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Genetics

Polyadenylylation helps regulate mRNA decay in *Escherichia coli*

[poly(A) polymerase/*trxA*/*ompA*/*lpp*/mRNA half-lives]

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ABSTRACT **As part of our genetic analysis of mRNA decay in *Escherichia coli* K-12, we examined the effect of the *pcnB* gene [encoding poly(A) polymerase I] on message stability. Eliminating poly(A) polymerase I (Δ*pcnB*) dramatically stabilized the *lpp*, *ompA*, and *trxA* transcripts. The half-lives of individual mRNAs were increased in both a Δ*pcnB* single mutant and a Δ*pcnB pnp-7 rnb-500 rne-1* multiple mutant. We also found mRNA decay intermediates in Δ*pcnB* mutants that were not detected in control strains. By end-labeling total *E. coli* RNA with [^{32}P]pCp and T4 RNA ligase and then digesting the RNA with RNase A and T_1, we showed that many RNAs in a wild-type strain contained poly(A) tails ranging from 10 nt to >50 nt long. When polynucleotide phosphorylase, RNase II, and RNase E were absent, the length (>100 nt) and number (10- to 20-fold) of the poly(A) tails increased. After transcription initiation was stopped with rifampicin, polyadenylylation apparently continued. Deleting the structural gene for poly(A) polymerase I (*pcnB*) reduced the amount of 3′-terminal poly(A) sequences by >90%. We propose a model for the role of polyadenylylation in mRNA decay.**

To study mRNA decay in *Escherichia coli* and its role in gene regulation, our laboratory used a genetic approach to examine RNases that might participate in mRNA decay (1–4). When Babitzke *et al.* (3) constructed a polynucleotide phosphorylase (PNPase), RNase II, RNase III, RNase E quadruple mutant, however, they found that RNA decay was only moderately affected. Their results made it clear that important aspects of mRNA decay were still not understood.

Consequently, we set out to examine additional genes that might be involved. The identification of *pcnB*, the structural gene for poly(A) polymerase I (PAP I) (5), has allowed us to determine whether polyadenylylation affects mRNA decay in *E. coli* the same way it does in eukaryotes (6). For many eukaryotic mRNAs, the degradation of poly(A) tails initiates mRNA decay (7–9). If it does so in *E. coli*, then, presumably, mRNA stability would increase in the absence of PAP I.

To test whether the degradation of poly(A) tails initiates mRNA decay in *E. coli*, a series of isogenic strains containing mutations in PAP I (10), RNase E (*rne*) (1), PNPase (*pnp*) (1), and RNase II (*rnb*) (1) were constructed. Northern analysis of the *trxA*, *ompA*, and *lpp* mRNAs showed that when polyadenylylation was almost completely eliminated, their half-lives always increased significantly and their decay patterns changed. We also determined the number and size of poly(A) tails in the total bacterial RNA population. In wild-type *E. coli*, poly(A) tails ranged from 10 to >50 nt. Where there was no PNPase and there was reduced RNase II activity, both the number and the size of the tails increased significantly. More than 90% of the poly(A) tails were absent in Δ*pcnB* mutants. We discuss the implications of these results below.

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MATERIALS AND METHODS

Bacterial Strains. All strains were derived from *E. coli* MG1693 (*thyA715*), provided by B. Bachmann (*E. coli* Genetic Stock Center, Yale University). SK5704 (*pnp-7 rnb-500 rne-1 thyA715*) (1) was constructed by phage P1-mediated transduction (11). SK7988 (Δ*pcnB thyA715*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1 thyA715*) were derived from MG1693 and SK5704, respectively. A kanamycin-resistance (Km^R) determinant in the Δ*pcnB* gene (10) was used as a selectable transduction marker. SK7988 and SK8901 each grew more slowly than their *pcnB*$^+$ parents. The absence of poly(A) polymerase in SK8901 did not affect the conditional lethality associated with inactivating RNase II, RNase E, and PNPase.

