JOURNAL OF BACTERIOLOGY, Aug. 1989, p. 4112-4120
0021-9193/89/084112-09$02.00/0

Vol. 171, No. 8

Stabilization of the 3′ One-Third of *Escherichia coli* Ribosomal Protein S20 mRNA in Mutants Lacking Polynucleotide Phosphorylase

GEORGE A. MACKIE

Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1

Received 16 December 1988/Accepted 1 May 1989

Mutations which largely inactivate polynucleotide phosphorylase and which render RNase II thermolabile exert two effects on the metabolism of the two nested mRNAs which encode ribosomal protein S20. (i) The lifetime of both mRNA species is extended 2.5-fold at 38°C in a strain harboring both mutations. (ii) A relatively stable truncated fragment of these mRNAs accumulates to significant levels in strains lacking polynucleotide phosphorylase. The truncated RNA (Po RNA) is 147 to 148 residues long and is coterminal with the 3′ ends of intact S20 mRNAs. Its 5′ end appears to be generated by endonucleolytic cleavage to the 5′ side of a G residue in the sequence AACCGAUC. The data are consistent with the hypothesis that S20 mRNAs can be degraded by alternative pathways. The normal pathway depends on functional polynucleotide phosphorylase and is concerted, since S20 mRNAs disappear without accumulation of detectable intermediates in the decay process. The slower alternative pathway is followed when polynucleotide phosphorylase is inactivated by mutation. This pathway is distinguished by segmental rather than concerted degradation of S20 mRNAs and involves at least one endonucleolytic cleavage. The 5′ two-thirds of S20 mRNAs decays significantly more quickly than the 3′ third in this latter mode of mRNA turnover.

The metabolic instability of most bacterial mRNAs was the first recognized property of these macromolecules (3, 7, 18). The basis for this property of mRNAs is still not understood, although the factors which determine the rate at which mRNAs turn over are frequently determinants of the level of expression of a given gene (10, 17, 31, 33, 35, 37, 38). A number of investigations have demonstrated that hairpin structures in bacterial mRNAs, such as the REP (repetitive extragenic palindrome) sequence (19) or rho-independent terminators, can confer increased stability on mRNAs or on segments of mRNAs which contain them (22, 25, 30, 32, 35, 37). Such structures would be expected to be relatively resistant to attack by known 3′ exonucleases, including RNase II and polynucleotide phosphorylase, enzymes whose substrate preference is for relatively unstructured RNAs (12, 23). Indeed, several researchers have shown that these 3′ exonucleases are directly involved in determining the chemical lifetime of total bacterial mRNA (15, 21). Nevertheless, these enzymes are not considered to be the primary enzymes in the degradation of mRNAs. Rather, it is believed that they are scavengers of fragments of mRNAs generated by one or more endonucleolytic cleavages which occur early in the pathway of mRNA turnover (2, 9, 15). Findings of differential segmental decay rates in the *lac* operon and *ompA* mRNAs (20, 40), of truncation of the leaders of both *ompA* and *bla* mRNAs (34), and of internal cleavages in several mRNA species, including *lac* mRNA, *trxA* mRNA, and *cat* mRNA (2, 9, 39), are consistent with endonucleolytic cleavages of mRNAs. The enzymes and the mechanism(s) by which these cleavages occur remain to be established.

The mRNAs for bacterial ribosomal protein S20 are attractive models for studying mRNA turnover. These mRNAs are monocistronic, small (356 and 447 residues), discrete, and moderately abundant and contain a stable stem-loop structure from a rho-independent terminator at their 3′ ends (24). Their stabilities are influenced by both 5′ and 3′ determinants in the mRNAs (25, 36). A number of point, deletion, or replacement mutations in the untranslated 5′ leader whose primary effect is on translational efficiency all exert a strong effect in *cis* on the stability of S20 mRNA (36; unpublished data; cf. reference 4). In addition, deletion of the normal rho-independent terminator and its replacement by fusion either to the gene encoding galactokinase or to 5S rRNA sequences and the *rrnB* transcriptional terminator reduce the stability of altered S20 mRNA by a factor of two to three (25, 36). These findings raise the question of whether there is a unique rate-limiting step or rather several alternative steps that initiate the degradation of S20 mRNAs. One approach to addressing this issue is to examine the stabilities of S20 mRNAs in strains deficient in 3′ exonucleases. If the normal pathway of degradation of S20 mRNAs simply involves 3′ exonucleolytic degradation, then the stabilities of these mRNAs ought to increase substantially in a mutant strain. Furthermore, if there are alternative pathways of degradation, even for the same mRNA species, these pathways can be characterized in the mutant host.

MATERIALS AND METHODS

Strains. The otherwise isogenic strains *Escherichia coli* SK5003 (Cm^r *pnp-7 rnb-500*), SK5004 (Cm^r *pnp-7*), SK5005 (Cm^r *rnb-500*), and SK5006 (Cm^r) were constructed by Donovan and Kushner (15) and generously provided by Sidney Kushner (University of Georgia, Athens). Strain RD100 (*rna pnp-7 sup met relA trpD9778 lacZ*) was constructed by Robert P. Dottin (Ph.D. thesis, University of Toronto, Toronto, Ontario, Canada, 1974) and obtained from M. L. Pearson (E. I. du Pont de Nemours & Co., Inc.). Plasmid pGM72 contains residues 92 to 550 of the S20 sequence (numbering as in Fig. 1) cloned into the polylinker of vector pKK223-3 (8) so that the coding sequences for ribosomal protein S20 are transcribed from the hybrid *tac* promoter in the vector and terminate at the S20 transcriptional terminator. Plasmid pGM75 consists of the same S20

