with 0.2% glucose (w/v) and all amino acids and vitamins (28,29), or in an LB medium at the temperature specified in the text.

**Functional lifetime of bulk mRNA**

Cells were grown in MOPS medium supplemented with glucose and amino acids lacking methionine and cysteine and then treated with rifampicin (500 μg/ml; zero time). At timed intervals, 1 ml aliquots were labelled for 1 min with 25 μCi [35S] methionine (>1000 Ci/mmole) at 37°C. Cells were then treated with excess cold methionine and sodium azide for 5 min and pelleted. Frozen cells were resuspended in 0.1 M Tris–HCl, pH 8, 0.4 M NaCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, lysed by sonication on ice, and total proteins were separated on 10% SDS-PAGE.

**RNA extraction and analysis**

Templates for the synthesis of the different RNA probes were obtained by PCR amplification using the primers described in Table 2. The upstream primer includes the T7 promoter. RNA were synthesized by T7 RNA polymerase yielding uniformly labelled RNA with [z-32P]UTP (30). 5S rRNA was probed with the 5'-labelled ACTACCATCAGGGCCTACGGC oligonucleotide.

DNA preparation, reverse transcription (using the 5'-CGGTATTCCAGACGGCTGA primer complementary to the glmS mRNA) and Northern blotting on 10% agarose formaldehyde gel were previously described (31,32).

**2D gel electrophoresis**

Three independent cultures of N3433 and IBPC 903 were grown in LB at 37°C. 50 ml were withdrawn from OD650 = 0.5. Soluble protein extracts were prepared from cells sonicated in water, 20 μg/ml DNase and RNase were added after centrifugation at 10000 g for 30 min. First-dimension electrophoresis was carried out on a non-linear immobilized pH 3–11 gradient IPG strip (18 cm BioRad), and the second dimension by 12% SDS-PAGE. The six samples were analyzed in parallel. Densitometric quantification of the six blue-collodial-stained 2D gels was performed using the PDQuestTM 2D Analysis software. The analysis of these gels revealed that expression of two polypeptides was greatly increased in the mutant. These protein spots were excised from gels for trypsin in-gel digestion and MALDI-TOF analysis.

**RESULTS**

**PAP I deficiency modifies gene expression**

The presence of 3' terminal hairpins has been shown to protect mRNAs against exonucleolytic degradation.

**TABLE 1. New strains were constructed by transduction of the ΔpcnB (KanR) and rng::CmR alleles of strains SK7988 and CA244 cafA, respectively, with bacteriophage P1.**

**TABLE 2. PCR primers. Italics correspond to RNA polymerase sequence promoter of the T7 phage.**

**Immunoblotting**

Total proteins were separated on 10% SDS-PAGE, the proteins were transferred to Hybond C-super (Amersham) by electroblocting. The membrane was incubated with polyclonal anti-GFAT (GFAT = human glucosamine-6P synthase enzyme) antibodies, which cross-react against E. coli GlmS. The bound antibodies were detected by reaction with [125I]-labelled protein A.

Cells were grown in a MOPS medium supplemented with glucose and amino acids lacking methionine and cysteine and then treated with rifampicin (500 μg/ml; zero time). At timed intervals, 1 ml aliquots were labelled for 1 min with 25 μCi [35S] methionine (>1000 Ci/mmole) at 37°C. Cells were then treated with excess cold methionine and sodium azide for 5 min and pelleted. Frozen cells were resuspended in 0.1 M Tris–HCl, pH 8, 0.4 M NaCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, lysed by sonication on ice, and total proteins were separated on 10% SDS-PAGE.

Gels were stained with Coomassie blue and dried, and the radioactive pattern was analyzed with a PhospholImage.
The fact that the addition of poly(A) tails facilitates exonucleolytic degradation of tightly folded RNA prompted us to investigate whether polyadenylation affects stability of normal cellular mRNAs that possess stable 3' terminal hairpins. To do this, we examined the functional half-life of mRNAs by comparing the incorporation of radiolabelled methionine into proteins at fixed times after inhibiting RNA synthesis with rifampicin (zero time), and their protein-synthesizing capacity was monitored after 1, 3, 5, 7 and 9 min by pulse-labelling for 1 min with radioactive methionine. Soluble proteins were analyzed on SDS-PAGE gels that were autoradiographed. The positions of polypeptides overproduced in the penB strain are indicated by an arrow for the 66-kDa protein and by asterisks.

