Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 3973–3977, April 1995
Biochemistry

Polyadenylylation destabilizes the *rpsO* mRNA of *Escherichia coli*

[RNA decay/poly(A) polymerase/poly(A) tails/3′ exoribonucleases/ribosomal protein S15]

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ABSTRACT **The *rpsO* mRNA, encoding ribosomal protein S15, is only partly stabilized when the three ribonucleases implicated in its degradation—RNase E, polynucleotide phosphorylase, and RNase II—are inactivated. In the strain deficient for RNase E and 3′-to-5′ exoribonucleases, degradation of this mRNA is correlated with the appearance of posttranscriptionally elongated molecules. We report that these elongated mRNAs harbor poly(A) tails, most of which are fused downstream of the 3′-terminal hairpin at the site where transcription terminates. Poly(A) tails are shorter in strains containing 3′-to-5′ exoribonucleases. Inactivation of poly(A) polymerase I (*pcnB*) prevents polyadenylylation and stabilizes the *rpsO* mRNA if RNase E is inactive. In contrast polyadenylylation does not significantly modify the stability of *rpsO* mRNA undergoing RNase E-mediated degradation.**

Posttranscriptional addition of a poly(A) tail at the 3′ end of mRNA, which now appears as a general characteristic of all living organisms, was first identified in eukaryotic cells (1, 2). There is considerable evidence that the long poly(A) tail of eukaryotic mRNAs is a structural determinant of their stability (3). More recently, poly(A) sequences were also described at the 3′ end of prokaryotic mRNAs (4–8) and the *pcnB* gene coding for poly(A) polymerase I (PAP-I) of *Escherichia coli* has been cloned and sequenced (9–11). The detection of a substantial level of PAP activity in cells in which *pcnB* was deleted led to the identification of a second PAP, PAP-II (12). Originally, a mutation in *pcnB* was shown to reduce the copy number of ColE1-type plasmids, whose replication is negatively controlled by the small antisense RNAI (13). Inhibition of pBR322 DNA replication in *pcnB* mutants was demonstrated to be the consequence of the accumulation of RNAI resulting from prolongation of its half-life (14, 15). This stabilization of RNAI is correlated with inhibition of the polyadenylylation occurring at its 3′ end (15).

We investigated whether polyadenylylation has an effect on the stability of the mRNA encoding ribosomal protein S15 of *E. coli*. The *rpsO* gene coding for this protein forms an operon with the downstream *pnp* gene coding for polynucleotide phosphorylase (PNPase) (16). Several *rpsO* mRNAs have been identified that correspond to monocistronic and dicistronic transcripts and to molecules resulting from their processing by RNase E and RNase III (17, 18). The most abundant is the P1-t1 monocistronic mRNA (hereafter referred to as P1-t1) extending from the promoter P1 upstream of *rpsO* to the Rho-independent transcription terminator t1 just downstream of the gene (19). The degradation of this transcript is initiated by a RNase E-dependent cleavage at the M2 site (Fig. 1), which removes the stabilizing 3′ hairpin structure of t1 and the processed message is rapidly degraded by PNPase and/or RNase II (19). The *rpsO* mRNA is dramatically stabilized in RNase E mutants (18). However, the rate of decay of P1-t1 remains significant in a strain deficient for RNase E, PNPase, and RNase II, suggesting that it can also be degraded by an alternative pathway (19).

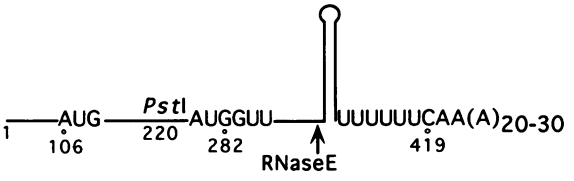

FIG. 1. Structure of the P1-t1 *rpsO* transcript. Numbering begins at the first nucleotide of the transcript. The coding sequence starts at position 106 and terminates 10 nt upstream of the M_2 RNase E cleavage site (arrow), which initiates the degradation of the mRNA. The *Pst* I site at position 220 is the restriction site where the PCR-amplified fragments are cleaved before cloning. The potential hairpin of the transcriptional terminator t1 and the nucleotides at the 3′ end of the P1-t1 mRNA are shown. C419 is the 3′-terminal nucleotide of the longer P1-t1 mRNA, which has been identified (17). The following AA sequence corresponds to residues encoded by the chromosome that are transcribed when RNA polymerase reads through t1 (17). The adenosine between brackets represents the stretch of 20–30 adenosine residues added posttranscriptionally at the 3′ end of the *rpsO* mRNA. The guanosine at position 282 locates the mRNA–poly(A) junction found in one particular clone.

