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Poly(A) RNA in *Escherichia coli*: Nucleotide sequence at the junction of the lpp transcript and the polyadenylate moiety

GONG-JIE CAO AND NILIMA SARKAR*

Department of Metabolic Regulation, Boston Biomedical Research Institute, Boston, MA 02114; and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

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ABSTRACT **Although it has been known for some time that bacterial mRNA molecules carry polyadenylate moieties at their 3′ ends, nothing is known about the molecular structure of bacterial poly(A) RNA. To define the polyadenylylation site of a specific bacterial mRNA, we took advantage of the presence of elevated levels of poly(A) RNA in cells of *Escherichia coli* deficient in exoribonucleases and synthesized DNA complementary to polyadenylylated lipoprotein mRNA, encoded by the *lpp* gene, by using avian myeloblastosis virus reverse transcriptase and an oligo(dT)-containing primer. The 5′-terminal portion of the cDNA was amplified by the polymerase chain reaction and appropriate oligonucleotide primers, and the amplified DNA was cloned in pUC18 and subjected to nucleotide sequence analysis. Four clones were found to contain the entire 3′-terminal coding region of lpp mRNA, with poly(A) attached to either of two sites in the downstream untranslated region of the transcript. In one type of clone, the polyadenylate moiety was attached at the putative transcription termination site of lpp mRNA, whereas other clones lacked the stem–loop structure of the ρ-independent transcription terminator and the polyadenylate moiety was attached to the residue just preceding the terminal stem–loop of the primary transcript. A model for the polyadenylylation of bacterial mRNA is proposed in which poly(A) polymerase and exonucleases compete for the 3′ ends of mRNA molecules.**

In recent years, much evidence has accumulated that most newly synthesized bacterial mRNA molecules resemble eukaryotic mRNA in that they contain poly(A) tracts at their 3′ ends (1–9). However, it has been difficult to characterize bacterial poly(A) RNA on the molecular level, primarily due to the great instability of prokaryotic mRNA, which makes the isolation of intact mRNA much more difficult than in eukaryotes. The recent discovery that the chemical half-lives of specific mRNA species were considerably increased in *Escherichia coli* strains with mutations in the genes for exoribonucleases (10–12) suggested a way to alleviate this problem. Indeed, the concentration of polyadenylylated mRNA in *E. coli* increased significantly under conditions when the exoribonucleases RNase II and polynucleotide phosphorylase were inactive. By taking advantage of the increased cellular concentration of poly(A) RNA in *E. coli* strains with defective ribonucleases and improved methods for the isolation of poly(A) RNA and for cDNA synthesis, we succeeded in cloning cDNAs corresponding to lpp mRNA. Nucleotide sequence analysis of several lpp cDNA clones allowed us to define the sites of polyadenylylation in a specific bacterial mRNA. The results presented in this paper show that poly(A) tracts are attached to specific positions in prokaryotic mRNAs and suggest a model for the role of exonucleases and poly(A) polymerase in the 3′ processing of mRNA in *E. coli*.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *E. coli* SK1695 [*thyA715*], SK5691 [*thyA715, pnp7*], SK5689 [*thyA715, rnb500*], and SK5726 [*thyA715, pnp7, rnb500*, pDK39 (Cm^r *rnb500*)] were gifts from S. Kushner (University of Georgia) (10). It should be noted that viability of the *pnp7 rnb500* double mutant, with a nonsense mutation in polynucleotide phosphorylase and a temperature-sensitive mutation in RNase II, required the presence of multicopy plasmid pDK39 carrying the *rnb500* mutation (10).

Materials. All restriction endonucleases were from New England Biolabs; bacterial alkaline phosphatase was from GIBCO/BRL; avian myeloblastosis virus (AMV) reverse transcriptase was from Life Sciences (St. Petersburg, FL); *Taq* (*Thermus aquaticus*) DNA polymerase was from Perkin–Elmer/Cetus; polyATtract mRNA isolation system II and RNasin were from Promega.

Oligonucleotide Synthesis. Oligonucleotides were synthesized by the phosphoramidite method with a MilliGen/BioSearch (Novato, CA) Cyclone DNA synthesizer. The oligonucleotides used for cDNA synthesis and DNA amplification were designed with restriction sites at the 5′ end to facilitate subsequent cloning.