RNA Isolation. Seven-milliliter samples were mixed with an equal volume of crushed frozen TM buffer (10 mM Tris, pH 7.2/5 mM $MgCl_2$) containing 20 mM NaN_3 and chloramphenicol at 0.4 mg/ml. The cells were then centrifuged and the pellet was suspended in 0.34 ml of TM buffer with lysozyme at 0.3 mg/ml and DNase I at 32 units/ml. After three freeze–thaw cycles, one-sixth volume of 20 mM acetic acid was added, followed by an equal volume of Catrimox-14 (Iowa Biotechnology, Oakdale, IA).

The resulting precipitate of protein, DNA, and RNA was spun in a microcentrifuge at medium speed to form a soft pellet. That pellet was completely suspended in 1 ml of 2 M LiCl in 35% ethanol. In this solution protein and DNA dissolve but RNA does not. The resulting RNA precipitate was centrifuged at high speed for 5 min to form a pellet. It was then suspended in 2 M LiCl in distilled water and centrifuged again. The pellet was washed with 70% ethanol and resuspended in distilled water. There was no difference between mRNA prepared by the phenol extraction procedure of Stubbs and Hall (12) and that prepared by the Catrimox-14 method in either Northern blots or primer extension analyses. There was, however, a better recovery of total RNA, including smaller species, when Catrimox-14 was used.

Determination of Poly(A) Tail Length. We adapted the methods of Ahlquist and Kaesburg (13) and Sachs and Davis (14) to label total RNA from the bacterial strains. Typically, between 3 and 20 μg of total RNA, depending on the strain, was 3′-end labeled with [^{32}P]pCp and T4 RNA ligase before digestion with 25 μg of RNase A and 400 units of RNase T_1 per sample. Poly(A) tails were resolved in 12% polyacrylamide sequencing gels containing 7 M urea. Estimates of sizes and relative numbers of poly(A) tails in various genetic backgrounds were derived from multiple independent determinations.

Digestion of Poly(A) Tails with RNase H. Total RNA isolated from SK5704 (*pnp-7 rnb-500 rne-1*) grown at 30°C was annealed with $(dT)_{20}$ at room temperature for 30 min and then digested with RNase H for 30 min at 37°C. Extensive digestion

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

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with RNase-free DNase removed the oligo(dT) before the poly(A) tails were sized as described in the assay above.

RNA Blotting Analysis. The method of Arraiano *et al.* (1) was used to make dot blots, except that the RNA was spotted onto Magnacharge membranes (Micron Separations Westboro, MA). In the [^{32}P]oligo(dT)/unlabeled oligo(dC) competition experiments, a 1000-fold excess of unlabeled oligonucleotide was added to both the prehybridization and the hybridization buffer. For Northern analyses, total RNA was denatured in formamide containing 0.3% xylene cyanol, 0.3% bromophenol blue, and 0.37% disodium EDTA. The RNA species were then separated in 5% or 6% polyacrylamide gels containing 7 M urea. After electrophoresis, the RNA was electroblotted onto Biotrans Plus membranes (ICN) according to the manufacturers' directions, probed with ^{32}P-labeled DNA fragments or oligonucleotides, and autoradiographed with Kodak XAR-5 x-ray film.

Determination of mRNA Half-Lives. The half-lives of individual full-length transcripts were determined with a PhosphorImager (model 400 series, Molecular Dynamics). The half-lives were determined by linear regression analysis.

RESULTS

Northern Analysis of Individual mRNAs. Our initial half-life determinations for total pulse-labeled RNA in SK5704 (*pnp-7 rnb-500 rne-1*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) suggested that the loss of PAP I had significantly affected mRNA decay (data not shown). We then used Northern analyses of total RNA isolated from these strains as well as from MG1693 (wild type) and SK7988 (Δ*pcnB*) to determine the fate of individual mRNAs. Three different mRNAs were tested: *trxA*, a short-lived message (1), and *lpp* (15) and *ompA* (16), two mRNAs with longer half-lives. As in earlier results (1), inactivation of RNase E, PNPase, and RNase II changed the decay rate of *trxA* (Fig. 1 *C* and *D*). The loss of these enzymes also changed the decay patterns of *lpp* (Fig. 1 *A* and *B*) and *ompA* (Fig. 1 *E* and *F*). In SK7988 (Δ*pcnB*), the decay pattern (*lpp*, Fig. 1*A*, and *trxA*, Fig. 1*C*) and rate (*lpp*, Fig. 1*A*) changed compared with the wild-type control.

