Stability of the Osmoregulated Promoter-Derived proP mRNA Is Posttranscriptionally Regulated by RNase III in *Escherichia coli*

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**ABSTRACT**

The enzymatic activity of *Escherichia coli* endo-RNase III determines the stability of a subgroup of mRNA species, including *bdm*, *betT*, and *proU*, whose protein products are associated with the cellular response to osmotic stress. Here, we report that the stability of *proP* mRNA, which encodes a transporter of osmoprotectants, is controlled by RNase III in response to osmotic stress. We observed that steady-state levels of *proP* mRNA and ProP protein are inversely correlated with cellular RNase III activity and, in turn, affect the proline uptake capacity of the cell. *In vitro* and *in vivo* analyses of *proP* mRNA revealed RNase III cleavage sites in a stem-loop within the 5’ untranslated region present only in *proP* mRNA species synthesized from the osmoregulated P1 promoter. Introduction of nucleotide substitutions in the cleavage site inhibited the ribonucleolytic activity and, in turn, affected the proline uptake capacity of the cell.

**IMPORTANCE**

Our results demonstrate that RNase III activity on *proP* mRNA degradation is downregulated in *Escherichia coli* cells under osmotic stress. In addition, we show that the downregulation of RNase III activity is associated with decreased RNA binding capacity of RNase III under hyperosmotic conditions. In particular, our findings demonstrate a link between osmotic stress and RNase III activity, underscoring the growing importance of posttranscriptional regulation in modulating rapid physiological adjustment to environmental changes.

The enzymatic properties and physiological roles of RNase III family enzymes are evolutionarily well conserved in both prokaryotes and eukaryotes (1–3). These enzymes are double-strand-specific endo-RNases that create 5’-phosphate and 3’-hydroxyl termini with two-nucleotide overhangs. Genome-wide analyses of *Escherichia coli* transcripts indicated that the abundance of a large number of mRNA species is regulated by RNase III (4, 5). The abundance of several *E. coli* mRNA transcripts is dependent on the endoribonucleolytic activity of RNase III, including *rnc* (6), *pnp* (7), *bdm* (8), *betT* (9), *corA* (10), *proU* (11), *mltD* (12), and *rrg* (4). Studies indicate that a subgroup of mRNA transcripts encoding factors associated with the cellular response of *E. coli* to osmotic stress is regulated by RNase III (8, 9, 11). The mechanisms of RNase III-mediated regulation of *bdm* (8), *betT* (9), and *proU* (11) have been identified. Specifically, RNase III controls the degradation of these mRNAs and their cleavage is significantly altered in *E. coli* cells exposed to osmotic stresses. The mRNA abundance of *proP*, another important osmoregulator, appeared to be dependent on the cellular RNase III concentration (8). However, the osmotic stress-induced RNase III-mediated regulation of *proP* has not been studied.

In *E. coli*, the *proP* gene encodes a low-affinity transporter of osmoprotectants, including proline and glycine betaine, which sense extracellular osmotic pressure and respond by maintaining membrane turgor pressure (13, 14). ProP is a member of the major facilitator superfamily and is an osmoprotectant proton symporter that is regulated by high osmotic pressure (15–17). *proP* is transcribed from two different promoters, an *rpoD*-dependent promoter, P1, and an *rpoS*-dependent promoter, P2. P1, which is similar to the *proU* promoter, is transiently induced upon subculture and is upregulated under hyperosmotic conditions, while P2 is induced by the stationary phase in the presence of Fis, a small, nucleoid-associated protein (18–20). Expression of *proP* is transcriptionally regulated by cyclic AMP receptor protein (CRP), an osmotic repressor of *proP* P1 transcription, and by Fis, an activator of *rpoS*-dependent promoter expression (15, 18, 20). Recent studies showed that lesions at *proQ*, which encodes an RNA chaperone, reduced the levels of ProP protein and activity through an unknown mechanism (21–24).

On the basis of previous findings that suggested an important role for RNase III in the posttranscriptional regulation of genes...
involved in the cellular response to osmotic stresses, we investigated the effect of RNase III activity on the expression of the proP gene, the protein product of which greatly contributes to the osmotic resistance of *E. coli*. Here, we provide direct evidence that RNase III controls the degradation of proP mRNA by cleaving a stem-loop in its 5′ untranslated region (UTR) present only in mRNA species synthesized from the osmoregulated P1 promoter, suggesting a physiological relationship between the regulation of RNase III activity and osmotic stress resistance.