Poly(A) RNA Isolation. Temperature-sensitive *E. coli* SK5726 was grown at 30°C in LB medium supplemented with thymine (50 μg/ml) and chloramphenicol (20 μg/ml). At midlogarithmic phase (A_{540} = 0.5), the culture was shifted to 44°C for 10 min, while a control culture was maintained at 30°C. Cells were then harvested and processed for lysis with proteinase K as described (2). The lysates were stored in 0.5 M NaCl at −70°C overnight and used for the isolation of poly(A) RNA with the Promega PolyATtract mRNA isolation system. Briefly, total RNA was hybridized with biotinylated $(dT)_{25}$ and the poly(A) RNA·$(dT)_{25}$-biotin hybrid was captured on streptavidin-substituted magnetic beads. The beads were washed, and bound poly(A) RNA was released by elution with water and precipitated with alcohol in the presence of 0.3 M sodium acetate.

cDNA Synthesis. Poly(A) RNA was reverse-transcribed by AMV reverse transcriptase (Life Sciences) using 5′-CTGCCTGCAGGATCCCCGGG$(T)_{20}$, which contains a *Bam*HI restriction site (underlined), as primer. Poly(A) RNA was denatured in water for 3 min at 80°C, cooled on ice, brought to 20 mM in CH_3HgOH, and incubated at room temperature for 7 min. 2-Mercaptoethanol was added to a final concentration of 65 mM and, after 5 min at room temperature, the mixture was used for cDNA synthesis. Incubation was in a volume of 80 μl containing 50 mM Tris·HCl (pH 8.3), 40 mM KCl, 7 mM $MgCl_2$, 5 mM dithiothreitol, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.1 mM [^{3}H]dTTP (2.5 mCi/μmol; 1 Ci = 37 GBq), 80 units of

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Abbreviation: AMV, avian myeloblastosis virus.

*To whom reprint requests should be addressed at: Department of Metabolic Regulation, Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA 02114.

RNasin, 9 μg of oligonucleotide primer, and 65 units of enzyme, for 3 min at 42°C and 57 min at 50°C. The amount of DNA synthesized was estimated by acid-insoluble radioactivity in a small sample of the incubation mixture. The remainder of the reaction mixture containing cDNA was directly used for amplification.

Specific Amplification of lpp cDNA. A sample of the cDNA solution (1 μl) was added to a reaction mixture (50 μl) containing 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, gelatin (10 μg/ml), all four dNTPs (each at 0.2 mM), 0.5 μg of each primer, and 2.5 units of *Taq* DNA polymerase. The cDNA corresponding to the *lpp* gene was amplified using specific primers AGCAACGCTAAAATCGATCAGC, an internal lipoprotein coding sequence for the 5′ end, and CTGCCTGCAGGATCCCCGG for the 3′ end, the latter containing a *Bam*HI site (underlined). Amplification was carried out for 40 cycles with the following parameters: denaturation for 1 min at 94°C, annealing for 2 min at 50°C, and extension for 2 min at 72°C. After these 40 cycles were completed, a final 12-min step at 72°C was done. A sample was electrophoresed on 3% agarose to analyze the reaction products.

Cloning of the Amplification Products. After the polymerase chain reaction (PCR), the amplification products were treated with proteinase K (50 μg/ml) for 30 min at 37°C, extracted with phenol/$CHCl_3$ in the presence of ethidium bromide and 5 M ammonium acetate, and precipitated with ethanol (13). The precipitated DNA was digested with *Pvu* II and *Bam*HI, fractionated by gel electrophoresis in 1% low-melting-temperature agarose, and the 0.28-kilobase band was excised and ligated to *Hin*cII- and *Bam*HI-digested pUC18 for transformation of *E. coli* DH5-α.

DNA Sequence Analysis. The nucleotide sequence of the cDNA clones in pUC18 was determined by the dideoxynucleotide chain-termination method using Sequenase Version 2.0 from United States Biochemical and 5′-GTTTTCCCAGTCACGAC and 5′-TCCGGCTCGTATGTTGTG as primers. Double-stranded templates were first denatured with alkali.