We demonstrate here that P1-t1 is polyadenylylated by PAP-I downstream of the hairpin structure of the transcriptional terminator and that the nonpolyadenylylated P1-t1 *rpsO* mRNA of a PAP-I$^-$ mutant is much more stable than the polyadenylylated species.

MATERIALS AND METHODS

Bacteria and Plasmids. Strains SK5665 (RNase E$^-$ exo$^+$) (20) and SK5704 (RNase E$^-$ exo$^-$) (20) were grown at 30°C in LB medium supplemented with thymine (50 μg/ml) and MG1693 (20) was grown in the same medium at 37°C. For cells transformed with pFB1, Bluescript II KS+ (Stratagene), and pRS415, ampicillin (500 μg/ml or 100 μg/ml, for *pcnB*$^-$ strains) was added to the medium.

The *pcnB80* allele encoding inactive PAP-I was P1-cotransduced with a *zad*::Tn*10* Tet^R transposon into strains SK5665, SK5704, and MG1693 transformed with plasmid pRS415 (21) coding for *lacZ*. *pcnB*$^+$ Tet^R monotransductants [IBPC670 (SK5665 *pcnB*$^+$; RNase E$^-$ exo$^+$ PAP-I$^+$), IBPC674 (SK5704 *pcnB*$^+$; RNase E$^-$ exo$^-$ PAP-I$^+$), and IBPC694 (MG1693 *pcnB*$^+$; RNase E$^+$ exo$^+$ PAP-I$^+$)] overproducing β-galactosidase from the multicopy plasmid were selected as blue colonies on 5-bromo-4-chloro-3-indolyl β-D-galactoside plates whereas *pcnB*$^-$ Tet^R cotransductants [IBPC667 (SK5665 *pcnB*$^-$; RNase E$^-$ exo$^+$ PAP-I$^-$), IBPC673 (SK5704 *pcnB*$^-$; RNase E$^-$ exo$^-$ PAP-I$^-$), and IBPC690 (MG1693 *pcnB*$^-$; RNase E$^+$ exo$^+$ PAP-I$^-$)] (90% of Tet^R transductants), which only contain low amounts of pRS415, were white. The pRS415 copy number characteristic of the *pcnB* alleles was

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Abbreviations: PAP, poly(A) polymerase; PNPase, polynucleotide phosphorylase.

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verified in preparations of plasmid DNA. Plasmid pFB1 was obtained by ligation in Bluescript II KS+ of the 1-kb *Sst* II–*Sst* II fragment of pB15.6 (22) carrying *rpsO*.

Preparation and Analysis of RNAs. Total RNAs were analyzed on Northern blots (19) or by extension by reverse transcriptase of the internal primer (5′-TGGTTGATCTGTGCAGTC, complementary to the *rpsO* mRNA between nt 201 and 218) annealed to 10 μg of total RNA (18). Amounts of *rpsO* RNAs and cDNAs were quantified with a PhosphorImager (Molecular Dynamics).

Preparation and Analysis of cDNAs. Five picomoles of the internal primer described above or 50 pmol of oligo(dT) primers [(dT)12-18 (Pharmacia), 3′-$(T)_{18}$GCCCTAGGGC harboring a *Bam*HI site (underlined), or 3′-$(T)_{18}$AGATCTCAGCTG harboring an *Xba* I site (underlined)] was mixed with 20 μg of total RNA previously denatured in water for 3 min at 100°C and chilled on ice. Hybridization was allowed to proceed for 10 min at 25°C in water and 10 min at 25°C in 80 mM KCl. RNA–primer hybrids were adjusted to 50 mM Tris·HCl, pH 8/10 mM $MgCl_2$/4 mM dithiothreitol/80 mM KCl/all four dNTPs (each at 0.5 mM) and sequentially incubated 10 min at 25°C, 10 min at 42°C, and 40 min at 50°C with 2 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). After ethanol precipitation, nucleic acids were resuspended in 20 μl of water, and 5-μl samples were separated on an 8% polyacrylamide/urea gel, transferred to nylon membranes, and probed for *rpsO* cDNAs. Southern blots were performed as described for Northern blots (19) except that the membranes were fixed in 0.4 M NaOH for 15 min and that hybridizations and washes were carried out at 53°C. The probe was an *rpsO* mRNA fragment (nt 70–400) transcribed from pEHa4 by SP6 RNA polymerase (19). Labeled DNA marker fragments (pBR322 cleaved by *Msp* I) were separated on the gels and transferred with the cDNAs.