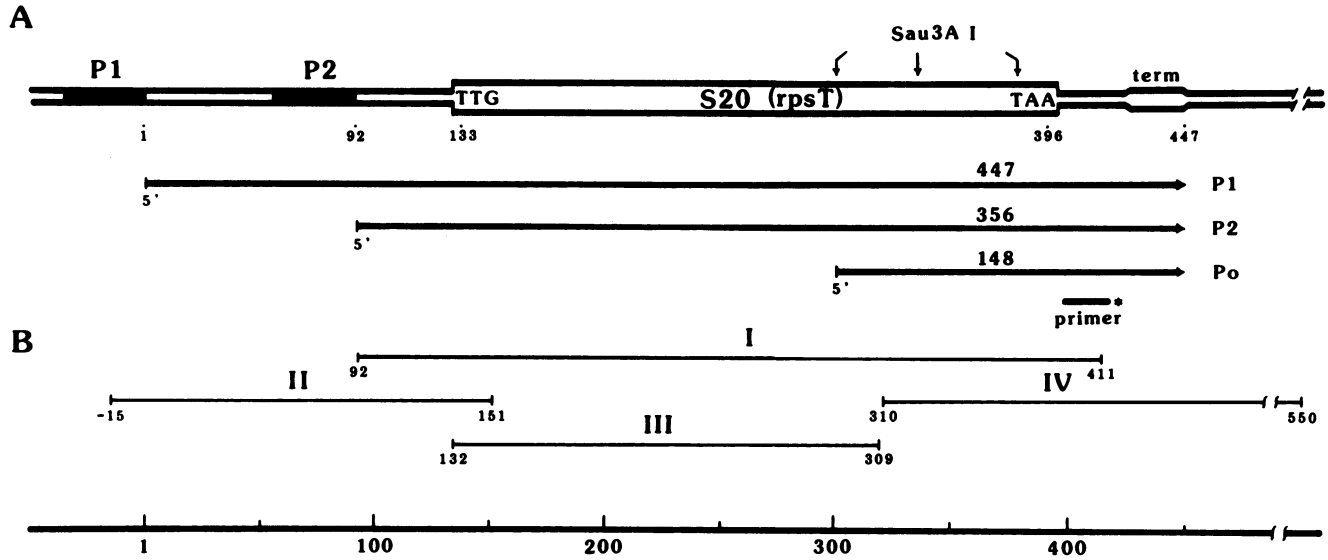

FIG. 1. Map of the gene for ribosomal protein S20. (A) The upper line represents the gene for ribosomal protein S20. P1 and P2 denote the positions of the two promoters (26), and term shows the position of a rho-independent terminator (24). The sequence coordinates are modified on the basis of previous publications (24). Residue 1 corresponds to the first residue transcribed from the P1 promoter (residue 141 in the numbering of reference 24). The short vertical arrows in panel A denote the positions of three *Sau*3AI restriction sites discussed in the text. The three horizontal arrows denote three major RNAs discussed in this report, initiated at the two promoters (P1 and P2 mRNAs) or by internal cleavage (Po RNA). The numbers above these arrows give their sizes in residues. (B) Lines I to IV denote the extents of probes derived from S20 DNA sequences. The coordinates of the terminal residues of each probe are indicated. Probes I and II are complementary RNAs synthesized in vitro with SP6 RNA polymerase (24, 25). Probes III and IV are duplex DNA fragments labeled by nick translation (27). The bottom line provides a scale in nucleotide residues.

sequences cloned into vector pEX-1, supplied by L. Passador and T. Linn and derived from pKK223-3 (8). In addition, the UUG initiation codon in pGM75 and its derivatives was replaced with AUG (cf. plasmid pGP13 in reference 36). The pEX-1 vector also contains the *lacI* gene (and the $lacI^q$ promoter mutation) to maintain repression of the cloned sequences in addition to the *tac* promoter and *rrnB* terminator. Deletions spanning the three *Sau*3AI sites in the S20 coding sequence have been previously described (14). Either of two such deletions was transferred, together with the S20 transcriptional terminator, to the pEX-1 vector by standard methods to construct plasmids pGM75Δ12 and pGM75Δ13 (see Fig. 5). Plasmid pGM78 contains residues 92 to 411 of the S20 sequence cloned into the polylinker of pEX-1. This plasmid lacks the S20 transcriptional terminator and in effect fuses S20 coding sequences to the *rrnB* terminator in the vector. The processing sites for the 5S rRNA sequence adjacent to the *rrnB* terminator are deleted from the construction so that 5S rRNA cannot be excised from transcripts containing it (8). The analogous construction in pKK223-3 is pCD6 (36).

Media. All derivatives of SK5006 were grown in LB medium (27) supplemented with 20 mg of chloramphenicol per liter or 40 mg of ampicillin per liter when required.

Extraction and analysis of RNA. RNA was extracted from cultures of exponentially growing cells by one of two means. Method I is based on the procedure of Dennis and Nomura (11) with previously described modifications (24, 25). In this method, cells are chilled and harvested by centrifugation before extraction of RNA. In method II, 2 ml of culture was pipetted directly into 1 ml of boiling 0.3 M sodium acetate (pH 6) containing 30 mM EDTA and 1.5% sodium dodecyl sulfate. After 60 s, the cleared cell lysate was chilled and extracted successively with phenol, phenol-chloroform-isoamyl alcohol (25:24:1), and ethyl ether. The final aqueous phase was precipitated with 2.5 volumes of ethanol. Nucleic acids were recovered by centrifugation, reprecipitated once from 0.25 M sodium acetate, washed once with 70% ethanol, and finally dissolved in 0.1 to 0.2 ml of sterile H_20. Yields of RNA were estimated spectrophotometrically.

S20 mRNAs were analyzed by three means. (i) Northern (RNA) blotting was performed as described previously (24), by using the probes diagrammed in Fig. 1. Samples of RNA were denatured with glyoxal, and the RNA species were resolved by electrophoresis on a 1.25% agarose gel. The RNAs were transferred to a nylon membrane (HyBond N; Amersham Corp.) by blotting. S20 mRNAs were visualized by being annealed to complementary probes prepared either by transcription of residues 92 to 415 by SP6 RNA polymerase in the presence of [α^{32}P]UTP to yield a single-stranded complementary RNA (24, 25) or by nick translation (27) of denatured duplex DNA fragments with [α^{32}P]dCTP. (ii) S1 protection experiments were done by the general method developed by Berk and Sharp (5). Total bacterial RNA (1 to 12 μg) was annealed to a probe spanning residues 307 (*Ava*II) to 550 (*Hin*fI) previously labeled with [α^{32}P]dCTP by repair at residue 307 on the 3′ end of the lower strand. The RNA-DNA duplexes so formed were digested with 15 U of S1 nuclease per ml for 30 min at 37°C as previously described (5). The resistant products were recovered and applied to an 8% sequencing gel along with markers derived from the same probe by chemical cleavage with formic acid (A+G) or hydrazine (C+T) (27). The labeled products were visualized by autoradiography. (iii) Primer extension was performed as follows. A synthetic primer (5′-AGCTTCAGCAAATTG GCG) was synthesized on an Applied Biosystems 380A DNA Synthesizer and purified by preparative polyacrylamide gel electrophoresis. A sample containing 25 pmol was labeled with [γ^{32}P]ATP by polynucleotide kinase in a buffer containing 50 mM Tris hydrochloride (pH 7.6), 10 mM $MgCl_2$, 0.1 mM spermidine, 0.1 mM EDTA, 5 mM dithiothreitol, and about 1 μM ATP. After inactivation of the

enzyme, samples (0.1 to 0.5 pmol, depending on the experiment) of the labeled primer were annealed for 10 min to 1 to 10 μg of total RNA in a volume of 12.5 μl by using the residual salts from the labeling reaction to buffer the incubation. The mixture was cooled to room temperature over a period of 45 to 60 min. Annealing was found to be independent of temperature between 24 and 70°C. Extension was performed in a final volume of 25 μl containing the following components: 50 mM Tris hydrochloride (pH 7.6), 75 mM KCl, 4 mM $MgCl_2$, 10 mM dithiothreitol, each of the four deoxynucleoside triphosphates (neutralized) at 0.5 mM, and 100 to 200 U of murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Inc.). Incubations proceeded for 30 min at 43°C, after which they were chilled and made 10 mM in EDTA and 2 M in ammonium acetate. After addition of 25 μg of carrier yeast RNA, the stopped incubation was extracted once with phenol-chloroform-isoamyl alcohol and the nucleic acids in the aqueous phase were precipitated with ethanol. The pellets were washed once with 70% ethanol, dissolved in buffered 80% formamide, heated briefly to 95°C, and applied to a standard 8% polyacrylamide sequencing gel. DNA sequence markers were generated by sequencing a cloned S20 sequence (residues −140 to 411 in Fig. 1) in M13mp8 with the same primer by the procedure of Biggin et al. (6).

Determination of mRNA lifetime. Exponential cultures were treated with rifampin to 200 mg/liter. Samples of 12.5 ml were withdrawn at intervals thereafter and quenched, and the RNA was extracted by method I, described above. Samples containing a known mass of total RNA were denatured, and S20 mRNAs were identified by RNA (Northern) blotting with probe I (Fig. 1B) as described in section i in the preceding paragraph. Autoradiograms were scanned by laser densitometry, and the areas under the peaks corresponding to intact P1, P2, and Po RNAs were normalized to those found in 5 μg of total RNA at time zero (25, 36).