Measurement of Degree of Polyadenylylation of Pulse-Labeled RNA. Exponentially growing cultures (2 ml) were pulse-labeled for 1 min with [5-^{3}H]uridine (50 μCi). Labeling was terminated by the addition of NaN_3 and chloramphenicol to final concentrations of 25 mM and 50 μg/ml, respectively, and rapid chilling in an ice/salt mixture. Cells were collected by centrifugation and lysed without prior washing as described (2). The degree of polyadenylylation was determined as the fraction of acid-insoluble radioactivity that bound to oligo(dT)-cellulose at 0.5 M NaCl and could be eluted by distilled H_2O (2).

RESULTS

Levels of Poly(A) RNA in Exoribonuclease Mutants. Whereas the synthesis of DNA complementary to poly(A) RNA of *Bacillus subtilis* proceeded without difficulty (3–5), total poly(A) RNA isolated from *E. coli* had only low template activity when incubated with oligo(dT) primer and reverse transcriptase. By reasoning that this difference could be due to higher levels of exoribonuclease activity in *E. coli* capable of degrading the 3′-terminal poly(A) moiety, we examined the levels of poly(A) RNA in strains of *E. coli* deficient in either or both of the major 3′-exonucleases polynucleotide phosphorylase and RNase II (10). Analysis of the degree of polyadenylylation of pulse-labeled RNA by its ability to bind to oligo(dT)-cellulose showed that the absence of polynucleotide phosphorylase alone had no significant effect, inactivation of RNase II in a temperature-sensitive mutant caused a nearly 3-fold increase in the degree of polyadenylylation, and an even greater increase (5-fold) was observed when both RNase II and polynucleotide phosphorylase were inactive (Table 1). No differences in poly(A) levels were observed in the four *E. coli* strains at a temperature (30°C) at which RNase II was not inactivated (data not shown).

cDNA Synthesis. In view of the increased levels of poly(A) RNA observed in *E. coli* SK5726 under conditions when both RNase II and polynucleotide phosphorylase were inactive, we examined the rates of cDNA synthesis using as template poly(A) RNA isolated either from cells grown at 30°C or after a temperature shift to 44°C to inactivate RNase II. Primer-dependent cDNA synthesis was >12 times higher with template poly(A) RNA from cells incubated 10 min at the nonpermissive temperature (Fig. 1). Similar results were obtained when $(dT)_{12\text{-}18}$ was used as primer in place of 5′-CTGCCTGCAGGATCCCCGGG$(T)_{20}$ (data not shown).

In the light of these results, poly(A) RNA for cDNA synthesis was isolated from cells of *E. coli* SK5726 10 min after shift to 44°C. An improved method for the isolation of poly(A) RNA from total RNA was used, which involved hybridization with oligo(dT)-biotin in solution and subsequent hybrid capture using streptavidin coupled to paramagnetic particles. Finally, a two-stage DNA synthesis at 42°C and 50°C was employed to give AMV reverse transcriptase maximal opportunity to traverse possible regions of secondary structure at the 3′ ends of mRNA.

Amplification of cDNA with a *lpp*-Specific Primer. To facilitate cloning of DNA complementary to polyadenylylated lpp mRNA, first-strand cDNA was amplified by the PCR using appropriate primers. These consisted of the *lpp* gene-specific primer AGCAACGCTAAAATCGATCAGC corresponding to a region well within the *lpp* coding sequence (see PCR primer 1, Fig. 3) as the 5′-end primer and CTGCCTGCAGGATCCCCGG as the 3′-end primer (see PCR primer 2, Fig. 3). Analysis of the amplification products by agarose gel electrophoresis of samples of the PCR products showed three bands between 200 and 260 base pairs, the size range expected for amplification products utilizing these primers (Fig. 2). Control experiments using a *lac*-specific primer yielded no bands, consistent with the absence of lac mRNA under the culture conditions used, whereas a *trxA*-specific primer re-

Table 1. Effect of mutations affecting exoribonucleases on mRNA polyadenylylation

Strain	Relevant allele(s)	Exoribonucleases absent	Degree of polyadenylylation of pulse-labeled RNA, % of total RNA
MG1693	Wild type	None	1.3
SK5691	*pnp7*	PNP	0.9
SK5689	*rnb500*	RNase II	3.1
SK5726	*pnp7 rnb500*/pGK39R::*rnb500*	PNP and RNase II	6.2

Strains listed were grown to midexponential phase at 30°C in LB medium. After transfer to 44°C for 10 min to promote inactivation of RNase II in the temperature-sensitive *rnb500* strains, pulse-labeling with [^{3}H]uridine, RNA extraction, and measurement of the degree of polyadenylylation were carried out. PNP, polynucleotide phosphorylase.