PCR Amplification and Identification of Polyadenylylation Sites. Five microliters of cDNA initiated with the oligo(dT) primers harboring the *Bam*HI and *Xba* I sites (see above) was amplified by PCR with the 5′-GCAAACGACACCGGTTCTAC oligonucleotide extending from positions 160 to 179 and the oligomer used to initiate cDNA synthesis. Amplification was carried out for 30 cycles in 50 μl of 10 mM Tris·HCl, pH 8.3/50 mM KCl/2.5 mM $MgCl_2$/gelatin (10 μg/ml)/all four dNTPs (each at 0.2 mM)/2.5 units of AmpliTaq (Appligene, Strasbourg, France). For the first 5 cycles, samples were denatured 1.5 min at 92°C, annealed 2 min at 36°C, and allowed to polymerize for 2 min at 72°C. For the 25 last cycles, denaturation lasted 1.5 min at 92°C, annealing lasted 2 min at 45°C, and polymerization lasted 2 min at 72°C. After ethanol precipitation, the amplification products were digested with *Pst* I and *Xba* I or *Bam*HI and fractionated on 8% polyacrylamide gels stained with ethidium bromide. Amplified cDNAs of ≈220 nt were extracted from gel slices and ligated in the appropriate sites of pT3T718U (Pharmacia) or pGEM3Z(f-) (Promega). Single-stranded DNAs produced in JM101TR were sequenced.

RESULTS

The Elongated *rpsO* mRNA Harbors a Poly(A) Tail at Its 3′ End. We have used RNase E-deficient strains (*ams1*) to study the decay of the P1-t1 mRNA independently of the rapid degradation initiated by the RNase E cleavage (19). In previous experiments (19), a fuzzy band of P1-t1 *rpsO* mRNA posttranscriptionally elongated by 20–30 nt was detected at 44°C in strain SK5704. This strain harbors the *pnp7* allele encoding inactive PNPase and the *rnb500* and *ams1* alleles encoding thermosensitive RNase II and RNase E, respectively (19). At 44°C, SK5704 cells were almost completely devoid of 3′-to-5′ exonucleolytic activity and the RNase E-dependent degradation pathway of *rpsO* mRNA was not operational. We refer to this strain as RNase E^- exo^-. The fact that the elongated P1-t1 mRNA species did not appear at 44°C in the isogenic strain SK5665 *ams1*, which contains the 3′-to-5′ exonucleolytic activity of PNPase and RNase II (referred to as RNase E^- exo^+), suggests that these species result from the addition of nucleotides at the 3′ extremity of the mRNA (19). Accordingly, we found that the 3′ mRNA fragments, which result from directed RNase H cleavage of the P1-t1 *rpsO* mRNA from the RNase E^- exo^- strain, were more heterogeneous and slightly longer than the equivalent RNase H 3′ RNA fragments originating from the RNase E^- exo^+ strain. In contrast, the 5′ P1-t1 fragments of the two strains resulting from these RNase H cleavages were identical in length (data not shown).

If elongation of P1-t1 results from the addition of adenosine residues at its 3′ end, it should be possible to initiate the synthesis of cDNA from an oligo(dT) primer by using reverse transcriptase. Reverse transcripts from such an experiment were analyzed on Southern blots, under denaturing conditions, and probed with an *in vitro*-synthesized *rpsO* mRNA fragment corresponding to the coding sequence of S15. We refer to cDNAs approximately the size of P1-t1 or slightly longer as P1-t1 cDNAs (Fig. 2 *a* and *b*). RNA of the RNase E^- exo^- strain supported the synthesis of oligo(dT)-primed P1-t1 cDNAs, whereas RNA from the isogenic RNase E^- exo^+ strain did not (Fig. 2*a*, lanes T). In contrast, similar amounts of *rpsO* cDNA were obtained when either RNA preparation was reverse-transcribed from a primer internal to the rpsO mRNA, indicating that the two mRNA preparations contain the same amount of *rpsO* mRNA (Fig. 2*a*, lanes int).