RESULTS

Characterization of a truncated fragment of S20 mRNA. To recover S20 mRNAs and any potential breakdown products rapidly with minimal handling of cultures before isolation of RNA, I devised a simple procedure for purification of cellular RNAs modelled after methods devised for extraction of RNA-primed nascent DNAs (29; see method II in Materials and Methods). In essence, cultures were pipetted directly into a boiling solution of buffered sodium dodecyl sulfate without prior chilling or centrifugation. The lysed cell suspension was extracted successively with phenol and phenol-chloroform-isoamyl alcohol to eliminate protein and most of the sodium dodecyl sulfate. Nucleic acids were recovered by precipitation with ethanol. The final preparation of RNA was sufficiently pure for use in RNA blotting and nuclease protection experiments. Similar results were obtained in the experiments discussed below with RNAs prepared by this or more traditional means (method I). It was my experience, however, that yields from culture to culture were more uniform with this method than with method I.

Strains carrying wild-type or mutant alleles of the genes that encode polynucleotide phosphorylase (15), RNase I (Dottin, Ph.D. thesis), and RNase II (15) were grown under conditions permissive for the *rnb-500* mutation and shifted to higher temperatures for 1 h before extraction of total RNA by the method outlined above. Samples of these RNAs were analyzed by RNA blotting (Fig. 2A). The most striking finding was the appearance of a short RNA (<200 residues) complementary to an S20 probe in the lanes containing RNAs extracted from strains harboring a mutant allele (*pnp-7*) in the gene for polynucleotide phosphorylase (Fig. 2A, lanes 3, 4, 7, 8, 10, and 11). Small amounts of a similarly sized RNA could be visualized after prolonged exposure of the lane containing RNA from parental strain SK5006 when it was grown at 28°C but not at higher temperatures (Fig. 2A, compare lanes 1, 5, and 9). While the short RNA was readily detected in RNA extracted from strain SK5004 (*pnp-7*) (Fig. 2A, lanes 3, and 7), its relative yield was highest in the double mutant SK5003 (*pnp-7 rnb-500*), which contains mutations in the genes for both polynucleotide phosphorylase and RNase II (Fig. 2A, lanes 4, 8, and 10). The presence of a mutation in the gene for RNase I in strain RD100 (*pnp-7 rna*; Fig 2A, lane 11) did not provide any enhancement of the yield of the short RNA over that provided by the single mutation in the gene for polynucleotide phosphorylase (Fig.

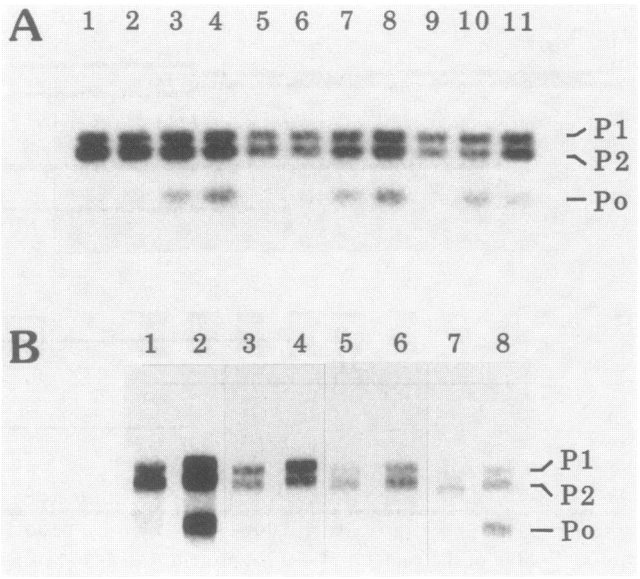

FIG. 2. Effects of mutations in genes for different RNases on steady-state levels of S20 mRNAs. Cultures of the strains indicated below were grown in LB medium, supplemented with chloramphenicol when appropriate, to an A_{600} of 0.2. Portions of each culture were maintained at 28°C (lanes 1 to 4) or shifted to 38°C (lanes 5 to 8) or 43°C (lanes 9 to 10). After 60 min of further growth, RNA was extracted from 2 ml of each culture by method II described in Materials and Methods. Strain RD100 was grown at 37°C throughout (lane 11). Samples containing 5 μg of total cellular RNA were analyzed by Northern blotting as outlined in Materials and Methods. RNA was extracted from the following strains: lane 1, SK5006; lane 2, SK5005 (*rnb-500*); lane 3, SK5004 (*pnp-7*); lane 4, SK5003 (*rnb-500 pnp-7*); lane 5, SK5006; lane 6, SK5005 (*rnb-500*); lane 7, SK5004 (*pnp-7*); lane 8, SK5003 (*rnb-500 pnp-7*); lane 9, SK5006; lane 10, SK5003 (*rnb-500 pnp-7*); lane 11, RD100 (*rna pnp-7*). The positions of S20 P1, P2, and Po (see the text) RNAs are indicated on the right. (B) Mapping of Po RNA. Total RNA (18 μg) from either SK5006 (lanes 1, 3, 5, and 7) or SK5003 (*rnb-500 pnp-7*; lanes 2, 4, 6, and 8) was denatured, resolved by electrophoresis, and blotted to Hybond N as described in Materials and Methods. Strips containing the equivalent of 3 μg of total RNA from either strain were annealed with one of the four probes whose extents are diagrammed in Fig. 1B. Strips in lanes 1 and 2 were annealed to probe I, those in lanes 3 and 4 were annealed to probe II, those in lanes 5 and 6 were annealed to probe III, and those in lanes 7 and 8 were annealed to probe IV. The positions of the three S20-specific RNAs are indicated on the right.

2A, compare lanes 11 and 7). The quantities of short S20 RNA in strains SK5006 and SK5003 (*pnp-7 rnb-500*) were estimated by densitometry. Its relative abundance was virtually equal to that of the more abundant P2 mRNA in strain SK5003 (*pnp-7 rnb-500*) but was 10-fold lower in parental strain SK5006. A thermosensitive mutation in RNase II in strain SK5005 (*rnb-500*) exhibited no effect on the sizes or relative abundances of S20 mRNAs by itself at 28°C (Fig. 2A, lane 2), 38°C (Fig. 2A, lane 6), or 43°C (data not shown). In contrast, a mutation in polynucleotide phosphorylase alone in strain SK5004 (*pnp-7*) was sufficient to lead to accumulation of truncated S20 mRNA (Fig. 2A, lanes 3 and 7). For this reason and to avoid possible complications in RNA metabolism caused by the heat shock response in cultures maintained at 43°C or higher, I performed most of the experiments at temperatures of less than 38°C, knowing that the mutant RNase II may retain significant residual activity (15).

RNA was also extracted from a set of strains described by Deutscher et al. (13) which are deficient in a number of RNases. A truncated S20 mRNA identical in size and relative intensity to that observed in strain SK5003 (*pnp-7 rnb-500*) in Fig. 2A was recovered from two strains containing a Tn*5* insertion in the gene for polynucleotide phosphorylase (28; data not shown). Thus, the occurrence of the S20 mRNA fragment is independent of the mutant *pnp* allele tested.

The short S20 mRNA fragment (hereafter called Po mRNA) could be a mixture of degraded S20 mRNAs or it could be a discrete entity. In the latter case, it would be expected to possess distinct boundaries. These possibilities were distinguished by determining whether Po RNA annealed to probes containing different portions of the S20 gene. RNA extracted from strain SK5006 (parental) or SK5003 (*pnp-7 rnb-500*) was resolved by electrophoresis, and the S20 mRNA species was identified after RNA blotting by annealing replicate blots with one of the four probes diagrammed in Fig. 1B. The data illustrated in Fig. 2B showed that Po RNA was detected by two of the four probes: the positive control (probe I in Fig. 1B) spanning residues 92 to 411 of the S20 sequence, including the entire structural gene (lane 2), and one spanning residues 307 to 550 (probe IV in Fig. 1B), including the distal one-third of the coding sequence and the entire 3′ noncoding sequence (lane 8). Two other probes encompassing either the 5′ portion of the gene (probe II; Fig. 2B, lanes 3 and 4) or the proximal two-thirds of the coding sequence (probe III; Fig. 2B, lanes 5 and 6) failed to detect the Po species in RNA extracted from either strain tested. Since hybrids of less than about 30 residues are not stable under the conditions of hybridization used in this experiment (unpublished data), Po RNA must map to the 3′ side of residue 277 in the S20 sequence. I conclude that Po RNA contains only a subset of the sequences in S20 mRNAs, specifically, those in the 3′ one-third.

