Polyadenylation Promotes Degradation of 3′-Structured RNA by the Escherichia coli Degradosome in Vitro*

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Polyadenylation contributes to the destabilization of bacterial mRNA. We have investigated the role of polyadenylation in the degradation of RNA by the purified Escherichia coli degradosome in vitro. RNA molecules with 3′-ends incorporated into a stable stem-loop structure could not readily be degraded by purified polynucleotide phosphorylase or by the degradosome, even though the degradosome contains active RhlB helicase which normally facilitates degradation of structured RNA. The exoribonucleolytic activity of the degradosome was due to polynucleotide phosphorylase, rather than the recently reported exonucleolytic activity exhibited by a purified fragment of RNase E (Huang, H., Liao, J., and Cohen, S. N. (1998) Nature 391, 99–102). Addition of a 3′-poly(A) tail stimulated degradation by the degradosome. As few as 5 adenosine residues were sufficient to achieve this stimulation, and generic sequences were equally effective. The data show that the degradosome requires a single-stranded “toehold” 3′ to a secondary structure to recognize and degrade the RNA molecule efficiently; polyadenylation can provide this single-stranded 3′-end. Significantly, oligo(G) and oligo(U) tails were unable to stimulate degradation; for oligo(G), at least, this is probably due to the formation of a G quartet structure which makes the 3′-end inaccessible. The inaccessibility of 3′-oligo(U) sequences is likely to have a role in stabilization of RNA molecules generated by Rho-independent terminators.

Polyadenylation of mRNA contributes to its stability and maturation and to the initiation of translation in eukaryotic cells (2–4). In prokaryotes, poly(A) polymerase activity was first identified over 3 decades ago (for a review of polyadenylation in bacteria, see Ref. 5). Nevertheless, only recently was polyadenylation considered to have a role in determining mRNA stability in bacteria. Two poly(A) polymerases have been cloned and characterized from Escherichia coli (6, 7), and several mRNA molecules have been shown to possess poly(A) tails (6, 8–12). Post-transcriptional addition of a poly(A) tail at the 3′-end of mRNA has been shown to destabilize certain RNA molecules (13, 14). Furthermore, disruption of the penB gene, encoding poly(A) polymerase I, results in increased stability of RNA I (12, 15) as well as lpp, ompA, rpsO, and trxA mRNAs in a pnp rnb rme background (9, 11). This is in contrast to the situation in eukaryotes, where polyadenylation stabilizes RNA.

In E. coli, degradation of mRNA is mediated by the concerted action of endo- and exoribonucleases. A large multienzyme complex (the degradosome) includes two of the most important ribonucleases, the endoribonuclease RNase E and the 3′ → 5′ exoribonuclease PNPase† (16–19), together with the DEAD box helicase RhlB, enolase, and a non-stoichiometric amount of polyphosphate kinase (19, 20). The roles of enolase and polyphosphate kinase in mRNA degradation are not yet clear. However, RhlB has a central role in the degradation of mRNAs with stable stem-loop structures (19). Purified PNPase is impeded by stem-loop structures in RNA, whereas PNPase in the degradosome can degrade structured RNA: in the degradosome, the RhlB helicase unwinds such structures in an ATP-dependent fashion to permit the passage of PNPase. Thus, the degradosome is generally efficient at degrading structured RNA. However, in this study, we show that the degradosome is inefficient in degrading RNA molecules with their 3′-ends incorporated into a stem-loop structure. Addition of a single-stranded 3′-poly(A) tail facilitated degradation, providing a “toehold” permitting the degradosome to initiate its attack on the 3′-end.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Growth Conditions, Plasmids, and Enzymes—The genotypes of the E. coli strains used in this study were as follows: BL21 (F−ompT rpsL(−156g) HB101 (F−Δgpt-proA62 leuB6 supE44 ara-14 galK2 lacY1 Δ(mcrC-mrr) rpsL20 (Stry+)-5tl-1 recA13). Cells were grown in LB medium with antibiotics as appropriate (21). Plasmids pCH77 (22), pPET-20b (Novagen), and pBluescript II KS(+) (Stratagene) have been described. DNA manipulations were performed as described (21), and enzymes were used as recommended by the manufacturers. Purified E. coli poly(A) polymerase was purchased from Cambio (Cambridge, United Kingdom). The degradosome was purified following the protocol described by Carposius et al. (16) as modified by Py et al. (19). Oligonucleotides and DNA Fragments for in Vitro Transcription—For in vitro RNA degradation assays, RNA was transcribed from DNA templates generated by the polymerase chain reaction (PCR). DNA fragments from the intergenic region of the malE-malF operon of E. coli were amplified using pCH77 as template (22) with the same 5′-primer A (5′-AACATTTAATAGCAACACTATAGG-3′) and each of the three alternative 3′-primers: 3′-primer i, 5′-TCTCGGATGCGACGAGCTGAGC-3′; 3′-primer ii, 5′-CTCGGATGCGACGAGCTGAGC-3′; and 3′-primer iii, 5′-CTCGGATGCGACGAGCTGAGC-3′. The PCR fragments generated (320, 355, and 355 bp, respectively) contained a 77 promoter at the 5′-end and could therefore be used directly for in vitro transcription (see below) to synthesize RNA molecules of 297, 312, and 312 nucleotides, respectively. All these RNAs also contained the A → C change indicated in Fig. 1. The 3′ termini of these