**MATERIALS AND METHODS**

**Strains and plasmids.** The *E. coli* strains and plasmids used in this study are listed in Table 1. *E. coli* strains BL2014 and BL20124 were constructed by deleting the *proP* gene (from the 5′ UTR to the 3′ end of the coding region) from the genomic DNA of MG1655 and BL2012, respectively, as previously described by Datsenko and Wanner (25). The PCR primers used in these experiments are listed in Table 2.

**PCR primers used.**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>proP 5′-UTR-F</td>
<td>5′-ATCTCTGTAACAAGCGTAGCNGTTT-3′</td>
</tr>
<tr>
<td>prop-108F</td>
<td>5′-GCTTACACCCTCCGGT-3′</td>
</tr>
<tr>
<td>proP 3′-UTR-F</td>
<td>5′-GGCCCCCTTTTCTGAAACTCG-3′</td>
</tr>
<tr>
<td>prop-5-UTR-F</td>
<td>5′-ATAAGACAGCGTCACATCAGG-3′</td>
</tr>
<tr>
<td>prop-3-end-His-R</td>
<td>5′-GTACACTGGGATCGCTGAATT-3′</td>
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**RESULTS**

**Semi-quantitative reverse transcription (RT)-PCR analysis.** Semi-quantitative reverse transcription (RT)-PCR was performed, and the results were analyzed as previously described (9, 12). Total RNA was isolated with an RNase Mini-prep kit (Qiagen, Valencia, CA, USA), and 1 μg of RNA was used for cDNA synthesis with a PrimeScript first-strand cDNA synthesis kit (TaKaRa, Otsu, Shiga, Japan). The primers used for RT-PCR were proP + 1 (5′-GTCAATTAAGCCTAACGCGAACGTAATT-3′) and proP + 309 (5′-GCCCGGAGACAGCGGTGAATTTGT-3′) for pProP, prop-R5′ (5′-TTTGCCCTACGGAGTACAGA-3′) and prop-R3′ (5′-TTGCTCGTACCGGCGGTGACTG-3′) for pnp, rpoS-O-5′RT (5′-GCTACTGGGATCGCTGTAATT-3′) and rpoS-3′RT (5′-GCCCGGAGACAGCGGTGAATTTGT-3′) for rpoS, and RNC cds-his (5′-TTCGCCCTTTTCTGAAACTCG-3′) for RNC.

**Western blot analysis.** Western blot analysis was performed as previously described (28). An anti-His tag monoclonal antibody was used to detect ProP-His, and polyclonal antibodies against RNase III and ribosomal protein S1 were also used. Specific proteins were imaged with a VersaDoc 100 (Bio-Rad, Hercules, CA) and quantified with Quantity One software (Bio-Rad, Hercules, CA).
RESULTS

RNase III negatively regulates proP expression. To investigate whether the absence of RNase III activity affects the abundance of proP mRNA, we measured the steady-state levels of proP mRNA in E. coli strains MG1655 and BL2012 by semiquantitative RT-PCR. Consistent with the microarray data from our previous study (8), rnc mutant cells showed a 2.0-fold increase in the amount of proP mRNA compared to that observed in wild-type cells (Fig. 1A). Western blot analysis also showed that the level of hexahistidine-tagged ProP expression from pProP4 in rnc mutant cells is approximately 3.5 times as high as that in wild-type cells (Fig. 1B). This enhanced ProP expression functioned as an adaptation reaction of E. coli cells exposed to high osmolarity, as indicated by the highest rate of proline uptake detected in rnc mutant cells in the presence of 0.5 M NaCl (Fig. 1C). Under high-osmolarity conditions, the external proline concentration of rnc-positive cells was approximately 4.24-fold higher than that observed in rnc mutant cells. These results imply that, in addition to transcriptional activation of proP expression under hyperosmotic conditions, RNase III is also associated with increased proP expression.