Termini of S20 Po RNA. Nuclease protection and primer extension experiments were used to delineate the 5′ and 3′ termini of Po RNA species more precisely. Initial experiments (data not shown) suggested that the 5′ end of this RNA mapped approximately 95 residues to the 5′ side of the termination codon. The results of a primer extension experiment using an end-labeled octadecameric primer complementary to residues 398 to 415 are illustrated in Fig. 3. RNAs from all strains containing the *pnp-7* allele (with or without an additional mutation in the gene for RNase II) displayed a pair of discrete bands corresponding to 5′ termini at residues

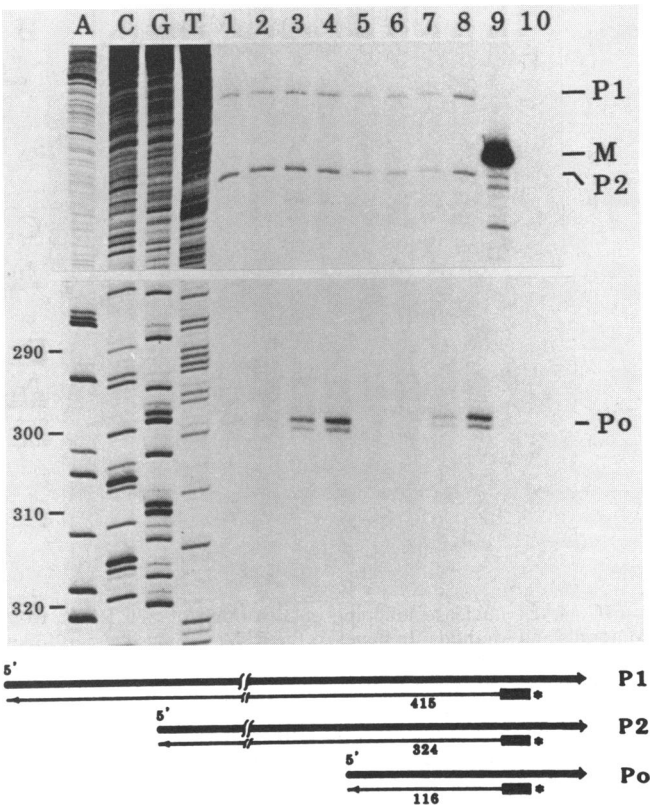

FIG. 3. Determination of the termini of S20 RNAs by primer extension. Total RNA (10 μg) extracted from one of the strains listed below was analyzed by primer extension as outlined in Materials and Methods. A schematic diagram of the design of the experiment is included at the bottom. RNAs were extracted from the following strains grown at the indicated temperatures in the 60 min immediately preceding extraction: lane 1, SK5006 (28°C); lane 2, SK5005 (*rnb-500*; 28°C); lane 3, SK5004 (*pnp-7*; 28°C); lane 4, SK5003 (*pnp-7 rnb-500*; 28°C); lane 5, SK5006 (38°C); lane 6, SK5005 (*rnb-500*; 38°C); lane 7, SK5004 (*pnp-7*; 38°C), lane 8, SK5003 (*pnp-7 rnb-500*; 38°C); lane 9, RNA (approximately 8 fmol) synthesized in vitro by SP6 RNA polymerase from a template spanning residues 92 to 411 as numbered in Fig. 1; lane 10, no template RNA. Lanes A, C, G, and T contained the products of a dideoxy sequencing reaction (6) performed with the same primer. Residues are numbered on the left as in Fig. 1. The letters on the right indicate the positions of full-length reverse transcripts corresponding to P1, P2, and Po RNAs and the SP6 marker (M) transcript.

300 and 301, the former being the more intense (Fig. 3, lanes 3, 4, 7, and 8). The shorter band may be artifactual because of premature termination of reverse transcription at the penultimate residue of Po RNA. The upper portion of the gel in Fig. 3 shows that all of the template RNAs tested in lanes 1 to 8 generated runoff transcripts of the size expected for P1 and P2 mRNAs. A control was performed with an in vitro-synthesized RNA as the template (Fig. 3, lane 9). Several bands indicative of strong stops are visible in this lane, but none comigrated with the bands produced by reverse transcription of total RNAs containing Po RNA.

Comparable results were obtained in nuclease protection experiments (data not shown). A probe spanning residues −140 to 415 and labeled at residue 415 was partially protected by RNA from strain SK5003 (*pnp-7 rnb-500*) but not by RNA from parental strain SK5006 to yield 5′ termini corresponding to residues 299 and 300 at S1 nuclease con-

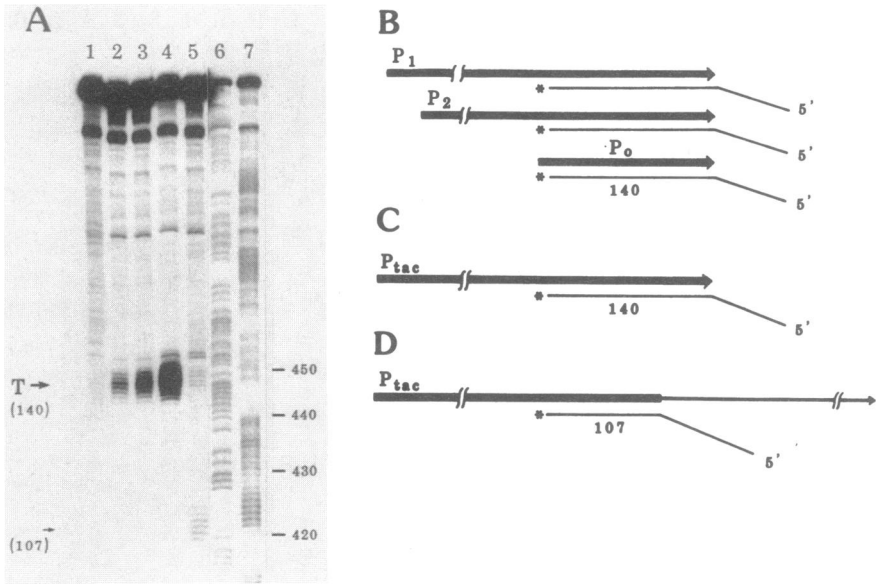

FIG. 4. S1 nuclease mapping of 3′ termini of S20 RNAs from different strains. Nuclease protection was performed as described in Materials and Methods. In panel A, the RNA samples were as follows: lane 1, no added RNA; lane 2, 12 μg of total RNA from strain SK5006 grown at 28°C; lane 3, 12 μg of total RNA from strain SK5003 (*pnp-7 rnb-500*) grown at 28°C; lane 4, 1 μg of total RNA from strain GM332; lane 5, 1 μg of total RNA from strain pGM325 (36). Lanes 6 and 7 contained the A+G and C+T reactions, respectively; these were visualized by longer exposure. The numbers on the right give the coordinates of the S20 sequence determined from the sequence markers. The thick arrow (T) on the left indicates the anticipated position of a protected probe of 140 residues corresponding to the 3′ ends of the S20 RNAs (panels B and C; see below), while the thin arrow indicates the expected position of a 107-residue protected fragment (D). Panels B, C, and D schematically show the protection of the probe by the RNAs used in lanes 2 and 3 (B), 4 (C), and 5 (D). The heavy lines denote S20 mRNAs, and the extension in panel D shows vector-derived sequences in plasmid pCD6 (32). The thin line represents the labeled strand of the probe, and the asterisk indicates the site of labeling. The numbers give the anticipated sizes (in residues) of the protected portions of the probes.

centrations of less than 50 U/ml. At somewhat higher concentrations, the major and minor 5′ ends of protected bands occurred at residues 300 and 301, respectively.