There were a few other differences in the pulse-labelled pattern of wt and penB strains. Two polypeptides whose expression is enhanced in the penB strain are indicated by asterisks (Figure 1A). It has been reported that overexpression of PAP I induces functional stabilization of meI and pnp mRNAs (33). However co-migration of the labelled proteins with a degradosome preparation did not detect any changes in expression of proteins corresponding to the sizes of these degradosome components.

In this work, we concentrate on the 66-kDa protein, which shows the strongest effect of penB-induced overproduction. In order to rule out possible strain or allele-specific effects of PAP I inactivation, we checked whether the 66-kDa polypeptide also accumulates in a strain carrying another penB allele and in different genetic backgrounds (Table 1 and Figure 1B). Indeed, the 66-kDa polypeptide accumulates in strains harbouring the penB::kanR (IBPC 903 and CA244-PAP) and the penB80 point mutation (IBPC 694), but is not detected in their penB+ parents. Moreover, this polypeptide is overproduced in the IBPC 903 mutant strain grown either in MOPS medium containing glucose and amino acids (Figure 1A) and in LB medium (Figure 1B). Strain N3433 and its penB::kanR derivative grown in LB medium were used in the experiments below.

**GlmS is upregulated in PAP I mutant**

In order to identify the 66-kDa polypeptide overproduced in the mutant, soluble proteins extracted from wild-type and penB strains were analyzed by 2D gel electrophoresis. Figure 2A shows that two polypeptides of about 66 kDa, migrating to adjacent positions on the gel, were much more abundant in the mutant than in the wild-type strain. Both spots were excised, and mass spectrometry identified these proteins as glucosamine-6-phosphate synthase (GlmS) (molecular weight 66.9 kDa), one of which may correspond to a modified GlmS form, although no modification of this protein was previously reported in *E. coli*. Densitometric quantification of the blue-colloidal-stained 2D gels revealed that the two spots of GlmS are 10- and 30-fold more abundant in the penB mutant compared to the wild-type strains. Finally, Western blotting with anti-GlmS antibodies confirmed that a 66-kDa polypeptide is produced much more efficiently in the PAP I mutant than in the wild-type strain (Figure 2B).
GlmS encodes the enzyme that synthesizes glucosamine-6-phosphate (GlcN6P), an essential precursor of lipopolysaccharides and peptidoglycan. The preceding gene on the chromosome, glmU, encodes the bifunctional enzyme glucosamine-1-phosphate acetyl transferase: UDP-GlcNAc synthetase (34,35), which synthesizes UDP-GlcNAc, the first dedicated component for the cell wall components. Previous work has shown that expression of both glmS and glmU is subject to regulation by the availability of amino sugars (35,36). Both genes are believed to be transcribed from two promoters located upstream of the first gene of the operon, glmU, and no promoter has been identified in the intergenic region.

The glmU-glmS primary transcript is processed in the intercistronic region

To investigate whether the overproduction of GlmS in cells lacking PAP I results from an accumulation of its mRNA, we compared the abundance of the glmS transcript in wild-type and PAP I-deficient strains. Total RNA was extracted from cells growing exponentially at 37°C, and amounts of this transcript were monitored on Northern blot. Probing for glmS detected a band of 1.9 kb that was about 16-fold more abundant in the pcnB strain than in the wild-type cells (Figure 3). A 1.9 kb transcript is sufficient to code for a protein of 66 kDa, but not for the glmU-glmS (glmUS) cotranscript. The very low amount of the 3.5-kb mRNA visible in Figure 3 led us to postulate that the expected glmUS cotranscript is very rapidly cleaved into the shorter 1.9-kb glmS mRNA detected in Figure 3.

We therefore investigated whether the endonucleases involved in mRNA maturation and degradation of E.coli mRNAs, namely RNase III (rnc), RNase E (rne) and RNase G (rng), cleave the glmUS cotranscript. Isogenic strains deficient for each of these RNases were used as well as a rne-rng double mutant. The effect of RNase E inactivation was examined by using the thermosensitive rne3071 allele. For comparison, RNAs from all strains were prepared from bacteria grown at 30°C (time 0) and 15 and 30 min after a temperature shift to 44°C required to inactivate thermosensitive RNase E. Total RNAs were then analyzed on Northern blots probed for glmS (Figure 4B). Neither the rnc105, nor the rng::CmR mutations allowed detection of the glmUS primary transcript or an increased level of glmS mRNA. In contrast, RNase E inactivation caused the accumulation of a long transcript with the size of the expected glmUS mRNA (3.5 kb). This result demonstrated that the glmUS cotranscript is cleaved by RNase E. Moreover, inactivation of RNase G in the absence of RNase E increases the level of the dicistronic transcript, suggesting that in the absence of RNase E there is some processing of the long primary transcript by RNase G but that normally rapid degradation by RNase E eliminates any effect of rng mutation. Probing the Northern blot for the glmU mRNA confirms that the full-length 3.5-kb transcript was only...
Figure 4. Fate of the transcripts of the \( glmU-glmS \) cotranscript. (A) Structure of the \( glmU-glmS \) transcript and of the processed mRNAs. The map of the \( glmU-glmS \) genes of \( E.coli \) shows the location of the 2 \( glmU \) promoters (arrows), of the transcription terminator (t) and the major RNase E processing site (black triangle). The structure of the primary transcript is indicated beneath the map, together with the mRNA species that were detected by Northern blot. The Northern blots do not distinguish between mRNAs starting at the two promoters. The small black box locates the oligonucleotide used in the primer extension experiment. Locations of the RNA used to probe for \( glmU \) and \( glmS \) are indicated by rectangles.