When similar experiments were performed with the same strains transformed with the pFB1 multicopy plasmid carrying *rpsO*, which overproduces P1-t1 (Fig. 3), oligo(dT)-primed P1-t1 cDNAs were generated from RNA of the RNase E^- exo^+(pFB1) strain (Fig. 2*b*, left lane). However, the signal was much weaker than with RNA of the RNase E^- exo^-(pFB1) strain (Fig. 2*b*, lanes T). We estimate that RNA of the RNase E^- exo^+(pFB1) strain is reverse-transcribed into P1-t1 cDNA 24 times less efficiently than RNA from the RNase E^- exo^-(pFB1) strain. The ratio of amount of oligo(dT)-primed P1-t1 cDNA to the amount of *rpsO* reverse transcripts primed with the internal primer (Fig. 2*a*, lanes int) measures the efficiency of oligo(dT)-primed reverse transcription of P1-t1 from a given RNA preparation. We verified on a Northern blot that overproduction of *rpsO* mRNA from pFB1 gives rise to elongated molecules in the RNase E^- exo^-(pFB1) cells but not in the RNase E^- exo^+(pFB1) cells (Fig. 3). The correlation between the detection of elongated P1-t1 mRNA on Northern blots and the ability of RNA preparations to support oligo(dT)-primed synthesis of P1-t1 cDNA (Fig. 2 *a* and *b*) strongly suggests that the elongated *rpsO* mRNA harbors a poly(A) 3′ extension. Moreover, the faint band of cDNA synthesized with the RNA template isolated from the RNase E^- exo^+(pFB1) strain (Fig. 2*b*, left lane) shows that the *rpsO* mRNA harbors a poly(A) tail even in the presence of exonucleases. However, in the RNase E^- exo^+ strain, which does not overproduce P1-t1, the length of poly(A) tails and/or the number of polyadenylylated mRNA molecules were probably not sufficient to support the synthesis of detectable amounts of P1-t1 cDNA (Fig. 2*a*, left lane).

cDNAs initiated by oligo(dT) primers hybridized to poly(A) tails downstream of P1-t1 are expected to be longer than P1-t1. In fact, reverse transcription of RNA from the RNase E^- exo^-(pFB1) strain (Fig. 2*b*) gave *rpsO* cDNAs of ≈460 nt, which are 40 nt longer than P1-t1. On the other hand, oligo(dT)-primed reverse transcription of total RNA from the RNase E^- exo^+(pFB1) strain produced a faint band of cDNA that migrated slightly faster in the gel as a molecule of 440 nt (Fig. 2*b*). These data suggest that the oligo(A) tails of the *rpsO*