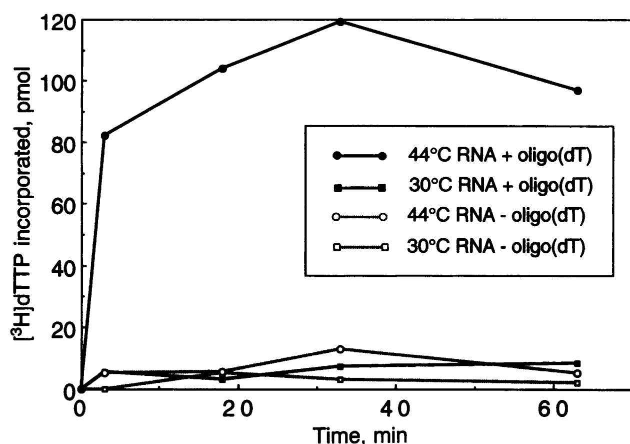

FIG. 1. Effect of temperature shift on the ability of poly(A) RNA isolated from cells with a temperature-sensitive RNase II to serve as template for oligo(dT)-dependent cDNA synthesis. RNA was isolated from *E. coli* SK5726 [*pnp7, rnb500*, pDK39 (Cm^r *rnb500*)] grown at 30°C before or after incubation at 44°C for 10 min to inactivate RNase II. Poly(A) RNA was isolated using magnetic streptavidin beads after hybridization with biotinylated oligo(dT) and poly(A) RNA samples corresponding to 10 mg of cells (dry weight) were used as template for DNA synthesis by using AMV transcriptase with or without oligo(dT) primers.

sulted in a diffuse band, possibly due to nonspecific amplification. The DNA corresponding to three bands obtained with the *lpp*-specific primer (Fig. 2, lane 3) was isolated after gel electrophoresis in low-melting-temperature agarose.

Cloning and Nucleotide Sequence Analysis of the Amplified cDNA. DNA isolated from each of the three PCR bands obtained after amplification with the *lpp* primers was cleaved with *Pvu* II and *Bam*HI restriction endonucleases and ligated into *Hin*cII- and *Bam*HI-digested pUC18 for cloning in *E. coli*. A total of 15 recombinant clones were obtained—6 from band I, 6 from band II, and 3 from band III—the bands being numbered according to increasing electrophoretic mobility. The nucleotide sequence of all 6 of the band I clones was determined, of which 4 were found to contain polyadenylylated *lpp*-specific sequences and 2 were found to contain unidentified polyadenylylated sequences. Only 3 of the 9 band II and band III clones were sequenced, all yielding unidentified polyadenylylated sequences, which may have been the result of unspecific priming and amplification. The sequences of the four *lpp*-specific clones derived from band I are shown in Fig. 3 and compared with the lpp mRNA sequence determined earlier by Inouye and coworkers (14). Clone 3 contained the entire 3′ end of the lpp mRNA molecule with polyadenylylation at the mRNA termination site. The other three clones (clones 1, 4, and 6) lacked the original 3′-terminal stem–loop structure of lpp mRNA but contained the complete C-terminal lipoprotein coding region, with the polyadenylylation site just before the onset of the stem–loop (Fig. 4).