The size of Po RNA and the position of its 5′ end in the S20 sequence suggested that the 3′ terminus of Po RNA would be coterminal with the 3′ ends of the two mature S20 mRNAs. To verify this point, the 3′ termini of S20 mRNAs from strains SK5003 (*pnp-7 rnb-500*) and SK5006 were compared with those of positive and negative controls. These reference RNAs were encoded by plasmids containing either the authentic S20 rho-independent terminator (plasmid pGM72 in strain GM332, the positive control) or a fusion of the S20 sequences (residues 92 to 411 in Fig. 1A) proximal to this terminator to the *rrnB* terminator (pCD6 in strain GM325, the negative control; see reference 36). RNAs from strains SK5003 (*pnp-7 rnb-500*), SK5006, and GM332 exhibited identical families of protected bands corresponding to 3′ termini ending close to residue 447, the most distal of a stretch of seven uridylic acid residues (Fig. 4, lanes 2–4). This set of bands was much fainter when the RNA providing protection was obtained from strain GM325 (Fig. 4, lane 5). The faint bands in this case were likely due to chromosomally encoded s20 mRNAs. No additional abundant 3′ termini were found in strain SK5003 (*pnp-7 rnb-500*). Other faint bands in lanes 2 to 5 of Fig. 4 are probably due to incomplete digestion of the probe (cf. lane 1) rather than to protection by additional 3′ termini. Indeed, most of the faint bands would correspond to endpoints beyond the rho-independent terminator, which defines the end of the S20 transcriptional unit and could not reflect RNA-dependent protection. Taking the data in Fig. 2–4 together shows that the Po RNA found in strains containing the *pnp-7* allele extends from residue 300 (or 301) to the normal 3′ terminus of S20 mRNAs at or near residue 447.

In view of the occurrence of specific cleavages in the leaders of *ompA* and *bla* mRNAs (34) and in *lac* mRNA (9), a search was made for additional 5′ termini in the 5′-untranslated region of S20 mRNAs both by S1 analysis and by extension by using a primer complementary to residues 164 to 181. A few minor termini were found among RNAs extracted from strains SK5006 to SK5003, but none was as intense as that of Po RNA (data not shown).

Role of distal sequences in the generation of Po RNA. We have previously exploited three *Sau*3AI sites in the distal portion of the S20 coding sequence (Fig. 1A) to construct internal deletions in the coding sequence for S20 (14). These deletions are in frame (and thus nonpolar) and do not alter either the extreme C terminus of S20 or the rho-independent terminator. Fortuitously, the first of these *Sau*3AI sites is coincident with the 5′ terminus of Po RNA. Thus, the new junctions created by the deletions retain the same sequence, 5′-AACCGAUC, found at the site of presumed cleavage of unaltered S20 mRNAs. Two such deletions were transferred to vector pEX-1 so that the gene for S20 is placed under control of the inducible *tac* promoter. Each construction was subsequently introduced into strains SK5005 (*rnb-500*) and SK5003 (*pnp-7 rnb-500*). The effects of these deletions on the accumulation of a truncated S20 mRNA were ascertained by using primer extension. Comparable results at lower resolution were obtained with RNA blotting (data not shown). Deletion of 30 residues (300 to 329 inclusive) in pGM75Δ13 encompassing the 5′ end of Po resulted in accumulation of a truncated RNA (denoted as Po′) in strain SK5003 (*pnp-7 rnb-500*) but not in strain SK5005 (*rnb-500*) (Fig. 5, compare

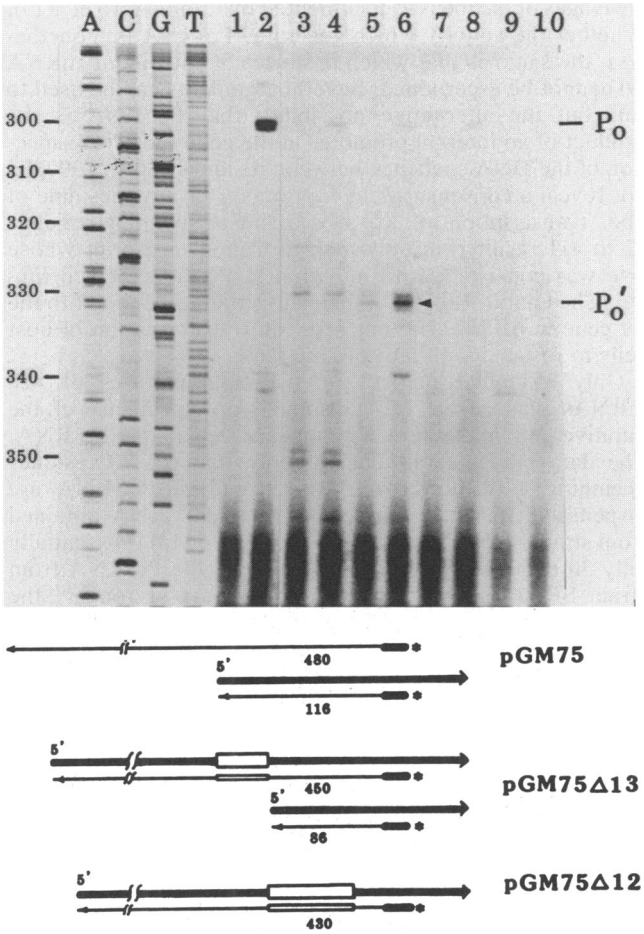

FIG. 5. Effects of deletions in the S20 structural gene on the generation of truncated S20 RNAs. Cultures of derivatives of strain SK5003 (*pnp-7 rnb-500*) (even-numbered lanes) or SK5005 (*rnb-500*) (odd-numbered lanes) were grown at 28°C to an A_{600} of 0.2. The cultures were shifted to 37°C for 40 min and then made 1 mM in isopropyl-β-D-thiogalactopyranoside. RNA was extracted from a 2-ml sample of each culture by method II (see Materials and Methods) 20 min later. Approximately 3 μg of total RNA from each culture was analyzed by primer extension as described in Materials and Methods. The markers in lanes A, C, G, and T are the same as in Fig. 3, except that storage before use accentuated ghost bands, particularly in lane T. The following plasmids served as the major source of S20 mRNA in vivo: pGM75 (lanes 1 and 2), pGM75Δ12 (lanes 3 and 4), pGM75Δ13 (lanes 5 and 6), and pGM78 (lanes 7 and 8). In lane 9, the RNA template was synthesized in vitro as for Fig. 3, lane 9. Lane 10 contained no RNA template. Po and Po′ on the right denote the positions of truncated S20 RNAs discussed in the text. The upper portion of the gel was cut off, so that the P1, P2, and Ptac extension products are not shown. The numbers on the left give the sequence coordinates read from the markers. The structures of the RNA templates and the anticipated extents of the major extension products are illustrated in the diagram at the bottom; internal deletions between *Sau*3AI sites are denoted by open boxes.

lanes 5 and 6). It is noteworthy that the truncated RNA (Po′) in Fig. 5, lane 6, was almost exactly 30 residues shorter than Po, essentially the size of the deletion. RNA from strains harboring a longer deletion of 48 residues (330 to 377 inclusive in pGM75Δ12) did not contain an allele-specific truncated mRNA fragment (Fig. 5, compare lanes 3 and 4). Nonetheless, the size of the truncated RNA in this case was also reduced by the size of the deletion. In contrast, a strain