(B) Northern blot analysis of transcripts of the \( glmU-glmS \) operon. Strains indicated at the top of the autoradiograph were grown at 30°C in LB medium and shifted to 44°C for the times indicated in minutes at the top of the autoradiograph. Total RNA (12 μg) were analyzed on Northern blot, which were probed for \( glmS \), \( glmU \) mRNAs and 5S rRNA. 9S rRNA precursors accumulate after RNase E inactivation. As reported previously, \( rne^{+} \) strains contain higher amounts of 5S rRNA after the shift to 44°C (51). (C) Overexposure of a Northern blot probed for \( glmU \) transcripts. (D) Primer extension analysis of the \( glmU-glmS \) intercistronic region. Total RNA (12 μg) from N3433 (wt) and IBPC 903 (\( pcnB \)) strains were used as templates for AMV reverse transcriptase with the 5' labelled \( glmS \) oligonucleotide (1 pmol). The extension products were analyzed on a 6% denaturing polyacrylamide gel. Molecular sizing markers are shown on the right side of the autoradiograph. The \( glmS \) primer was used with the Sequenase kit (Amersham) on a PCR amplified fragment to precisely locate the 5' extremities of the reverse transcriptase reactions (data not shown).
surprisingly, thermal inactivation of RNase E leads not only to the accumulation of the uncleaved glmUS mRNA but also to an increased level of the downstream glmS monocistronic mRNA. The detection of the glmS monocistronic transcript implies that residual cleavage still occurs in the intergenic region of the glmUS transcript after the temperature shift that inactivates RNase E. RNase G is not responsible for this cleavage since the 1.9-kb glmS transcript was still detected in the double rne-rng strain. We previously observed residual processing of another RNase E site after thermal inactivation, implying that thermal inactivation of RNase E carrying the rne3071 mutation is not complete (31). Alternatively, the glmS transcript could be due to another unidentified endonuclease, or to the presence of a glmS-specific promoter located near the intergenic region. We note that the monocistronic glmS transcript is somewhat diffuse in the rne and rne-rng strains, which might be consistent with it being due to something other than cleavage at the major RNase E site. The observation that the glmS transcript is less abundant in rnc cells than in wt while no glmUS transcript is detected (Figure 4B lanes 1–3 and 13–15, respectively) suggests that RNase III may indirectly participate to glmS stability.

To localize the processing site that generates the 1.9-kb glmS mRNA, primer extension was performed with an oligonucleotide hybridizing to the beginning of the glmS structural gene. Three 5’ extremities were located, all mapping in the glmU-glmS intergenic region. The most abundant (band a on Figure 4D) is located just downstream of the stop codon of glmU mRNA. The AU-rich sequence surrounding this extremity (AAAAAGUGA↓UUCU) suggests that it might be generated by RNase E. Two minor 5’ ends located in the intergenic region were also observed when PAP I was inactive. One (band b) maps 20nt downstream of the major cleavage (band a) in the sequence CACAU↓GGG, and the other (band c) maps in a GC-rich sequence that does not conform to the usual RNase E cleavage site sequence (Figure 4D).