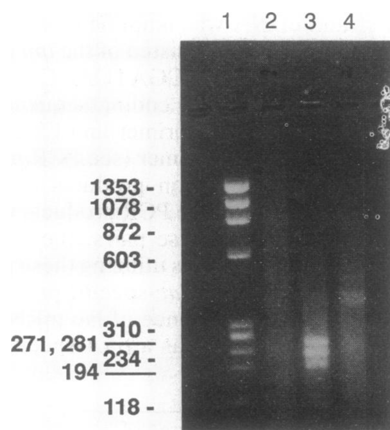

FIG. 2. Agarose gel electrophoresis of the products of PCR amplification of cDNA. The PCR was carried out with cDNA prepared from poly(A) RNA isolated from *E. coli* SK5726 after a shift to 44°C (see Fig. 1), by using one general primer (Fig. 3, PCR primer 2) and one gene-specific primer. Samples of the reaction products were electrophoresed in 3% agarose gels and stained with ethidium bromide. Lanes: 1, *Hae* III digest of bacteriophage ΦX174 DNA; 2, product obtained with a *lacA*-specific primer (5′-ACGTCTG-GATCGGAAGTCATGTGGT-3′); 3, products obtained with a *lpp*-specific primer (5′-AGCAACGCTAAAATCGATCAGC-3′; Fig. 3, PCR primer 1); 4, products obtained with a *trxA*-specific primer (5′-GACACGGATGTACTCAAAGCGGACG-3′).

DISCUSSION

The cloning and sequence analysis of DNA complementary to the lpp mRNA of *E. coli* show at the molecular level that bacterial mRNAs are post-transcriptionally polyadenylylated. The polyadenylate moieties found associated with the 3′ end of lpp mRNA occur at either of two sites, neither of which corresponds to a run of deoxyadenylate residues in genomic DNA. They are thus not encoded by the *E. coli lpp* gene but rather must be synthesized post-transcriptionally like the poly(A) tracts found in eukaryotic mRNA. The facts that cDNA synthesis was oligo(dT)-dependent and that the site of polyadenylylation in one clone corresponded to the putative transcription termination site that occurs in a region of the transcript entirely devoid of deoxyadenylate residues rule out artifacts such as reiteration of adenylate residues by "slippage" in the course of transcription, cDNA synthesis, or amplification.

Evidence that the presence of 3′-terminal polyadenylate moieties is a natural attribute of *E. coli* mRNA with a metabolic role comes from the observations that mutants defective in 3′-exoribonucleases had significantly higher degrees of mRNA polyadenylylation (Table 1) and that RNA isolated after inactivation of RNase II at the nonpermissive temperature had a much enhanced capacity to serve as template for oligo(dT)-dependent cDNA synthesis (Fig. 1). Indeed, all earlier attempts to clone cDNA corresponding to specific *E. coli* mRNAs from strains containing normal complements of exoribonucleases had failed, and the success of the experiments described in this paper depended to a large measure on the availability of exonuclease-deficient *E. coli* mutants (10–12).

The identification of two classes of polyadenylylated lpp mRNA, one in which the poly(A) moiety is attached to the stem–loop structure found by Inouye and coworkers (14) at the end of the primary lpp transcript (class I) and the other that lacks the 3′-terminal stem–loop structure (class II), suggests that the terminal processing of bacterial mRNA involves the competition between polyadenylylation and exonucleolytic degradation, as illustrated in Fig. 5. The primary transcript can be either polyadenylylated at its 3′ end or degraded by 3′-exonucleases, even though the latter process would be expected to be retarded by the terminal stem–loop structure (e.g., ref. 15). However, the primary transcript and its polyadenylylation product can also be cleaved by an endonuclease at a specific site at the base of the terminal stem–loop structure or both. A precedent for such an endonucleolytic cleavage is the removal of the terminal stem–loop structure from the ribosomal protein S15 mRNA by RNase E (16), an enzyme that has also been implicated in the control of mRNA stability (10, 17, 18). The cleaved transcript, no longer protected from 3′-exonucleolytic degradation by the terminal secondary structure, would be highly

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                                         PvuII
Lipoprotein     S  S  N  A  K  I  D  Q  L  S  S  D  V  Q  T  L  N  A  K  V  D
E. coli lpp     TCCAGCAACGCTAAAATCGATCAGCTGTCTTCTGACGTTCAGACTCTGAACGCTAAAGTTGAC
                   (PCR primer #1) --->