The loss of PAP I caused a more dramatic change when the three mRNAs were examined in SK5704 (*pnp-7 rnb-500 rne-1*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*). In all cases the full-length transcripts were stabler and there were more large breakdown products (Fig. 1 *B, D,* and *F*). The presence of large breakdown products of *ompA* 2 hr after shift to 44°C was particularly striking (Fig. 1*F*).

Half-Lives of *lpp, ompA,* and *trxA* in Various Genetic Backgrounds. Half-lives for the full-length transcripts in Fig. 1 were determined with a Molecular Dynamics series 400 PhosphorImager. In all cases, inactivation of RNase E, PNPase, and RNase II led to increases in the half-lives of the full-length transcripts (*lpp*, 9.6 min vs. 25 min; *ompA*, 14 min vs. 16 min; *trxA*, 1.8 min vs. 8.2 min). More important, removal of PAP I led to even more dramatic changes in the half-lives (*lpp*, 25 min vs. 31 min; *ompA*, 16 min vs. 37 min; *trxA*, 8.2 min vs. 12 min).

Detection of Poly(A) Tails in Total *E. coli* RNA. Based on results from Fig. 1, we decided to reexamine the extent and nature of polyadenylylation in *E. coli*. Total RNA was isolated from SK5704 (*pnp-7 rnb-500 rne-1*) grown at 30°C to midlogarithmic phase. After the 3′ ends were labeled with [^{32}P]pCp and T4 RNA ligase, many high-molecular-weight species appeared in the 12% polyacrylamide gel (Fig. 2, lane 1). After they were treated with RNase T_1, which degrades RNA after G residues, the labeled species were partially degraded (Fig. 2, lanes 2 and 3). There was more complete cleavage with RNase A, which cuts after C and U residues (Fig. 2, lanes 4 and 5), but unless both RNase A and RNase T_1 were used, degradation into a ladder of A-containing homopolymers (Fig. 2, lane 6) was not complete. A DNA sequencing ladder from the plasmid pBluescript (Stratagene) roughly indicated that the poly(A) sequences ranged from ≈10 to >50 nt (Fig. 2, lanes 7–10). A computer search for polyadenylate stretches in the *E. coli* genome indicated only 1 hexadecamer, 2 decamers, and 53 nonamers in >3 megabases of sequenced DNA. This result suggested that the species observed in Fig. 2 arose posttranscriptionally.