**Polyadenylation promotes degradation of the glmS transcript**

We next investigated whether the abundance of the glmS mRNA in the pcnB strain reflected the stabilization of this molecule. For this purpose, we measured the half-life of the glmS transcript in wild-type and pcnB strains at 37°C. The very low level of the bicistronic transcript detected under these conditions prevents bias due to the production of new glmS molecules during the experiment. The intracellular concentration of the monocistronic glmS mRNA was followed versus time after rifampicin addition (Figure 5A and B). The same Northern blot was probed for 5S rRNA to normalize the data. Figure 5A and B show that the glmS mRNA was more stable in the pcnB strain where its half-life was about 2.95 ± 0.17 min. The very low amount of glmS mRNA in the wild-type strain disappeared very rapidly; it was no longer detected 1.5 min after rifampicin addition. Its half-life was estimated to be 0.72 ± 0.17 min. The relatively short half-life of the glmS transcript in the pcnB strain agrees with the functional half-life measurement showing that GlmS synthesis was already considerably reduced 3 min after inhibition of transcription (Figure 1A). These data clearly demonstrate that PAP I inactivation stabilizes the glmS monocistronic mRNA. However, the ratio of half-lives is only a factor of about four, which is much less than the ratio of the abundance of the glmS transcript levels observed in the pcnB and the wild-type strains (about 16-fold) (Figures 3 and 5A) or of the GlmS protein on 2D gels (Figure 2A). It is clear that the pcnB mutation has some additional effect on the level of the glmS transcript. Whether this involves the RNase E processing of the bicistronic transcript or activation of additional transcription from the glmU promoters or a glmS specific promoter has not been clarified.

**Poly(A)polymerase specifically affects the stability of the glmS mRNA**

A typical Rho-independent terminator is located after the glmS gene, and one interpretation of the data described above is that polyadenylation facilitates exonucleolytic degradation of the 3’-terminal G-C rich hairpin of the transcription terminator, which otherwise protects the glmS transcript. Surprisingly, however, the glmUS cotranscript, which should have the same 3’ terminal-protecting motif, is much less abundant than the glmS mRNA in the absence of PAP I. RNAs extracted from wild-type cells and isogenic bacteria deficient for RNase E or PAP I or both RNase E and PAP I were analyzed on Northern blots and probed for glmS and glmU mRNAs (Figure 4B and C). The fact that the amount of the dicistronic mRNA detected in the rne-pcnB strain was...
slightly more abundant than in rne cells suggested that the long primary transcript was, to some extent, sensitive to poly(A)-assisted exonucleolytic decay. In agreement with this hypothesis, a smear of glms mRNAs, ranging from 1.9 to 3.5 kb, which presumably results from exonucleolytic degradation of the cotranscript, was detected when PAP I was active but not when it was inactive. However, the monocistronic glms mRNA accumulated much more strongly than the dicistronic transcript under identical conditions (i.e. after thermal inactivation of RNase E in the penB strain). These data reinforce the above observation that thermal inactivation of rne3071 is not complete or that some other mechanism exists to generate the 5' end of the glms mRNA. Irrespective of its origin, these data demonstrated that the monocistronic glms mRNA is much more sensitive to poly(A)-dependent degradation than the glmsUS cotranscript. Moreover, accumulation of both the glms monocistronic and the glmsUS dicistronic transcripts in a penB strain after RNase E inactivation confirms (see above) that they are both degraded by RNase E. Another unexpected observation was that the glms mRNA, which accumulates in the penB-rne+ strain growing at 30°C (Figure 4B, lane 16) and 37°C (Figure 3), disappeared after the temperature shift to 44°C (Figure 4B, lanes 17–18), suggesting that the RNase E-mediated degradation of glms mRNA is more efficient at 44°C than at 37°C.

Degradation of the glmU transcript

Cleavage of the glmsUS cotranscript would be expected to generate a glmU-specific transcript that is not detected in the wt strain (Figure 4B, lane 13). However in the rne strain after temperature shift, an additional transcript of about 1.5 kb is observed with the glmU probe (Figure 4B, lanes 11–12 and 4C lanes 2–3). Again, this transcript must be either due to residual RNase E activity in the thermal inactivated cultures, or the result of another endonuclease. As the transcript is only slightly more abundant in the rne- penB strain than the rne strain (Figure 4C, lanes 5–6 and 2–3, respectively), it is not dependent on polyadenylation for its degradation, unlike the glms monocistronic transcript.

DISCUSSION

We present here evidence that poly(A)polymerase can negatively affect gene expression. Previously, it was thought that the role of the poly(A)-dependent pathway was to increase the turnovers of defective tRNAs (37) and non-processed RNA precursors (38), and to clear the cell of endonucleolytically produced, tightly folded mRNA fragments resistant to further degradation by exonucleases (39,40). Here, we show that overproduction of glucosamine-6P synthase, an essential enzyme in E.coli in the absence of exogenous aminosugars, occurs upon inactivation of PAP I, and that this accumulation is correlated with the accumulation of the glms mRNA and the reduction in its decay-rate. This led us to conclude that poly(A)-dependent degradation of the functional glms mRNA determines the yield of GlmS. In agreement with our observation, glms ORF was recently shown, using macroarrays, to be highly polyadenylated when PAP I is overproduced (41). One cannot exclude, however, that polyadenylation acts indirectly on GlmS expression, e.g. by modulating the stability of a small regulatory RNA or another trans-acting factor.