                PvuII
Lipoprotein     Q  L  S  N  D  V  N  A  M  R  S  D  V  Q  A  A  K  D  D  A  A
E. coli lpp     CAGCTGAGCAACGACGTGAACGCAATGCGTTCCGACGTTCAGGCTGCTAAAGATGACGCAGCT
cDNA Clone 3       CTGAGCAACGACGTGAACGCAATGCGTTCCGACGTTCAGGCTGCTAAAGATGACGCAGCT

Lipoprotein     R  A  N  Q  R  L  D  N  M  A  T  K  Y  R  K-COOH
E. coli lpp     CGTGCTAACCAGCGTCTGGACAACATGGCTACTAAATACCGCAAGTAATAGTACCTGTGAAGT
cDNA Clone 3    CGTGCTAACCAGCGTCTGGACAACATGGCTACTAAATACCGCAAGTAATAGTACCTGTGAAGT
cDNA Clone 6    .............................................................AA
cDNA Clone 4    .............................................................AA
cDNA Clone 1    .............................................................AA

                 <------ ------•------ ------>
E. coli lpp     GAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGCG
cDNA Clone 3    GAAAAATGGCGCACATTGTGCGCCATTTTTTAAAAAAAAAAAAAAAAAAAAAACCCGGGGATC(pUC18)
cDNA Clone 6    AAAAAAAAAAAAAAAACCCGGGGATC(pUC18)                            BamHI
cDNA Clone 4    AAAAAAAAAAAAAAAAACCCGGGGATC(pUC18)
cDNA Clone 1    AAAAAAAAAAAAAAAAAAAAACCCGGGGATC(pUC18)
                                           BamHI
3'-end of lpp-mRNA                                              GGCCCCTAGGACGTCCGTC-5'
(Nakamura et al., 1980):..CACAUUGUGCGCCAUUUUU(UU)               <---(PCR primer #2)
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FIG. 3. Nucleotide sequence alignment of four lpp cDNA clones with the *E. coli lpp* gene. The sequence of the *lpp* gene (14) is shown in italic type, and the sequence of cDNA clone 3 is shown over the entire range determined. The sequences of the other clones are shown only where they differ from that of the genomic DNA. Also shown is the nucleotide sequence of the 3′ end of lpp mRNA determined by Inouye and coworkers (14). The putative ρ-independent transcription terminator is indicated by the arrows. The double-underlined sequences correspond to the PCR primers used.