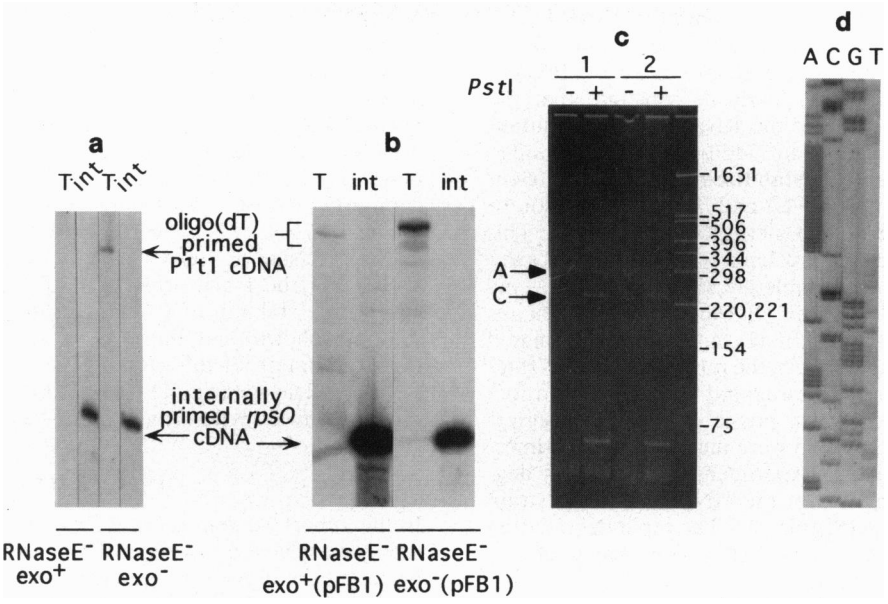

FIG. 2. Characterization of polyadenylylated P1-t1 mRNA. RNA isolated from strains RNase E⁻ exo⁺ and RNase E⁻ exo⁻ (*a*) and from the same strains transformed with pFB1 (*b*) was used as reverse-transcription templates. The primers were $(dT)_{12-18}$ (*a*, lanes T), the oligo(dT) harboring an *Xba* I cloning site (*b*, lanes T), or the internal primer (*a* and *b*, lanes int). cDNAs were analyzed on Southern blots and quantified. The cDNAs synthesized from RNA of the RNase E⁻ exo⁻ strain containing pFB1 were amplified by PCR using the *Bam*HI (c1) or the *Xba* I (c2) primers and the PCR primer described in *Materials and Methods*. The amplified fragment (*c*, arrow A) gives a shorter fragment of ≈225 nt after *Pst* I cleavage (*c*, arrow C). Migration and lengths of marker fragments are shown (*c*, right lane). (*d*) Sequence of the mRNA–poly(A) junction of 32 of the 33 sequenced cDNAs.

mRNA are added downstream of t1 and are ≈20 nt shorter in the RNase E⁻ exo⁺ strain than in the RNase E⁻ exo⁻ strain.

The Main Polyadenylylation Site Is Located Downstream of the 3′-Terminal Hairpin of the Terminator. To determine the sites of poly(A) addition precisely, the *rpsO*–poly(A) junctions of the cDNAs were amplified by PCR and the resulting DNAs were cloned and sequenced. For this purpose RNA prepared from the RNase E⁻ exo⁻(pFB1) strain was used as template to synthesize cDNA initiated with a DNA primer consisting of a stretch of 18 thymidines fused at its 5′ end to a 10- or 12-nt sequence containing a *Bam*HI or *Xba* I cleavage site, respectively. Then the same primers were used in combination with a second primer hybridizing to *rpsO* for PCR amplification of the cDNA (Fig. 2*c*). Identification on a polyacrylamide gel of a major band of amplified cDNA (band A) indicates that a large fraction of poly(A) tails was inserted at a unique site or at very close sites. As expected, cleavage of this fragment at the unique *Pst* I site of *rpsO* gave a fragment of ≈225 nt (Fig. 2*c*, band C) that was cleaved by *Bam*HI or *Xba* I and purified on a polyacrylamide gel before being cloned and sequenced (Fig. 2*d*).

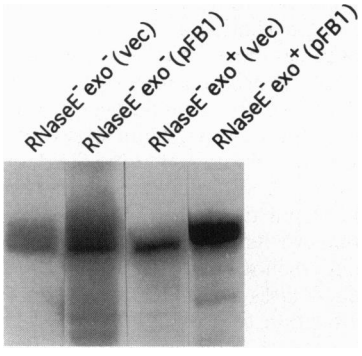

FIG. 3. Overproduction of elongated P1-t1 *rpsO* mRNA in RNase E⁻ exo⁻ mutant. Bacteria growing exponentially at 30°C were shifted to 44°C 15 min before RNA extraction to inactivate RNase E and exonucleases. Samples of total RNA (5 μg) isolated from the RNase E⁻ exo⁻ and RNase E⁻ exo⁺ strains containing the Bluescript II KS+ vector (vec) or the pFB1 plasmid overproducing *rpsO* P1-t1 mRNA (pFB1) were analyzed on Northern blots and probed for *rpsO* mRNA. Lane RNase E⁻ exo⁻(vec), which contains 55 times less P1-t1 *rpsO* mRNA than lane RNase E⁻ exo⁻(pFB1), is displayed at a 50 times higher intensity than the three other lanes to show the fuzzy band of elongated P1-t1. The difference of overproduction observed in cells transformed with pFB1 [55 times in the RNase E⁻ exo⁻(pFB1) strain and 6 times in the RNase E⁻ exo⁺(pFB1) strain] could be related to the difference in the steady-state level of *rpsO* mRNA in these two strains at 44°C (19).