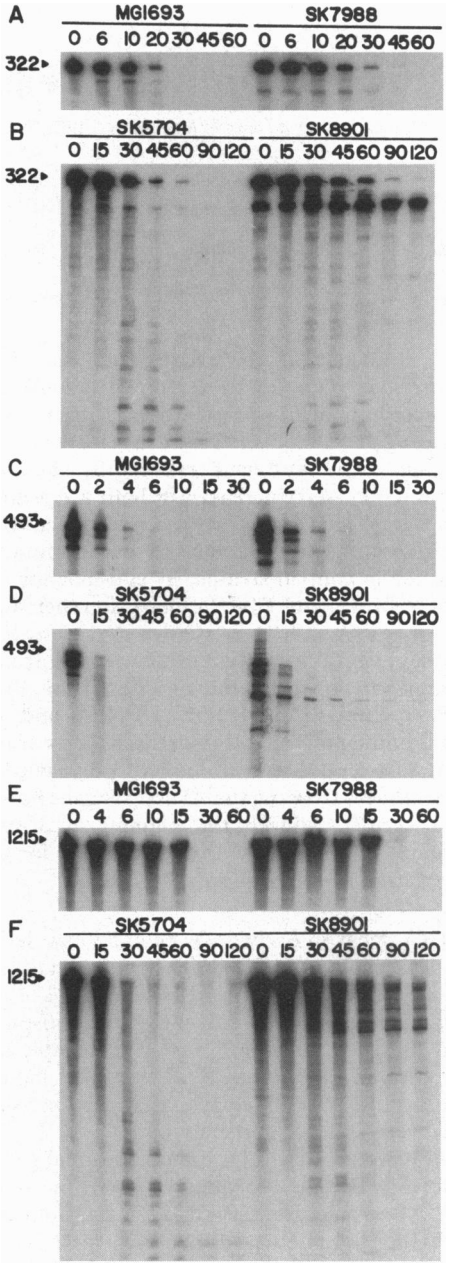

FIG. 1. Northern analysis of *lpp* (5 μg of RNA per lane) (*A* and *B*), *trxA* (7 μg) (*C* and *D*), and *ompA* (5 μg) (*E* and *F*) mRNAs. MG1693 (wild type), SK7988 (Δ*pcnB*), SK5704 (*pnp-7 rnb-500 rne-1*), and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) were grown at 30°C in L broth to 50 Klett units (no. 42 green filter), then shifted to 44°C after rifampicin (500 μg/ml; solubilized in dimethylsulfoxide) and nalidixic acid (20 μg/ml) were added. Samples were removed at the times indicated (minutes after shift) and total RNA was extracted as described in *Materials and Methods*. The *lpp* and *trxA* gels were 6% polyacrylamide and the *ompA* gels were 5% polyacrylamide; all gels contained 7 M urea. After electrophoresis, RNA was transferred by electroblotting to a Biotrans Plus nylon membrane and hybridized with ^{32}P-labeled DNA fragments. Sizes and positions of the full-length transcripts are indicated.

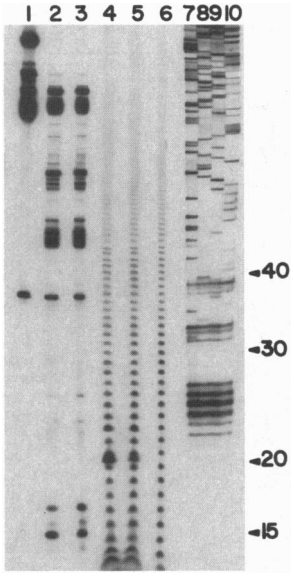

FIG. 2. Poly(A)-tail sizing assay. Three micrograms of total RNA from SK5704 grown at 30°C was labeled with [^{32}P]pCp and T4 RNA ligase. Lanes: 1, no RNase treatment; 2, 10 units of RNase T_1; 3, 20 units of RNase T_1; 4, 2.5 μg of RNase A; 5, 5.0 μg of RNase A; 6, 20 units of RNase T_1 and 5.0 μg of RNase A. A DNA sequencing ladder from pBluescript was used as a size marker (lanes 7–10). Lanes 1–3 contained 1/50th the amount of RNA used in lanes 4–6. For subsequent sizing experiments (Figs. 3–5), 400 units of RNase T_1 and 25 μg of RNase A were used to ensure complete digestion.

Sensitivity of Poly(A) Tails to RNase H Digestion. To confirm the existance of poly(A) species, we digested RNA from SK5704 (*pnp-7 rnb-500 rne-1*) with RNase H before 3′-end labeling with RNA ligase. Neither RNase H nor oligo(dT) alone significantly changed the pattern of poly(A) oligomers compared with the untreated control. However, when the RNA was treated with RNase H in the presence of oligo(dT) before [^{32}P]pCp labeling, almost all of the species of >20 nt were absent and a large smear of radioactivity appeared in the range of <10 nt (data not shown).

Northern Analysis of Total *E. coli* RNA with an Oligo(dT) Probe. We also determined the fate of polyadenylylation following cessation of transcription initiation and inactivation of PNPase, RNase II, and RNase E. After rifampicin was added and the temperature was shifted to 44°C, total RNA was isolated at various times from SK5704 (*pnp-7 rnb-500 rne-1*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) and separated in a 6% polyacrylamide gel as described in *Materials and Methods*. After electroblotting, the membrane was probed with a ^{32}P-labeled $(dT)_{20}$ in the presence of a 1000-fold excess of nonradioactive $(dC)_{20}$.