vulnerable to degradation by 3′-exonucleases such as polynucleotide phosphorylase or RNase II and, again, competition will ensue between degradation and polyadenylylation. The postulated competition between exonucleases and poly(A) polymerase for the endonucleolytic cleavage product is consistent with the observation that strains of *E. coli* lacking the two exonucleases (10) have higher cellular concentrations of polyadenylylated mRNA (Table 1 and Fig. 1). Such competition could play an important role in the control of mRNA half-life, which would be highly dependent on the ratio of 3′-exonuclease and poly(A) polymerase activities. At this time, it is not known whether polyadenylylation of *E. coli* mRNA increases its resistance to exonucleolytic attack. However, studies with a 3′-exonuclease from *Bacillus brevis* (19), an enzyme that may be functionally equivalent to *E. coli* RNase II (20), has shown poly(A) RNA to be relatively resistant to exonucleolytic degradation (9).

```
                                        U
                                      A   U
                                      C - G
                                      A - U
                                      C - G
                                      G - C
                                      C - G
                                      G - C
Class I mRNA  (cDNA Clone 3)          G - C
                                      U - A
                                      A - U
                                      A - U
lipoprotein                           A - U
tyr-arg-lys-COOH                      A - U
UACCGCAAGUAAUAGUACCUGUGAAGUG          A - U  UAAAAAAAAAAAAAAAAAAAAA-OH

Class II mRNA  (cDNA clones 1, 4, and 6)

lipoprotein
tyr-arg-lys-COOH
UACCGCAAGUAAUAGUACCUGUGAAAAAAAAAAAAAAAAAAAAA-OH

                                        U
                                      A   U
                                      C - G
                                      A - U
                                      C - G
Primary transcript                    G - C
                                      C - G
(Nakamura et al., 1980)               G - C
                                      G - C
                                      U - A
                                      A - U
                                      A - U
lipoprotein                           A - U
tyr-arg-lys-COOH                      A - U
UACCGCAAGUAAUAGUACCUGUGAAGUG          A - U  UU-OH
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FIG. 4. Comparison of the 3′-terminal portion of two classes of polyadenylylated lpp mRNA molecules found with the primary lpp transcript determined by Inouye and coworkers (14).

The fact that lpp mRNA is one of the most stable and abundant mRNA species in *E. coli* (14) undoubtedly contributed to our success in cloning its cDNA. Indeed, lpp mRNA is the only bacterial mRNA that has been purified and sequenced (21). It is interesting that these classical studies on lpp mRNA (21) revealed only full-length mRNA molecules corresponding to our class I clones, whereas, in the experiments described here, the majority of the cloned cDNAs were class II molecules in which the terminal stem–loop structure is missing (Fig. 4). A possible explanation of this difference is that the lpp mRNA studied in this investigation was derived from a *pnp rnb* strain under conditions when both polynucleotide phosphorylase and RNase II were inactive, allowing the accumulation of RNA molecules truncated by the postulated endonuclease (Fig. 5). In contrast, the studies of lpp mRNA by Inouye and coworkers (21) used a strain with a normal complement of exonucleases, where the endonucleolytic cleavage product may be primarily degraded and the major lpp mRNA will be the residual full-length molecules. The reason for the failure of these investigators (21) to detect polyadenylylated lpp mRNA molecules is not clear but may be related to our observation that poly(A) RNA preferentially fractionates into the phenol phase during the phenol extraction procedure commonly used for RNA isolation (2).