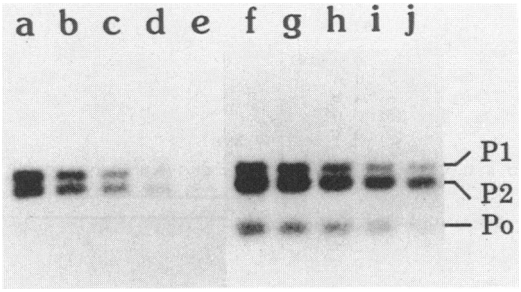

FIG. 6. Analysis of the decay of S20 mRNA species by RNA blotting. Cultures of strains SK5006 (lanes a to e) and SK5003 *pnp-7 rnb-500* (lanes f to j) were grown in LB medium containing 20 mg of chloramphenicol per liter at 28°C to an A_{600} of 0.2. The cultures were shifted to 38°C for 60 min, at which time rifampin was added to 200 mg/liter and RNA was extracted as outlined in Materials and Methods. Samples containing 8 μg were analyzed by RNA blotting, S20 RNAs were detected by being annealed to probe I (Fig. 1B), and the hybrids were visualized by autoradiography (12 h of exposure for lanes a to e and 5 h for lanes f to j). Samples were taken at the following times: a, 0 min; b, 1.5 min; c, 3.0 min; d, 4.5 min; e, 6.0 min; f, 0 min; g, 1.5 min; h, 4.5 min; i, 7.5 min; j, 10.5 min.

containing a replacement of the distal stem-loop structure of the S20 terminator (residues 412 to 550) with a sequence encompassing part of 5S rRNA and the *rrnB* transcriptional terminator in pGM78 failed to yield any detectable truncated S20 mRNA species either in the primer extension assay (Fig. 5, lanes 7 and 8) or in an RNA blotting assay (data not shown).

Effect of the *pnp-7* allele on the stability of S20 mRNAs. The lifetimes of the two mature S20 mRNAs in cultures of SK5003 (*pnp-7 rnb-500*) and SK5006 treated with rifampin were determined by RNA blotting as described in Materials and Methods (25, 36). An example of one such blot is shown in Fig. 6. No alteration of the size of the P1, P2, or Po RNA species concomitant with decay could be detected at this level of resolution. The half-life of P2 mRNA was 118 s in strain SK5006 grown at 28°C and shifted to 38°C for 60 min before treatment with rifampin, while that of P1 mRNA was 90 s (Table 1). These values are in good agreement with those measured previously in strain C600 *galK*, namely, 116 and 73 s (25). These half-lives were extended about 2.5-fold in strain SK5003 (*pnp-7 rnb-500*) to 310 s for P2 mRNA and to 220 s for P1 mRNA (Table 1). Measurement of the half-life of Po RNA in this strain was subject to error caused by the diffuse nature of the band. Its initial rate of decay corresponded to a half-life of 420 s (Table 1). This is probably an overestimate, however, because Po RNA likely accumulated during treatment with rifampin via decay of P1 and P2 (cf. reference 16).

TABLE 1. Half-lives of S20 mRNA species in strains SK5003 (*pnp-7 rnb-500*) and SK5006[a]

Strain	Half-life (s)		
	P1 mRNA	P2 mRNA	Po RNA
SK5006	90	118	ND[b]
SK5003	220	310	>420[c]

[a] RNA was isolated from cultures of strains SK5006 and SK5003 (*pnp-7 rnb-500*) grown as described in the legend to Fig. 6. The half-lives of intact P1 and P2 mRNAs, as well as that of Po RNA, were estimated by Northern blotting, as described in Materials and Methods, using a complementary RNA probe (probe I in Fig. 1B).

[b] ND, Not detectable.

[c] This estimate is not a true half-life for reasons discussed in the text.

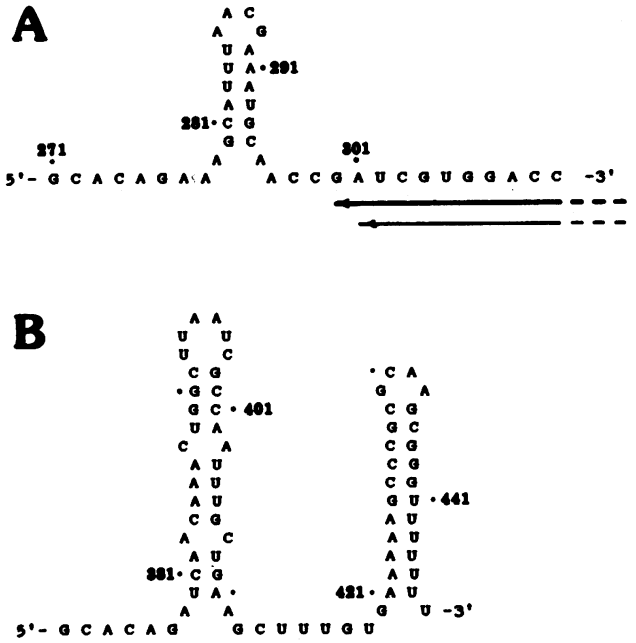

FIG. 7. Potential secondary structures in S20 mRNAs. (A) Sequence and possible folding of residues 271 to 310 of S20 mRNAs near the 5′ terminus of Po RNA. The heavier arrow below the sequence denotes the major product of the primer extension experiment in Fig. 3, while the lighter arrow shows the minor product. (B) Possible folding of residues 373 to 447 at the 3′ end of S20 mRNAs and Po RNA. The termination codon occurs at residues 394 to 396 at the apex of the left stem-loop structure.

I did not measure the half-lives of S20 mRNAs in strain SK5005 (*rnb-500*) or SK5004 (*pnp-7*) containing a single mutation. The relative intensities of the P1 and P2 mRNAs in strain SK5004 (*pnp-7*) were almost identical to the levels in double mutant SK5003 (*pnp-7 rnb-500*), and in both cases, they were higher than in strains SK5005 (*rnb-500*) and SK5006 (Fig. 2A, compare lanes 7 and 8 with 5 and 6). This finding is consistent with the conclusion that the mutation in polynucleotide phosphorylase is the primary cause of the increase in the half-lives of S20 mRNAs in strain SK5003 (*pnp-7 rnb-500*). Whether complete inactivation of RNase II alone would also lead to an increase in the life time of S20 mRNAs at physiological temperatures (and accumulation of Po RNA) cannot be assessed in this set of strains which retain significant levels of RNase II activity (15).

DISCUSSION

The most striking and unanticipated result of this investigation is the finding of a truncated S20 RNA (Po RNA) in strains carrying the *pnp-7* allele in the gene for polynucleotide phosphorylase. The 3′ end of Po RNA appears to be identical to those of mature S20 mRNAs. This RNA possesses a major 5′ terminus corresponding to residue 300 in S20 mRNA and a minor terminus at the adjacent residue 301 (Fig. 7A). The minor terminus may be a result of incomplete runoff by reverse transcriptase.

It seems most likely that the 5′ terminus of Po RNA arises through the action of a relatively specific endo-RNase on at least one of the mature S20 mRNAs. The specificity of the cleavage between residues 299 and 300 (CpG) (Fig. 7A) does not, however, correspond to that of any known endo-RNase in *E. coli* (reviewed in reference 12) or to any of the cleavages of *lac* mRNA documented by Cannistraro et al (9). Whether the activity which generates Po RNA is, nonetheless, the same as that which produces 5′ ends in *lac* mRNA (9) cannot be ascertained. Several arguments can be used to rule out the alternative possibility that Po RNA is the product of an internal promoter in the gene for S20. Inspection of the DNA sequence between residues 260 and 300 did not reveal a consensus *E. coli* promoter or any facsimile of one. Transcription in vitro of a fragment spanning residues 12 to 411 resulted in only a single runoff transcript whose size was consistent with initiation at P2, as expected (unpublished data). Likewise, fusion of residues 92 to 411 to the *tet* gene in pBR322 did not result in transformation of host cells to resistance to tetracycline (26).