The analysis revealed significant hybridization, with species from 100 nt to >2000 nt (Fig. 3*A*, SK5704). There was little hybridization in the isogenic strain missing PAP I (Fig. 3*A*, SK8901). Surprisingly, hybridization increased 15 min after the shift to 44°C (Fig. 3*A*, SK5704, 15 min). Even 2 hr after the temperature shift and the inhibition of new transcription, there was hybridization approximately equivalent (Fig. 3*A*, SK5704, 120 min) to that seen before the temperature was shifted (Fig. 3*A*, SK5704, 0 min). Total RNAs from MG1693 (wild type) and SK7988 (Δ*pcnB*) were also compared as described in Fig. 3*A*. As the data in Fig. 4 (lanes 2 and 3 vs. lanes 6 and 7) would lead one to expect, the hybridization to $(dT)_{20}$ seen with MG1693 RNA was significantly lower than that seen with SK5704 RNA and barely visible with SK7988 RNA (data not shown).

Control blots were also hybridized with either ^{32}P-labeled $(dA)_{20}$, ^{32}P-labeled $(dC)_{20}$, or ^{32}P-labeled $(dG)_{20}$ probe. The

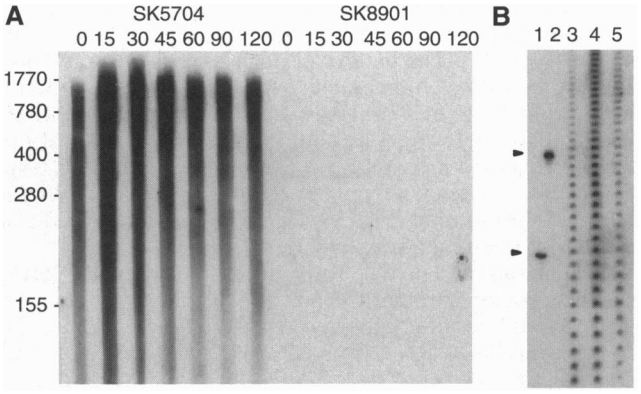

FIG. 3. (*A*) Northern analysis of total *E. coli* RNA using a $(dT)_{20}$ probe. SK5704 (*pnp-7 rnb-500 rne-1*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) were grown and treated as described in Fig. 1. RNA was electrophoresed through a 6% polyacrylamide gel containing 7 M urea. Five micrograms of RNA was loaded in each lane. After electrophoresis, RNA was transferred by electroblotting to a nylon membrane. The membrane was hybridized with ^{32}P-labeled $(dT)_{20}$ in the presence of a 1000-fold excess of nonradioactive $(dC)_{20}$. Sizes of RNA standards (nt) are indicated at left. (*B*) Number and length of poly(A) tails in SK5704. Poly(A) tails were measured as described in Fig. 2. Lanes: 1, $(dA)_{20}$; 2, $(dA)_{30}$; 3, 3 μg of total RNA from SK5704 time zero in *A*; 4, 3 μg of total RNA from SK5704 15 min after shift to 44°C (*A*); 5, 3 μg of total RNA from SK5704 120 min after shift to 44°C (*A*).

pcnB$^+$ and *pcnB*$^-$ strains showed comparably low levels of hybridization. Hybridization did not increase when the cultures were shifted to 44°C (data not shown).

To determine whether the size or number of the poly(A) tails changed during the time-course experiment (Fig. 3*A*), we

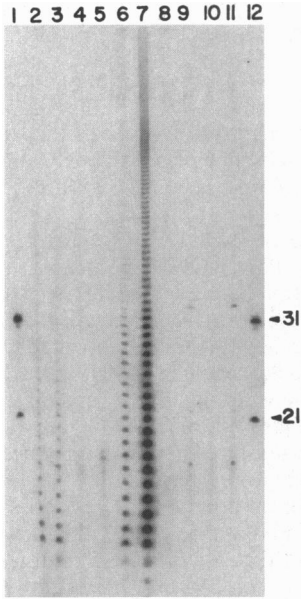

FIG. 4. Comparison of number and distribution of poly(A) tails in strains of *E. coli*. Total RNA isolated from MG1693 (wild type), SK7988 (Δ*pcnB*), SK5704 (*pnp-7 rnb-500 rne-1*), and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) was 3′-end labeled as described in Fig. 2. Lanes: 1 and 12, $(dA)_{20}$ and $(dA)_{30}$; 2, 20 μg of total RNA from MG1693 grown at 30°C; 3, 20 μg of total RNA from MG1693 10 min after shift to 44°C; 4, 20 μg of total RNA from SK7988 grown at 30°C; 5, 20 μg of total RNA from SK7988 10 min after shift to 44°C; 6, 3 μg of total RNA from SK5704 grown at 30°C; 7, 3 μg of total RNA from SK5704 10 min after shift to 44°C; 8, 3 μg of total RNA from SK8901 grown at 30°C; 9, 3 μg of total RNA from SK8901 10 min after shift to 44°C; 10, as lane 8 but with 6 μg of total RNA; 11, as lane 9 but with 6 μg of total RNA.