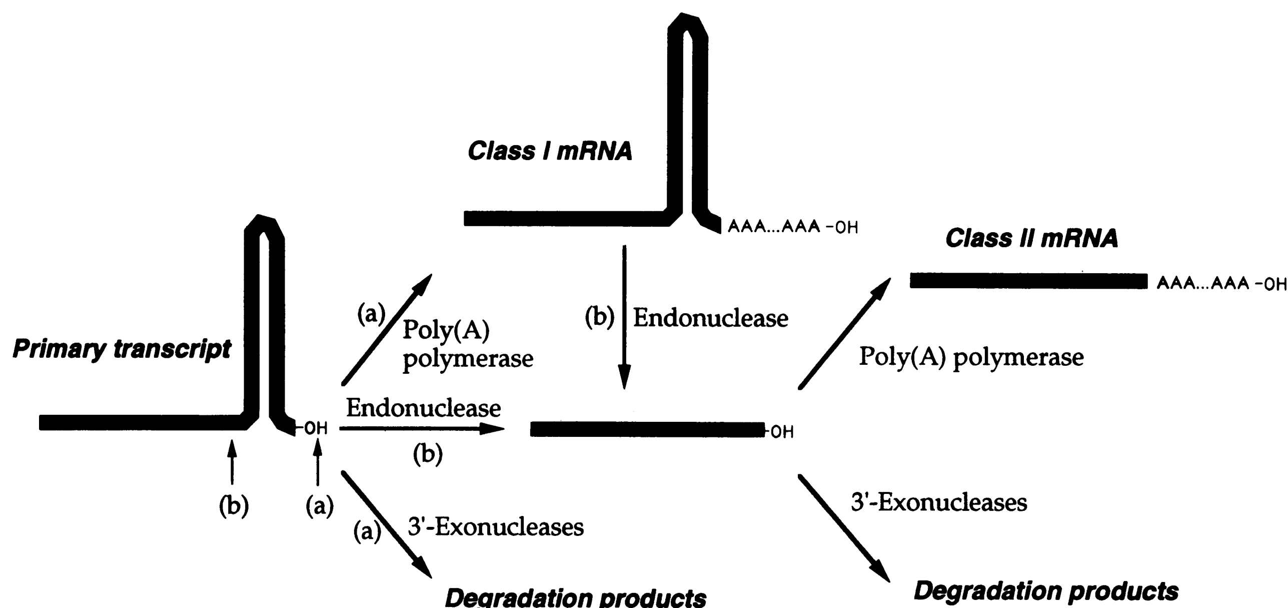

FIG. 5. Postulated sequence of steps in the polyadenylylation of *E. coli* lpp mRNA. The arrows marked (a) and (b) indicate the primary sites of polyadenylylation and endonucleolytic cleavage, respectively, of the primary transcript.

Our results suggest that 3′ processing of bacterial mRNAs may be quite complex, a conclusion that is also borne out by the results of others. mRNA encoded by the *trp* operon appears to be terminated in a ρ-dependent manner *in vivo* and is then processively degraded from the 3′ end to the base of a stem–loop structure that can function as a ρ-independent terminator *in vitro* (22). However, it has been pointed out that the ability of such stem–loop structures to impede digestion by exonucleases *in vitro* is insufficient to account for the increased *in vivo* mRNA half-lives and that additional stabilizing factors must be involved in the control of mRNA stability (23). The results reported in this paper suggest that one such factor may be polyadenylylation. The pattern of RNA processing observed here is probably not unique to *E. coli*, for class II polyadenylylated transcripts have also been identified in *B. subtilis* (unpublished results). The availability of strains of *E. coli* lacking 3′-exonucleases (10) and the recent cloning and disruption of the gene for a poly(A) polymerase (unpublished results) will make possible the genetic analysis of 3′ processing of bacterial mRNAs and may finally provide some direct insights into the biological function of mRNA polyadenylylation.

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1. Sarkar, N., Langley, D. & Paulus, H. (1978) *Biochemistry* **17**, 3468–3474.
2. Gopalakrishna, Y., Langley, D. & Sarkar, N. (1981) *Nucleic Acids Res.* **9**, 3545–3554.
3. Gopalakrishna, Y. & Sarkar, N. (1982) *Biochemistry* **21**, 2724–2729.
4. Gopalakrishna, Y. & Sarkar, N. (1982) *J. Biol. Chem.* **257**, 2747–2750.
5. Karnik, P., Gopalakrishna, Y. & Sarkar, N. (1986) *Gene* **49**, 161–165.
6. Taljanidisz, J., Karnik, P. & Sarkar, N. (1987) *J. Mol. Biol.* **193**, 507–515.
7. Karnik, P., Taljanidisz, J., Sasvari-Szekely, M. & Sarkar, N. (1987) *J. Mol. Biol.* **196**, 347–354.
8. Gopalakrishna, Y. & Sarkar, N. (1983) *Arch. Biochem. Biophys.* **224**, 196–205.
9. Gopalakrishna, Y. & Sarkar, N. (1981) *Biochem. Biophys. Res. Commun.* **103**, 454–460.
10. Arraiano, C. M., Yancey, S. D. & Kushner, S. R. (1988) *J. Bacteriol.* **170**, 4625–4633.
11. Mackie, G. A. (1989) *J. Bacteriol.* **171**, 4112–4120.
12. Plamann, M. D. & Stauffer, G. V. (1990) *Mol. Gen. Genet.* **220**, 301–306.
13. Crowe, J. S., Cooper, H. J., Smith, M. A., Sims, M. J., Parker, D. & Gewert, D. (1991) *Nucleic Acids Res.* **19**, 184.
14. Nakamura, K., Pirtle, R. M., Pirtle, I. M., Takeishi, K. & Inouye, M. (1980) *J. Biol. Chem.* **255**, 210–216.
15. Belasco, J. G. & Higgins, C. F. (1988) *Gene* **72**, 15–23.
16. Regnier, P. & Hajnsdorf, E. (1991) *J. Mol. Biol.* **217**, 283–292.
17. Ono, M. & Kuwano, M. (1979) *J. Mol. Biol.* **129**, 343–357.
18. Babitzke, P. & Kushner, S. R. (1991) *Proc. Natl. Acad. Sci. USA* **68**, 1–5.
19. Sarkar, N. & Paulus, H. (1975) *J. Biol. Chem.* **250**, 684–690.
20. Deutscher, M. P. & Reuven, N. B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3277–3280.
21. Takeishi, K., Yasumara, M., Pirtle, R. & Inouye, M. (1976) *J. Biol. Chem.* **25**, 6259–6266.
22. Mott, J. E., Galloway, J. L. & Platt, T. (1985) *EMBO J.* **4**, 1887–1891.
23. McLaren, R. S., Newbury, S. F., Dance, G. S. C., Causton, H. C. & Higgins, C. (1991) *J. Mol. Biol.* **221**, 81–95.