Only a limited number of structural features of S20 mRNAs play a role in determining the specificity of the putative endonuclease which appears to generate Po RNA. The data presented in Fig. 5 imply that the 30 residues beginning 4 residues from the 5′ end of Po RNA are dispensable to its formation, since the Po′ RNA obtained from strain SK5003 *pnp-7 rnb-500* (pGM75Δ13) is essentially only 30 residues shorter than the prototype Po RNA from strain SK5003 (*pnp-7 rnb-500*) and occurs at roughly the same abundance. At least one of the internucleotide bonds cleaved in the formation of Po′ RNA, namely CpG, is the same as that for Po RNA. A major determinant of the specificity of the putative endonuclease appears, therefore, to be the sequence context at or near the site of cleavage. It is also possible that the short stem-loop structure to the 5′ side of the cleavage (Fig. 7A) forms part of the recognition sequence of the enzyme. Replacement of the S20 terminator with sequences from the vector, including a strong rho-independent terminator (plasmid pGM78), abolished the accumulation of truncated S20 RNA (Fig. 5, lanes 7 and 8). The data do not permit a distinction between a requirement for the integrity of the 3′ end of S20 mRNAs for the formation of Po RNA as opposed to a requirement for its stability. The tandem stem-loop structures which can fold from the sequences proximal to the 3′ end of Po RNA (and S20 mRNAs) are the most obvious structural features of this region of S20 mRNAs (Fig. 7B). These two RNA hairpins would be expected to confer resistance to 3′ exonucleases, such as RNase II and polynucleotide phosphorylase (12, 23, 30). Thus, a major reason for the accumulation of Po RNA in strains SK5003 (*pnp-7 rnb-500*) and SK5004 (*pnp-7*) is simply that they lack the enzyme(s) to degrade this RNA efficiency. By implication, these strains lack the means to degrade the 3′ portion of complete S20 mRNAs as well.

In strains containing wild-type polynucleotide phosphorylase, S20 mRNAs decay without accumulation of abundant discrete intermediates (concerted or all-or-nothing decay), as shown for *ompA* mRNA (40). Any intermediates in the decay process are apparently too ephemeral to be detected. The commitment step in initiating the decay of S20 mRNAs must depend on functional polynucleotide phosphorylase, because the reduction in its activity by mutation in strains SK5004 (*pnp-7*) and SK5003 (*pnp-7 rnb-500*) clearly results in significant increases in the abundances of intact S20 mRNAs in both strains as well as in the half-lives of intact P1 and P2 mRNAs in strain SK5003 (*pnp-7 rnb-500*). What might the commitment step be? The simplest model is that degradation of S20 mRNAs can be accomplished by polynucleotide phosphorylase itself (or in concert with RNase II), with or without prior internal cleavage of these mRNAs by endonucleases. Thus, the limiting step would be its binding to the 3′ end(s) of mRNA in a manner which allows it to

initiate processive 3′-to-5′ phosphorolysis of its substrate. Intact S20 mRNAs are potentially highly structured at their 3′ termini (Fig. 7B). They should, therefore, serve as poor substrates for this enzyme (12, 23). A second explanation is that unwinding of S20 mRNAs preliminary to the action of polynucleotide phosphorylase (or other RNases) constitutes the normal commitment step in the degradation of S20 mRNAs. Third, if commitment involves one or more endonucleolytic cleavages, as suggested by several researchers (1, 2, 9, 39), then all of the RNA segments so generated must disappear at similar rates (within the limits of detection) to simulate concerted decay. This could be achieved if polynucleotide phosphorylase (with or without RNase II and/or other 3′ exonucleases) is in sufficient excess to attack new 3′ ends as quickly as they are generated. This third model cannot, however, easily account for the stabilization of intact S20 mRNAs in strains such as SK5003 (*pnp-7 rnb-500*). Finally, polynucleotide phosphorylase may act as a cofactor for the synthesis, binding, or activity of the true initiating enzyme. Tests to distinguish among these possibilities using S20 mRNAs synthesized in vitro are being undertaken.

When polynucleotide phosphorylase is inactivated by the *pnp-7* mutation, S20 mRNAs display differential segmental decay, demonstrated for *lac* (20) and *bla* (40) mRNAs, rather than concerted decay. In these circumstances, the decay of S20 mRNAs appears to be initiated by one or more endonucleolytic cleavages, the most 3′ of which occurs on the 5′ side of residue 300 (and possibly of residue 301). After cleavage at or near residue 300, the 3′ one-third of S20 mRNAs (i.e., Po RNA) remains intact for reasons discussed above, while the 5′ two-thirds is degraded quickly. This degradation may be accomplished by nucleases other than RNase II or polynucleotide phosphorylase, although I cannot rule out the possibility that the residual activity of these enzymes in strain SK5003 (*pnp-7 rnb-500*) is sufficient to catalyze the degradation of both the 5′ and 3′ portions of S20 mRNAs.

One implication of these results is that there is no universal pathway for turnover of mRNA, even for a single mRNA species. Both endo- and exo-RNases can be required for degradation of mRNAs (1, 2, 9, 15, 21). Which type of enzyme acts first undoubtedly depends strongly on the primary sequence and secondary structure of the particular mRNA substrate. As the size of an mRNA increases, the ratio of potential endonucleolytic sites to 3′ ends increases. This obviously favors initial endonucleolytic cleavages of mRNA, as suggested by several authors (1, 2, 9, 39), particularly since the extreme 3′ end may be sequestered by RNA polymerase. The small size of S20 mRNAs makes them ideal substrates for experiments in vitro in which the steps in their decay can be determined more precisely and ultimately reconstructed.

ACKNOWLEDGMENTS

I thank my colleagues Murray Deutscher, Sidney Kushner, Tom Linn, Lou Passador, and Mark Pearson for the gift of strains. S. D. Dunn, G. Chaconas, and a reviewer made valuable comments on the manuscript.

I thank the Medical Research Council of Canada for financial support. The oligonucleotides used in this work were synthesized at a facility established by the Academic Development Fund of the University of Western Ontario.

ADDENDUM IN PROOF

Mapping of the 5′ terminus of the Po RNA by primer extension has been repeated with four modifications to the procedure described in Materials and Methods: (i) the labeled primer was purified by gel filtration to eliminate salts and unincorporated [^{32}P]ATP before annealing to RNA; (ii) 0.2 M sodium acetate was substituted for 2 M ammonium acetate for precipitation of the products of primer extension; (iii) the primer was phosphorylated at its 5′ end before synthesis of the marker sequence ladder to ensure that primer extension products and chain-terminated markers of the same length would display equivalent electrophoretic mobilities; and (iv) synthesis of the sequence markers was catalyzed by T7 DNA polymerase rather than by the Klenow fragment. The first two steps reduce interference in the separation of the products by salt, while the latter two improve the reliability of the markers. As a consequence the major 5′ end of Po RNA maps unambiguously to residue 301 (A) and the minor terminus maps to residue 302 (U), i.e., one residue to the 3′ side of the termini shown in Fig. 7A. The preferred site of cleavage to generate Po RNA occurs, therefore, at a GpA dinucleotide rather than at a CpG dinucleotide as stated in the text.