We also demonstrate that the glms monocistronic mRNA, stabilized in PAP I-deficient cells, likely results from the endonucleolytic maturation of the glmsUS dicistronic primary transcript. Although the dicistronic transcript was only detected in cells lacking RNase E, the fact that the monocistronic glms transcript is still produced in these bacteria suggests that glmsUS could be processed by another enzyme. The 5' mRNA extremity mapping just downstream of the UGA stop codon suggests that ribosome stalling could be implicated in the mechanism of glmsUS RNA processing (42–45).

Interestingly, in spite of the fact that the glms and glmsUS mRNAs have identical 3' terminal structures, we found that the primary transcript is much less sensitive to the poly(A)-assisted exonucleolytic route of decay than the monocistronic processed molecule. An important question arising is how the poly(A)-assisted machinery of decay recognizes its targets and, in the case described above, how it distinguishes the glms monocistronic mRNA from glmsUS primary transcript and other primary transcripts. The fact that the glms and glmsUS mRNA correspond to the 3' part of the dicistronic transcript implies that it is the nature of the 5' part of the RNA that determines its sensitivity to this pathway. One possibility is that, as described earlier for RNA I, the 5' monophosphorylated terminus generated by RNase E facilitates polyadenylation of the glms mRNA and thus promotes its degradation (22). Such a model could explain why the RNase E-processed mRNA is more sensitive to the poly(A)-dependent pathway than the primary transcript harbouring a 5' triphosphate extremity. At the moment, the mechanism by which the 5' end of the long 1.9-kb glms mRNA could affect a reaction occurring at its 3' end is not known. A greater accessibility of the 3' end has been proposed to explain why the precursor of a mutated tRNA5'P is degraded by a poly(A)-dependent pathway (37). One can imagine that refolding of the glms mRNA consecutive to the processing of the primary transcript improves accessibility of PAP I and/or exonucleases at its 3' end. It is possible, for example, that translation of glms is coupled to that of the upstream gene glmU and that a reduction of ribosome loading of the glmU message consecutive to the removal of the upstream glmU cistron modifies the folding of the molecule.

It is worth mentioning here that the 5' monophosphate extremity that may trigger the poly(A)-dependent decay of the glms mRNA may also promote its degradation by RNase E (46). The fact that the glms mRNA accumulates in the absence of PAP I at 30°C, 37°C but not at 44°C is consistent with the idea that the efficiency of its degradation by RNase E increases at high temperature. In contrast, this observation indicates that the contribution of the poly(A)-dependent pathway to the decay of this message increases at low temperature. It therefore appears
that RNase E exerts a predominant role in mRNA decay around the optimum temperature of growth and that the poly(A)-dependent decay may substitute for RNase E at lower temperatures. The idea that poly(A)-dependent decay can substitute for RNase E is also supported by earlier data showing that this pathway becomes effective on mRNAs that are no longer degraded by RNase E (25–27).

GlmS catalyzes the conversion of fructose-6-phosphate into glucosamine-6-phosphate, which then undergoes sequential transformations leading to the formation of UDP-N-acetylglucosamine, the major intermediate in the biosynthesis of all amino-sugar-containing macromolecules in the cell (47). Regulation of glmS expression occurs both at the transcriptional level (36) with NagC acting as both an activator and a repressor of the glmUS operon in E.coli (35) and at the posttranscriptional level (present work). Interestingly, the expression of the glmS gene of Bacillus subtilis is also subject to a regulatory mechanism involving the processing and the degradation of its mRNA. However, in these bacteria lacking orthologs of both RNase E and PAP I (48,49), an alternate strategy has emerged. A new class of ribozyme that cleaves the messenger of the glmS mRNA was discovered, which is activated by GlcN6P (50). In spite of some sequence homology in the glmU-glmS intergenic region in bacterial species closely related to E.coli, in-line probing did not reveal any structural changes or self-cleavage activity when the entire E.coli intergenic glmU-glmS region was incubated with either Glc6P or GlcN6P (Elaine Lee and Ron Breaker, personal communication). It therefore appears that different strategies exist that allow sensitive control of glmS mRNA and hence protein levels despite the fact that the machineries of mRNA processing and degradation are very different. Such pathways may present selective advantages that could explain their conservation in Gram-positive and Gram-negative bacteria.

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