Thirty-two out of the 33 clones analyzed exhibited a poly(A) tail beginning just after the cytidine that follows the stretch of uridines immediately downstream of the terminator hairpin (Figs. 1 and 2*d*). They might correspond to polyadenylylation starting at this cytidine or at the following two adenosines that are encoded by the genome (Fig. 1). Most of the cloned poly(A) stretches were 18–20 nt long and three of them are >25 nt. The observation that two different primers, sharing an oligo(dT) stretch but differing in sequence, generated nearly exclusively cDNA initiated at the same site indicates that they annealed to the template through their oligo(dT) region. Moreover, there is no adenosine-rich region downstream of the polyadenylylation site that could account for hybridization of the oligo(dT) primer to the read-through *rpsO* transcripts extending downstream of t1 (17). Interestingly, one of the cloned cDNAs reflects the synthesis of a long poly(A) tail of at least 63 residues from a site within the coding sequence of *rpsO* (Fig. 1).

Polyadenylylation of the *rpsO* mRNA Is Controlled by the *pcnB* Gene. If the 3′ extension of P1-t1 corresponds to polyadenylylation by PAP-I, the modification should not be observed in a strain lacking this enzyme. To check this hypothesis, after a shift to 44°C, P1-t1 mRNA was isolated from a RNase E⁻ exo⁻ PAP-I⁻ derivative harboring the *pcnB80* allele (13) and analyzed on Northern blots. Rifampicin was added to the culture at the time of the shift to measure the half-life of the P1-t1 mRNA under conditions that prevent the heat shock response (see below). As observed (19), the temperature shift induced the appearance of a fuzzy band of P1-t1 *rpsO* mRNA

in the RNase E⁻ exo⁻ control strain containing PAP-I (Fig. 4*a*). In contrast, the size of P1-t1 was not modified for 20 min after the temperature shift in the RNase E⁻ exo⁻ PAP-I⁻ mutant (Fig. 4*a*). These data clearly demonstrate that the elongation of P1-t1 observed in the RNase E⁻ exo⁻ mutant results from the PAP-I-dependent addition of poly(A) tails.

The P1-t1 *rpsO* Transcript Is Stabilized in a Strain Deficient for PAP-I. It is striking in Fig. 4 *a* and *b* that inactivation of PAP-I also has a dramatic effect on the stability of P1-t1. This mRNA, which had a half-life of 2.2 min in the RNase E⁻ exo⁻ strain at 44°C, was almost completely stable in the isogenic RNase E⁻ exo⁻ PAP-I⁻ strain. Because the fuzzy bands resulting from the elongation of P1-t1 could be underestimated when quantified on Northern blots, the relative amount of *rpsO* mRNA 5′ extremities was also estimated by primer extension. In spite of small discrepancies, possibly due to transcripts extending downstream of t1 that were included in the primer-extension quantification, this experiment confirms that degradation of the *rpsO* mRNA in the RNase E⁻ exo⁻ strain requires the activity of PAP-I (Fig. 4*c*). The rapid degradation observed just after the shift to 44°C (Fig. 4) probably reflects the kinetics of RNase E inactivation.

If the above conclusion that P1-t1 harbors poly(A) tails even in the presence of 3′-to-5′ exonucleases is correct, inactivation of PAP-I in the RNase E⁻ exo⁺ strain should also stabilize P1-t1, as in the RNase E⁻ exo⁻ PAP-I⁻ strain. To verify this prediction, the decay rates of P1-t1 were compared in the RNase E⁻ exo⁺ PAP-I⁻ transductant harboring the *pcnB80* allele and the RNase E⁻ exo⁺ PAP-I⁺ isogenic strain. As expected, *rpsO* mRNA was significantly more stable in the PAP-I-deficient strain than in the PAP-I⁺ cells (its half life was >15 min vs. ≈4 min) (Fig. 4*b*), thereby showing that PAP-I plays a role in the degradation of the P1-t1 mRNA even if