3′-end labeled the RNA samples from 0 min, 15 min, and 120 min with [^{32}P]pCp and T4 RNA ligase and digested them with RNases A and T_1. The pattern of poly(A) sequences at 0 min (Fig. 3*B*, lane 3) was comparable to those seen with other RNA preparations from SK5704 (Figs. 2 and 4). As predicted from the Northern blot (Fig. 3*A*), both the size and number of the poly(A) oligomers increased 15 min after the shift to 44°C (Fig. 3*B*, lane 4). The size and number of poly(A) sequences in the 2-hr post-shift sample (Fig. 3*B*, lane 5) were almost identical to those of the 0-min sample (Fig. 3*B*, lane 3).

Comparison of Poly(A) Tails in Various Genetic Backgrounds. To confirm that the poly(A) sequences observed in Figs. 2 and 3 resulted from posttranscriptional addition by PAP I, we isolated RNA from MG1693 (wild type), SK7988 (Δ*pcnB*), SK5704 (*pnp-7 rnb-500 rne-1*), and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*), labeled it with [^{32}P]pCp and T4 RNA ligase, and digested it with RNases A and T_1. Since the *rnb-500* and *rne-1* alleles encode thermolabile enzymes, we took two samples of each strain, one at 30°C and one 10 min after a shift to 44°C, to inactivate RNase II and RNase E. Poly(A) tails from between 10 and 50 nt were present in the wild-type strain (Fig. 4, lanes 2 and 3). Without PAP I (SK7988) only a few labeled species appeared (Fig. 4, lanes 4 and 5). In comparison, removing PNPase and reducing the level of RNase II in SK5704 led to a ≥10-fold increase in the number of poly(A) tails (Fig. 4, lane 6). Twenty micrograms of RNA was loaded in lane 2 compared with 3 μg in lane 6. When RNase II and RNase E were inactivated by shifting to 44°C, polyadenylylation was further enhanced (Fig. 4, lane 7). Discrete bands of up to 100 nt long could be seen. More importantly, without PAP I most of the species were absent (Fig. 4, lanes 8–11). Several distinct oligonucleotides seen in SK7988 (Fig. 4, lanes 4 and 5) also appeared in SK8901 (lanes 8–11). Although the decrease in polyadenylylation seen here is greater than that observed by Kalapos *et al.* (17), their assay did not measure poly(A) tail length but simple incorporation of [^{32}P]AMP into acid-insoluble material.

DISCUSSION

Our results confirmed our hypothesis that in *E. coli* mRNAs are stabler in the absence of polyadenylylation (Fig. 1). Fig. 5 describes a model that accounts for how poly(A) tails can initiate mRNA decay.

In the absence of PAP I, mRNAs are less susceptible to nucleolytic decay. Poly(A) tails, then, must serve as the targets for binding by RNA decay complexes, which can mediate phosphodiester bond cleavages either endo- or exonucleolytically (Fig. 5). The poly(A) tails are probably better substrates for 3′ → 5′ exonucleases, such as PNPase, than the stem–loop structures normally found at the end of many transcripts. When PNPase binds to the 3′ poly(A) tail it also brings RNase E, an endonuclease, since these two enzymes apparently exist as a complex within the cell (18).

Once RNase E is bound to a potential substrate by its association with PNPase, it could, at a distance, generate a discrete decay product, leaving the 5′ end intact. The 3′ ends of the *trxA* (2) and *cat* (19) mRNAs seem to be shortened in just this way. As an added benefit, such a mechanism would also remove 3′-terminal stem–loops associated with REP sequences or Rho-independent terminators. Each endonucleolytic cleavage would provide a new 3′ terminus for polyadenylylation and would recycle the PNPase/RNase E complex.