Identification of putative RNase III cleavage sites in proP mRNA. Previous S1 nuclease mapping and primer extension analyses of proP mRNA showed several uncharacterized proP mRNA species with 5′ ends that were mapped between the proP P1 and P2 promoters (Fig. 2) (18, 19). These proP mRNA species were thought to result from either primer extension artifacts or transcription from other start sites. However, on the basis of our observation of RNase III-dependent proP mRNA abundance, we hypothesized that they may represent mRNA species that were cleaved by RNase III. To test whether the region between the P1 and P2 promoters contains cis-acting elements that are responsive to RNase III, we performed a primer extension analysis with a

![Image](http://jb.asm.org/article-figures/FIG1.png)

**FIG 1** Downregulation of proP expression by RNase III. (A) Semiquantitative RT-PCR analysis of proP mRNA. The effects of rnc deletion on proP mRNA levels were measured by using total RNA prepared from E. coli strains MG1655 (wild type, WT) and BL2012 (rnc). The strains were grown in M63 medium supplemented with 22 mM glucose and 0.17 M NaCl until an OD_{600} of 0.6 was reached. The abundance of M1, the RNA component of RNase P, was measured as an internal standard to evaluate the total amount of mRNA in each reaction mixture. (B) Western blot analysis of the ProP protein. E. coli strains BL2014 and BL2012 harboring the pProP4 plasmid, which contains the gene encoding hexahistidine-tagged ProP, were grown as described above. The amounts of ProP, RNase III, and ribosomal protein S1 were analyzed by Western blot assay. The S1 protein was used as an internal standard to evaluate the amount of cell extract loaded in each lane. The same membrane that was probed for His-tagged ProP was also probed with polyclonal antibodies to RNase III and S1. (C) Effects of RNase III activity on proline uptake as determined by colorimetric analysis of external proline. Strains BL2012 and BL2012 harboring either pPM30 or pProP3 were incubated at 37°C in modified Davis minimal medium containing 1 mM proline. Proline uptake was measured in the absence of NaCl (0 hr) and again after 3 h of incubation in the presence of 1 M NaCl, and the final cell density was adjusted to an OD_{600} of 0.5. The external proline content was determined as described by Nagata et al. (33).
5′-end 32P-labeled primer (proP-125R) that was designed to hybridize to a region downstream of the P2 promoter. Total RNA was purified from wild-type and rnc mutant cells grown in Luria-Bertani medium. In the lane loaded with the reaction mixture containing total RNA from wild-type cells, we observed one cDNA band, which we designated the B site (Fig. 3A). For a higher-resolution analysis of the cDNA bands, the reaction was performed with total RNA prepared from E. coli cells overexpressing proP mRNA. In this reaction mixture, we observed two major cDNA bands extended from proP mRNA. In this reaction mixture, we observed two major cDNA bands extended from proP mRNA that appeared to be RNase III dependent (Fig. 3B). One band corresponded to the B site, while the other was designated the A site. These bands were not present in the lane loaded with the reaction mixture containing total RNA extracted from cells that overexpressed proP mRNA. In this reaction mixture, we observed two major cDNA bands extended from proP mRNA that appeared to be RNase III dependent (Fig. 3B). One band corresponded to the B site, while the other was designated the A site. These bands were not present in the lane loaded with the reaction mixture containing total RNA extracted from cells that overexpressed proP mRNA. In this reaction mixture, we observed two major cDNA bands extended from proP mRNA that appeared to be RNase III dependent (Fig. 3B). One band corresponded to the B site, while the other was designated the A site. These bands were not present in the lane loaded with the reaction mixture containing total RNA extracted from cells that overexpressed proP mRNA. In this reaction mixture, we observed two major cDNA bands extended from proP mRNA that appeared to be RNase III dependent (Fig. 3B). One band corresponded to the B site, while the other was designated the A site. 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In this reaction mixture, we observed two major cDNA bands extended from proP mRNA that appeared to beRNase III cleavage determines proP mRNA stability in vivo. To test whether RNase III cleavage regulates the stability of proP mRNA, we randomly introduced nucleotide substitutions at cleavage site B in the proP overexpression plasmid (pProP4) and analyzed five of these clones (Fig. 4A). Various levels of ProP protein expression were observed among the clones (Fig. 4B). To examine the relationship between the steady-state levels of proP mRNA and ProP protein expression, total RNA was isolated from these clones and a Northern blot assay was performed to measure the steady-state levels of proP mRNA (Fig. 4C). The increase in proP mRNA abundance resulting from nucleotide substitutions at cleavage site B accordingly affected ProP protein expression levels. Clone 5 showed the most dramatic increase in proP mRNA abundance (6.65-fold increase). This clone contained two nucleotide substitutions (C→111A and C→110G) that completely disrupted the base pairing between cleavage sites A and B. To test whether the increased steady-state levels of mutant proP mRNA were a consequence of decreased RNase III cleavage, we performed a primer extension analysis. The results demonstrated alterations in the abundance and cleavage patterns of the cDNAs produced from the mutant proP mRNA to those produced from the wild-type proP mRNA (Fig. 4D). cDNA bands corresponding to cleavage site B in the mutant proP mRNA of clones 1, 2, and 3, which contain C→110U, C→111U C→110A, and C→110G, respectively, exhibited a shift of one or two nucleotides. Clones 4 and 5, which contain the C→111U C→110G and C→111A C→110G mutations, respectively, did not show cleavage products from the B site. Cleavage products from the A site in mutant proP mRNA were absent from clone 5. Although the effects of these mutations on RNase III-mediated cleavage of proP mRNA differed, the ratios of the total intensity of the cDNA bands corresponding to the RNase III cleavage products to that of the putative P1 transcriptional initiation site were inversely correlated with the steady-state levels of proP mRNA and ProP protein expression levels. In addition, analysis of mutant proP mRNA from clones 4 and 5 indicated that RNase III cleavage at site A (not A and B) is the rate-limiting step for proP mRNA degradation in vivo, as abolishment of RNase III cleavage activity at site B was sufficient to stabilize proP mRNA (Fig. 4D). Next, the half-lives of these proP mRNAs were measured to test whether the increased steady-state levels of proP mRNA are a consequence of increased proP mRNA stability. Wild-type proP mRNA and a mutant proP mRNA transcribed from clone 4 were used for these experiments since this mutant mRNA appears to form a stem-loop with a stability similar to that of the wild-type mRNA (ΔG = -70.36 versus -77.81 kcal/mol), and it exhibited a significant increase in mRNA abundance. The half-life of the mutant mRNA was 2.7-fold higher than that of the wild type (~0.75 min versus ~2.00 min), indicating a good correlation between proP mRNA abundance and stability. These results suggest that proP mRNA stability is regulated by RNase III cleavage.
incomplete 3