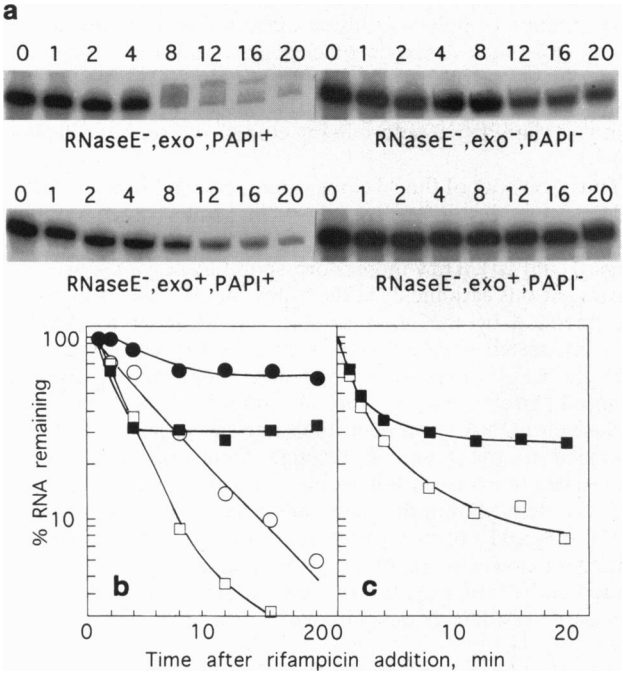

FIG. 4. Stabilization of nonpolyadenylylated mRNA. Bacteria growing at 30°C were shifted to 44°C at time 0 to inactivate RNase E and RNase II at the same time that rifampicin (500 μg/ml) was added to inhibit initiation of transcription. Total RNA was prepared at times indicated in min above each lane, and aliquots were analyzed on Northern blots (*a*) and by primer extension (data not shown). Relative amounts of P1-t1 *rpsO* mRNA deduced from Northern blots (*b*) and of P1 extremities deduced from primer extension (*c*) were quantified and plotted as a function of time after rifampicin addition. □, RNase E⁻ exo⁻ PAP-I⁺; ■, RNase E⁻ exo⁻ PAP-I⁻; ○, RNase E⁻ exo⁺ PAP-I⁺; ●, RNase E⁻ exo⁺ PAP-I⁻.

poly(A) tails are shortened or partly removed by 3′-to-5′ exonucleases.

Inactivation of PAP-I Does Not Alter *rpsO* mRNA Stability in Strains Containing RNase E. The demonstration that the polyadenylylated *rpsO* mRNA can be degraded in the absence of RNase E raises the question of the contribution of the poly(A)-dependent degradation of the *rpsO* mRNA in strains containing RNase E. Comparison of the decay rates of P1-t1 in the MG1693 RNase E⁺ exo⁺ PAP-I⁺ strain and in the isogenic RNase E⁺ exo⁺ PAP-I⁻ strain harboring *pcnB80* shows that the inactivation of PAP-I does not significantly modify the stability of P1-t1 in strains containing RNase E (data not shown) and, therefore, that under these conditions, the degradation of the *rpsO* mRNA mainly depends on the RNase E cleavage (Fig. 1) that exposes the upstream message to the exonucleolytic activity of PNPase and RNase II (19).

DISCUSSION

In this report we demonstrate that the *rpsO* mRNA of *E. coli* is polyadenylylated. Several mRNAs originating from different bacteria have previously been reported to be polyadenylylated (4–8). However, we show that the polyadenylylated and the nonadenylylated forms of the same mRNA, both present in a preparation of total RNA, can be distinguished on the basis of their length by Northern blot analysis. In addition, a band of oligo(dT)-primed specific cDNAs slightly longer than the nonpolyadenylylated form of the mRNA was characterized. The accumulation and increase in length of the polyadenylylated mRNA in a strain deficient in 3′-to-5′ exonucleases and the disappearance of the longer polyadenylylated mRNA in the PAP-I-deficient strain are compelling evidence that *rpsO* mRNA is polyadenylylated.