Inactivating both RNase E and PNPase increases mRNA stability (Fig. 1 and ref. 1). The absence of PAP I (Fig. 1) further stabilizes mRNAs. The existence of a second multiprotein complex containing an endonuclease different from RNase E (Fig. 5) could explain these facts. Such a complex would contain an additional 3′ → 5′ exonuclease or a poly(A)-binding protein.

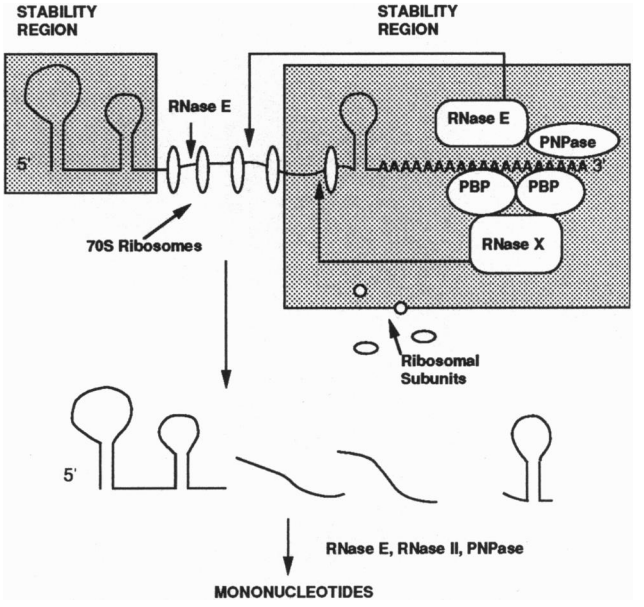

FIG. 5. Model for mRNA decay in *E. coli*. The boxed shaded areas represent stability regions that can affect mRNA half-life. The poly(A)-binding proteins (PBP) and RNase X, shown bound to the poly(A) tail, are hypothesized. RNase X could bind directly to the poly(A) tails. As illustrated, both RNase E and RNase X can act at a distance to introduce endonucleolytic cleavages into the mRNA. RNase E may also act independently of its association with PNPase. Breakdown products are converted to mononucleotides primarily by the action of PNPase and RNase II. The model is not drawn to scale.

The fate of the *ompA* mRNA (Fig. 1 *E* and *F*) supports this hypothesis. An unusual secondary structure at the 5′ end of the *ompA* message contributes to the normal half-life of the mRNA (20). Although inactivation of RNase E, PNPase, and RNase II barely changed the *ompA* message half-life (MG1693, 14 min; SK5704, 16 min), without PAP I a 2.3-fold increase occurred (SK5704, 16 min; SK8901, 37 min) and the mRNA decay pattern changed significantly (Fig. 1*F*). By anchoring an alternative RNA decay complex, the poly(A) tail would contribute to the overall decay even when the 5′ stabilizing element was still intact.

RNA decay is not completely blocked even when there is no RNase E and PAP I, so there must be additional, less efficient ways, to at least partially degrade mRNAs. For example, in SK8901 (Δ*pcnb pnp-7 rnb-500 rne-1*) the full-length *lpp* transcript was slowly converted to a very stable, slightly smaller polynucleotide that did not appear to decay further (Fig. 1*B*). In contrast, with some mRNAs, such as *trxA* (Fig. 1*D*), the alternative decay process was more effective. Thus (Fig. 5), certain endonucleases must have access to mRNAs whether or not an exonuclease is attached to the 3′ poly(A) tail.

In *E. coli* the presence of poly(A) tails destabilized the messages we tested. It may be, however, that polyadenylylation can also stabilize certain mRNAs in *E. coli*. That significant polyadenylylation remained 120 min after cessation of new transcription (Fig. 3) suggests that some RNA species are in fact very stable. Since the Northern data suggest that the actual mRNA species remaining after 120 min are relatively small (Fig. 1), the larger species (Fig. 3*A*) could arise from polyadenylylation of structural RNAs such as 16S and 23S ribosomal RNAs. Xu *et al.* (21) have already shown that RNA I, a structural RNA involved in plasmid ColE1 replication, is stabilized in the absence of polyadenylylation.