and gel picture. Other minor cleavage products indicated by asterisks might have been produced by RNase III digestion of RNA transcripts containing an

using size markers generated by alkaline hydrolysis (Hydrolysis) and RNase T1 digestion. Positions of G residues are numbered 1 to 17 in the secondary structure

secondary structure of proP

mRNA. The model hairpin was used for the in vitro cleavage assays presented in Fig. 2E and F. It is shown in the right panel. (E, F) In vitro cleavage of the model proP hairpin RNA. One picomole of 3'-end 32P-labeled (E) or 3'-end 32P-labeled (F) proP model hairpin was incubated with 0.05 pmol of purified RNase III in a cleavage buffer with (III + Mg2+) or without (III) MgCl2. Samples were withdrawn at the indicated time intervals and separated by 12% PAGE in gels containing 8 M urea. Cleavage products (A and B) were identified by using size markers generated by alkaline hydrolysis (Hydrolysis) and RNase T1 digestion. Positions of G residues are numbered 1 to 17 in the secondary structure and gel picture. Other minor cleavage products indicated by asterisks might have been produced by RNase III digestion of RNA transcripts containing an incomplete 3' or 5' end.

FIG 3 Identification of putative RNase III cleavage sites in proP mRNA in vitro and in vivo. (A) Primer extension analysis of endogenously expressed proP mRNA. (B) Primer extension analysis of heterologously overexpressed proP mRNA. Total RNA was prepared from MG1655 and BL21(DE3) (A) or those harboring pProP3 (B), which were grown at 37°C in Luria-Bertani (LB) medium supplemented with 0.3 M NaCl. Total RNA (40 or 80 μg) was hybridized with a 5'-end 32P-labeled primer (proP-125R). Synthesized cDNA products were analyzed by 12% polyacrylamide gel electrophoresis (PAGE). Sequencing ladders were produced with the same primer used for cDNA synthesis, and a PCR product encompassing the proP gene was used as the template. Putative transcription initiation sites derived from the P1 and P2 promoters are identified as TIS (P1) and TIS (P2), respectively. *, +, no expression; +, endogenous expression; +++, overexpression; **, cDNA bands synthesized in a proP mRNA-independent manner under high-osmolarity conditions. (C) Primer extension analysis of a proP transcript synthesized in vitro. One picomole of full-length proP transcript synthesized from the P1 transcript start site was incubated with or without 0.09 pmol of purified RNase III for 10 min in cleavage buffer with MgCl2. RNA samples were purified by phenol-chloroform extraction and ethanol precipitation and then hybridized with a 5'-end 32P-labeled primer (proP-125R). Lane m contains the reaction mixture used in the last lane of Fig. 3B and was used to compare cDNA products synthesized from the RNase III-cleaved synthetic proP mRNA in vitro. Synthesized cDNA products were analyzed by 12% PAGE. (D) Predicted secondary structure of proP mRNA. The secondary structure was deduced with the M-fold program (40). The model hairpin RNA used for the in vitro cleavage assays presented in Fig. 2E and F is shown in the right panel. (E, F) In vitro cleavage of the model proP hairpin RNA. One picomole of 5'-end 32P-labeled (E) or 3'-end 32P-labeled (F) proP model hairpin was incubated with 0.05 pmol of purified RNase III in a cleavage buffer with (III + Mg2+) or without (III) MgCl2. Samples were withdrawn at the indicated time intervals and separated by 12% PAGE in gels containing 8 M urea. Cleavage products (A and B) were identified by using size markers generated by alkaline hydrolysis (Hydrolysis) and RNase T1 digestion. Positions of G residues are numbered 1 to 17 in the secondary structure and gel picture. Other minor cleavage products indicated by asterisks might have been produced by RNase III digestion of RNA transcripts containing an incomplete 3' or 5' end.