Our data also demonstrate that polyadenylylation takes place downstream of the hairpin structure of the transcription terminator t1. Nearly all the poly(A) stretches that we have found are 3′ adjacent to the $(U)_6C$ sequence that was reported to be the main 3′ extremity of the *rpsO* mRNA on the basis of S1 nuclease mapping (17). Unlike previous reports on *lpp* mRNA and ColE1 plasmid RNAI (4, 15), we did not find poly(A) tails fused immediately upstream of the t1 hairpin. The fact that we characterized polyadenylylation sites of *rpsO* mRNA in a strain deficient for RNase E might explain why we did not find polyadenylylation at 3′ ends generated by RNase E (18), in particular, at the M2 site just upstream of t1 (Fig. 1). The lack of 3′-to-5′ exoribonucleases able to remove nucleotides at the 3′ end of P1-t1 could account for the striking homogeneity of the polyadenylylation sites that we have characterized. Another possibility is that the CA sequence and the upstream hairpin are preferentially recognized by PAP-I (15). Finally, the step of purification of amplified cDNA homogeneous in size that we have included in our cloning strategy could have excluded shorter or longer amplified *rpsO* cDNA polyadenylylated at sites upstream or downstream of t1. Indeed, the identification of a mRNA–poly(A) junction in the coding sequence of *rpsO* indicates that polyadenylylation might occur at other sites. The long stretch of 63 adenosines of this cDNA probably explains why it migrated in a polyacrylamide gel with the major band of amplified cDNAs that we have analyzed.

The lengths of poly(A) stretches of amplified cDNAs initiated by an oligo(dT) primer able to anneal to any stretch of 16–18 adenosines within poly(A) tails do not allow one to determine the length of these poly(A) tails. However, the size of polyadenylylated mRNAs detected on Northern blots indicates that most poly(A) tails range between 20 and 30 nt in a strain deficient in exonucleases. Identification of a clone harboring 63 adenosines shows that many more adenosine residues can be added by PAPs. That cDNAs are 20 nt shorter when they are synthesized on a RNA template isolated from

the exo$^+$ strain instead of RNA from an exo$^-$ strain (Fig. 2*b*, lanes T) indicates that the length of poly(A) tails in the exo$^+$ strain might range between 1 and 10 nt.

PAP-I is responsible for the polyadenylylation of P1-t1. The disappearance of the elongated *rpsO* mRNA in the PAP-I-deficient strain suggests that PAP-II cannot polymerize adenosine residues at the 3′ end of the *rpsO* mRNA as efficiently as PAP-I even in the absence of active PAP-I. However, it cannot be excluded that PAP-II could add few adenosine residues and produce slightly elongated molecules that are not detected by Northern blot analysis.

The fact that cDNAs are shorter and less abundant in the RNase E$^-$ exo$^+$ strain than in the isogenic exo$^-$ strain confirms that 3′ exonucleases are involved in the degradation of poly(A) tails (4). Moreover, the absence of elongated *rpsO* mRNA in both the RNase E$^-$ RNase II$^-$ (*ams–rnb*) and the RNase E$^-$ PNPase$^-$ (*ams–pnp*) strains (19) suggests that PNPase and RNase II are both able to degrade poly(A) tails. On the other hand, the instability of P1-t1 in a RNase E$^-$ exo$^-$ PAP-I$^+$ strain implies that ribonucleases distinct from RNase E, PNPase, and RNase II require a poly(A) tail to degrade the *rpsO* mRNA.

The finding that PAP-I inactivation does not significantly modify the stability of the *rpsO* mRNA in a RNase E$^+$ strain raises the question of the role of polyadenylylation in the degradation of this mRNA. A possibility is that poly(A) tails affect the decay of *rpsO* RNA fragments resulting from the rate-limiting RNase E endonucleolytic cleavage (19). Polyadenylylation might destabilize full-length transcripts of *E. coli*, which are inefficiently attacked by endonucleases.

The stabilizing and destabilizing effects of poly(A) tails on eukaryotic and prokaryotic mRNAs, respectively, could be related to differences in length and to the protecting effect of poly(A) binding proteins (23). Possibly, RNA decay is initiated by degradation of poly(A) tails in both systems (23). In any case, the discovery that poly(A) tails modify the stability of mRNA in both kinds of cells reinforces the interest of the *E. coli* model to understand the molecular basis of mRNA stability.

We thank M. Grunberg-Manago and M. Springer for encouragements and discussions, C. A. Ingle for suggestions, and M. Dreyfus and R. D'Ari for critical reading of the manuscript. This work was funded by Centre National de la Recherche Scientifique (Unité de Recherche Associée 1139), University Paris 7, European Union (Human Capital and Mobility), and the Ministère de la Recherche et de l'Enseignement Superieur (fellowship to F.B.).

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