In contrast to *Saccharomyces cerevesiae*, where most poly(A) tails are 70–90 nt long (14), in *E. coli* the average tail length in wild-type cells is probably 15–40 nt, a number that agrees with earlier work (22). There were polyadenylate sequences of

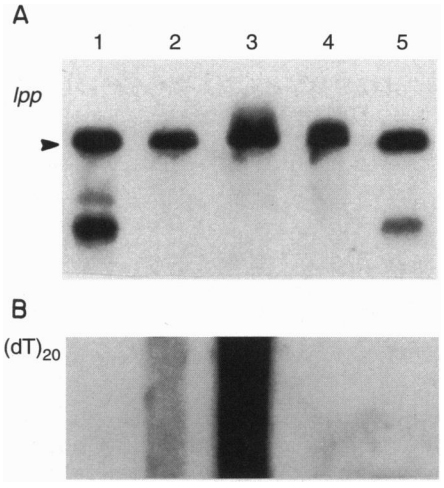

FIG. 6. Northern analysis of *lpp* mRNA before and after RNase H treatment. SK5704 (*pnp-7 rnb-500 rne-1*) and SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*) RNA samples from Fig. 1 were electrophoresed through a 5% polyacrylamide gel containing 7 M urea and electroblotted to a nylon membrane. The membrane was hybridized with ^{32}P-labeled $(dT)_{20}$ (*B*). Following stripping, the membrane was hybridized with ^{32}P-labeled *lpp* DNA (*A*). The same region of the blot is shown in *A* and *B*. Different amounts of RNA were loaded in each lane to equalize the levels of full-length *lpp* mRNA, indicated by the arrowhead. Lanes: 1, 1 μg of SK8901 30-min RNA; 2, 0.5 μg of SK5704 0-min RNA; 3, 6 μg of SK5704 30-min RNA; 4, 6 μg of SK5704 30-min RNA treated with RNase H and $(dT)_{20}$; 5, 0.5 μg of SK8901 0-min RNA.

>50 nt, but not many. Moreover, that the number of poly(A) tails increased 10- to 20-fold when there was reduced RNase II activity and no PNPase showed that these two 3′ → 5′ exonucleases were the major degradative enzymes for the poly(A) tails. In fact, PNPase apparently was the primary enzyme involved (J.A.C. and S.R.K., unpublished work). These enzymes may thus function as the prokaryotic equivalent of the poly(A) nucleases observed in eukaryotic cells (23–25). Whether their role is to limit the number or the length of the poly(A) tails is unclear.

One prediction of the model described in Fig. 5 is that mRNA species isolated from SK5704 (*pnp-7 rnb-500 rne-1*) should be 10–100 nt longer than those isolated from SK8901 (Δ*pcnB pnp-7 rnb-500 rne-1*). Cao and Sarkar (26) have shown that in MG1693 only 1.3% of total pulse-labeled RNA is polyadenylylated. The level of polyadenylylation increases to 6.2% in a *pnp-7 rnb-500* double mutant. From these numbers, it would seem that at any given time only a small percentage of a specific mRNA is polyadenylylated. Thus the apparent lack of a change in size, particularly in Fig. 1*B*, is not surprising. However, when *lpp* mRNA was isolated from either SK5704 or SK8901 and examined under slightly different electrophoretic conditions, the presence of larger species was detected in the *pcnB*$^+$ strain (Fig. 6*A*, lane 3). Upon treatment with oligo(dT) and RNase H, the *lpp* transcript from SK5704 had a migration pattern similar to that isolated from SK8901 (Fig. 6*A*, lane 4). Similar results were also observed with *trxA* (data not shown).

Evidence for polyadenylylation in *E. coli* has been available for many years. As early as 1962, August *et al.* (27) identified a PAP activity in *E. coli*. Since 1975 many reports have indicated that *E. coli* mRNAs contain poly(A) tails (22, 26, 28–33). While this work was generally ignored, and it was assumed that polyadenylylation was unique to eukaryotes, the identification of the structural gene for PAP I (*pcnB*) (5) has now made it possible to firmly establish polyadenylylation as an important biological process in prokaryotes.

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