FIG 4 Introduction of mutations at the putative RNase III cleavage sites inhibits RNase III-mediated cleavage of proP mRNA. (A) Secondary structures of the proP model hairpin RNAs containing the nucleotide substitutions of each mutant proP mRNA are shown. The mutated nucleotides are those within the rectangles. WT, wild type. (B) Effects of RNase III cleavage site mutations on ProP protein levels. E. coli BL2014 strains harboring either pProP4 or one of five mutant clones with the indicated substitution(s) (mutant clone 1, C→H11002111U; mutant clone 2, C→H11002110U and C→H11002111A; mutant clone 3, C→H11002111G; mutant clone 4, C→H11002110U and C→H11002111G; or mutant clone 5, C→H11002110A and C→H11002111G) were grown as described in the legend to Fig. 2B. The amounts of ProP, RNase III, and ribosomal protein S1 were analyzed by Western blot assay. (C) Effects of RNase III cleavage site mutations on steady-state levels of proP mRNA, as determined by Northern blot assay. Total RNAs were prepared from strain MG1655 cells harboring either pProP4 or one of five mutant clones grown as described above. The
sults indicated that RNase III cleavage activity at the stem-loop is largely responsible for proP mRNA abundance.

**Osmoregulation of proP expression by RNase III.** Previous studies have demonstrated the RNase III activity-mediated osmoregulation of the expression of several osmosensing factors, including Bdm, ProU, and BetT (8, 9, 11). On the basis of these results, we investigated the relationship between the osmoregulation of RNase III activity and proP expression levels by measuring the half-lives of proP mRNAs under high-osmolarity conditions. The results of a semiquantitative RT-PCR analysis showed that in wild-type E. coli, the half-life of proP mRNA under high-osmolarity conditions was approximately twice as long as that under normal conditions (1.5 min versus 3.2 min) (Fig. 5A). Steady-state levels of proP mRNA were also approximately 1.4 times as high under high-osmolarity conditions, showing a correlation between the half-life and abundance of proP mRNA. In rnc mutant cells, the half-life of proP mRNA also differed slightly under high-osmolarity conditions (3.4 min versus 4.4 min under normal and high-osmolarity conditions, respectively), and the steady-state levels of proP mRNA were approximately 1.1 times as high under high-osmolarity conditions (Fig. 5A). The increased steady-state levels of proP mRNA observed in rnc mutant cells under high-osmolarity conditions may reflect the activation of proP mRNA transcription from P2, which does not contain RNase III cleavage sites, as has been previously shown (15, 18, 19). A small decrease in the half-life of proP mRNA in rnc mutant cells under high-osmolarity conditions suggests that there may be some rnc-independent components of the regulation of proP mRNA degradation. One such candidate is ProQ, an RNA chaperone protein that affects the expression and activity of ProP via an unknown mechanism (21–24). We tested this possibility by measuring the half-life of proP mRNA in response to hyperosmotic stress in the presence and absence of functional ProQ. The results did not indicate ProQ involvement in the RNase III-mediated posttranscriptional regulation of proP expression, as ProQ-dependent changes in the half-life of proP mRNA were not observed (Fig. 5B).