LITERATURE CITED

1. **Apirion, D.** 1972. Degradation of RNA in *Escherichia coli*. A hypothesis. Mol. Gen. Genet. **122**:313–322.
2. **Arraiano, C. M., S. D. Yancey, and S. R. Kushner.** 1988. Stabilization of discrete mRNA breakdown products in *ams pnp rnb* multiple mutants of *Escherichia coli* K-12. J. Bacterial. **170**:4625–4633.
3. **Astrachan, L., and E. Volkin.** 1958. Properties of ribonucleic acid turnover in T2-infected *Escherichia coli*. Biochim. Biophys. Acta **29**:3–11.
4. **Belasco, J. G., G. Nilsson, A. von Gabain, and S. N. Cohen.** 1986. The stability of *E. coli* gene transcripts is dependent on determinants localized to specific mRNA segments. Cell **46**:245–251.
5. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease digested hybrids. Cell **12**:721–732.
6. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
7. **Brenner, S., F. Jacob, and M. Meselson.** 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature (London) **190**:576–581.
8. **Brosius, J., and A. Holy.** 1984. Regulation of ribosomal RNA promoters with a synthetic *lac* operator. Proc. Natl. Acad. Sci. USA **81**:6929–6933.
9. **Cannistraro, V. J., M. N. Subbarao, and D. Kennell.** 1986. Specific cleavage sites for decay of *Escherichia coli* mRNA. J. Mol. Biol. **192**:257–274.
10. **Cole, J. R., and M. Nomura.** 1986. Changes in the half-life of ribosomal protein messenger RNA caused by translational repression. J. Mol. Biol. **188**:383–392.
11. **Dennis, P. P., and M. Nomura.** 1975. Regulation of the expression of ribosomal protein genes in *Escherichia coli*. J. Mol. Biol. **97**:61–76.
12. **Deutscher, M. P.** 1985. *E. coli* RNases: making sense of alphabet soup. Cell **40**:731–732.
13. **Deutscher, M. P., G. T. Marshall, and H. Cudny.** 1988. RNase PH: an *Escherichia coli* phosphate-dependent nuclease distinct from polynucleotide phosphorylase. Proc. Natl. Acad. Sci. USA **85**:4710–4714.
14. **Donly, B. C., and G. A. Mackie.** 1988. Affinities of ribosomal protein S20 and C-terminal deletion mutants for 16S rRNA and S20 mRNA. Nucleic Acids Res. **16**:997–1010.
15. **Donovan, W. P., and S. R. Kushner.** 1986. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **83**:120–124.
16. **Ebbole, D. J., and H. Zalkin.** 1988. Detection of *pur*-operon

attenuated mRNA and accumulated degradation intermediates in *Bacillus subtilis*. J. Biol. Chem. **263**:10894–10902.
17. **Gottesman, M., A. Oppenheim, and D. Court.** 1982. Retroregulation: control of gene expression from sites distal to the gene. Cell **29**:727–728.
18. **Gros, F., H. Hiatt, W. Gilbert, C. G. Kurland, R. W. Risebrough, and J. D. Watson.** 1961. Unstable ribonucleic acid revealed by pulse-labelling of *Escherichia coli*. Nature (London) **190**:73–77.
19. **Higgins, C. F., G. Ferro-Luzzi Ames, W. M. Barnes, J. M. Clement, and M. Hofnung.** 1982. A novel intercistronic regulatory element of prokaryotic operons. Nature (London) **298**: 760–762.
20. **Kepes, A.** 1967. Sequential transcription and translation in the lactose operon of *Escherichia coli*. Biochim. Biophys. Acta **138**:107–123.
21. **Kinscherf, T. G., and D. Apirion.** 1975. Polynucleotide phosphorylase can participate in decay of mRNA in *Escherichia coli* in the absence of ribonuclease II. Mol. Gen. Genet. **139**: 357–362.
22. **Klug, G., C. W. Adams, J. Belasco, B. Doerge, and S. N. Cohen.** 1987. Biological consequences of segmental alterations in mRNA stability: effects of deletion of the intercistronic hairpin loop region of the *Rhodobacter capsulata puf* operon. EMBO J. **6**:3515–3520.
23. **Littauer, U. Z., and H. Soreq.** 1982. Polynucleotide phosphorylase, p. 517–553. *In* P. Boyer (ed.), The enzymes, vol. 15. Academic Press, Inc., New York.
24. **Mackie, G. A.** 1986. Structure of the DNA distal to the gene for ribosomal protein S20 in *Escherichia coli* K12: presence of a strong terminator and an IS1 element. Nucleic Acids Res. **14**:6965–6981.
25. **Mackie, G. A.** 1987. Posttranscriptional regulation of ribosomal protein S20 and stability of the S20 mRNA species. J. Bacteriol. **169**:2697–2701.
26. **Mackie, G. A., and G. D. Parsons.** 1983. Tandem promoters in the gene for ribosomal protein S20. J. Biol. Chem. **258**:7840–7846.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **McMurry, L. M., and S. B. Levy.** 1987. Tn*5* insertion in the polynucleotide phosphorylase (*pnp*) gene in *Escherichia coli* increases susceptibility to antibiotics. J. Bacteriol. **169**:1321–1324.
29. **Miyamoto, C., and D. T. Denhardt.** 1977. Evidence for the presence of ribonucleotides at the 5′ termini of some DNA molecules isolated from *Escherichia coli polAex2*. J. Mol. Biol. **116**:681–707.
30. **Mott, J. E., J. L. Galloway, and T. Platt.** 1985. Maturation of *Escherichia coli* tryptophan operon mRNA: evidence for 3′ exonucleolytic processing after rho-dependent termination. EMBO J. **4**:1887–1891.
31. **Newbury, S. F., N. H. Smith, and C. F. Higgins.** 1987. Differential mRNA stability controls relative gene expression within a polycistronic operon. Cell **51**:1131–1143.
32. **Newbury, S. F., N. H. Smith, E. C. Robinson, I. D. Hiles, and C. F. Higgins.** 1987. Stabilization of translationally active mRNA by prokaryotic REP sequences. Cell **48**:297–310.
33. **Nilsson, G., J. G. Belasco, S. N. Cohen, and A. von Gabain.** 1984. Growth-rate dependent regulation of mRNA stability in *Escherichia coli*. Nature (London) **312**:75–77.
34. **Nilsson, G., U. Lundberg, and A. von Gabain.** 1988. *In vivo* and *in vitro* identity of site specific cleavages in the 5′ non-coding region of *ompA* and *bla* mRNA in *Escherichia coli*. EMBO J. **7**:2269–2275.
35. **Panayotatos, N., and K. Truong.** 1985. Cleavage within an RNase III site can control mRNA stability and protein synthesis *in vivo*. Nucleic Acids Res. **13**:2227–2240.
36. **Parsons, G. D., B. C. Donly, and G. A. Mackie.** 1988. Mutations in the leader sequence and initiation codon of the gene for ribosomal protein S20 (*rpsT*) affect both translational efficiency and autoregulation. J. Bacteriol. **170**:2485–2492.
37. **Schmeissner, U., K. McKenney, M. Rosenberg, and D. Court.** 1984. Removal of a terminator structure by RNA processing regulates *int* gene expression. J. Mol. Biol. **176**:39–53.
38. **Singer, P., and M. Nomura.** 1985. Stability of ribosomal protein mRNA and translational feedback regulation in *Escherichia coli*. Mol. Gen. Genet. **199**:543–546.
39. **Subbarao, M. N., and D. Kennell.** 1988. Evidence for endonucleolytic cleavages in decay of *lacA* and *lacI* mRNAs. J. Bacteriol. **170**:2860–2865.
40. **von Gabain, A., J. G. Belasco, J. L. Schottel, A. C. Y. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.