These results indicate that the increased steady-state levels and half-life of proP mRNA may be associated with decreased RNase III activity, as previously reported (8, 9). In order to determine the basis of the decreased RNase III activity observed under hyperosmotic conditions, we analyzed the RNA bound to RNase III by in vivo cross-linking and immunoprecipitation of RNase III (Fig. 5C). The amount of the RNase III substrates, such as the proP and pnp mRNAs, which were cross-linked to RNase III under hyperosmotic conditions was approximately 1.4- to 4-fold lower than that precipitated under normal conditions. The amount of rpsO mRNA, which is not an RNase III substrate (8, 34, 35), was not significantly different. These results indicate that the decreased RNase III cleavage activity detected under hyperosmotic conditions is related to the decreased RNA binding capacity of RNase III.

**DISCUSSION**

The regulation of proP expression at the transcriptional level under different osmolarity conditions has been well studied (15, 16, 18, 21, 22, 36, 37). These studies showed that the proP P1 promoter rapidly and transiently responds to alterations in the osmolarity of the culture medium due to the activities of CRP and osmoprotectants (15, 19, 38). In this study, we identified an additional proP expression regulatory pathway involving RNase III at the posttranscriptional level. Here, we demonstrated that RNase III cleavage of the stem-loop in the 5′ UTR determines the decay rate of proP mRNA synthesized from the osmoregulated P1 promoter and affects ProP protein levels and proline uptake capacity (Fig. 2 and 3). This observation led us to speculate that subtle conformational changes in the RNase III-targeted stem-loop region could be induced by regulatory factors under various osmotic stress conditions, which may alter the RNase III ribonucleolytic activity (20, 21). Another possibility is that the increased cellular levels of proline under conditions of hyperosmotic stress alter RNase III activity on proP mRNA. However, we were not able to detect significant changes in the in vitro RNase III activity on the model proP hairpin RNA (see Fig. S1 in the supplemental material). It is still possible that ProQ and/or proline play a role in the RNase III-mediated posttranscriptional regulation of proP expression under other conditions. When E. coli cells are exposed to high-salt conditions, transcription from the proP P1 promoter is induced for a brief period of time (~5 min) and is then dramatically repressed after 15 min, the time point that coincides with the osmolarity-dependent binding of the CRP to a site within the proP P1 promoter (15). Our present study explains how the increased steady-state levels of proP mRNA resulting from this burst of proP transcription are maintained under hyperosmotic conditions, as RNase III cleavage of proP mRNA is downregulated in hyperosmotic media. Our analysis of the mRNA bound to RNase III suggested the basis for decreased RNase III activity under hyperosmotic conditions (Fig. 5C), which did not appear to be specific to the mRNA species involved in osmoregulation. Previous studies indicated that RNase III activity in E. coli could also be regulated under other conditions, such as bacterial phage infection, cold stress, and aminoglycoside antibiotic stress (4, 32, 39). These studies identified various protein regulators of RNase III, including T7 protein kinase and YmdB. However, these regulators do not appear to be involved in the osmoregulation of RNase III activity (8) and we are currently investigating the factors involved in osmoregulation.
The importance of the regulation of RNase III activity in the expression of osmosensing genes such as proU (11) and betT (9) under various osmolarity conditions has been recently recognized. Similarly, we showed that the expression of one of these important osmosensing genes, proP, is posttranscriptionally regulated by RNase III activity. Because the regulation of osmosensing factors needs to be rapidly controlled, posttranscriptional regulation of gene expression by RNase III activity has advantages over the de novo synthesis of factors that regulate the transcription of osmosensing genes. In addition, the downregulated RNase III activity observed under high osmolarity maintains the induced expression levels of osmoreponsive genes, resulting in the survival of E. coli cells upon osmotic shock. Taken together, the results suggest that the RNase III-mediated osmoregulatory pathway is largely responsible for the modulation of the expression of typical osmosensing and osmoreponsive genes, including bdm, proU, betT, and proP. Consequently, this pathway regulates cell survival upon sudden changes in environmental osmotic pressure.

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