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‡ The abbreviations used are: PNPase, polynucleotide phosphorylase; PCR, polymerase chain reaction; bp, base pair(s).
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RNAs ended precisely at the 3'-base of the malE-malF stem-loop structure ("stem") (3'-primer i) with a 3'-A<sub>m</sub> tail ("stemA<sub>m</sub>"), 3'-primer ii), or with 15 nucleotides of the original malE-malF sequence downstream of the stem-loop ("stemN<sub>15</sub>"). To transcribe RNA molecules with 5' or 10 adenosine residues or 15 guanosine or 15 uridine residues downstream of the stem-loop, derivatives of 3'-primer ii were used in which the T<sub>A</sub> sequence (boldface) was exchanged for the appropriate C or A nucleotides. Two DNA fragments in which the malE-malF intergenic region was shortened at the 5'-end by 86 nucleotides, compared with the fragments described above, were amplified using 5'-primer B (5'-AAATAATTAC-GAGCCATTCGCTCGACGTCAG-3') together with each of 3'-primers ii and 3'-iii to generate 3'-A<sub>m</sub> or 3'-N<sub>15</sub> tails downstream of the stem-loop, respectively. These PCR products (250 bp) also contained a T7 promoter at the 5'-end such that in vitro transcription generated RNA molecules of 228 bp (named "stemA<sub>m</sub>" and "stemN<sub>15</sub>").

A DNA fragment in which the stem-loop structure of the malE-malF region was replaced by the terminator stem-loop of the lpp gene (for the mRNA sequence, see Ref. 22) was amplified using 5'-primer A (see above) and the 3'-primer 5'-AAAAAAATGGCCACAATGTCGGC-CATTTTTCTTCTACAGCATTACCTTGGTAT-3', also containing a T7 promoter at the 5'-end such that in vitro transcription generated RNA molecules of 228 bp (named "lpp-stem").

**In Vivo Transcription—Radiolabeled RNA was synthesized in vitro from linearized, gel-purified DNA templates using bacteriophage T7 RNA polymerase (Pharmacia) and [α-<sup>32</sup>P]CTP (400 Ci/mmol; Amersham) as described previously (22).** The reaction products were diluted to 70 μl in water, extracted with phenol/chloroform, and eluted through a phenol column (Stratagene) to separate unincorporated nucleotides. The RNA was precipitated with ethanol and resuspended in water (MilliQ). The RNA products stemA<sub>m</sub>, stemU<sub>m</sub>, and stemN<sub>15</sub> migrated as broad bands (especially stemA<sub>m</sub>, in Fig. 8, band e), probably because runoff transcription does not stop precisely at the base of the DNA template due to the weak deoxyribo(T) polarity because runoff transcription does not stop precisely at the end of any in vitro transcription gave a 243-nucleotide RNA molecule (named "lpp-stem").

**Polyadenylation of mRNA—In vitro transcription, radiolabeled RNA was incubated with E. coli poly(A) polymerase and 5 mM ATP in the buffer recommended by the manufacturer (Cambio) for 20 min at 37 °C.** The reaction mixture was extracted with phenol/chloroform and further treated as described above (see "In Vitro Transcription").

**In Vitro RNA Degradation Assays—In vitro RNA degradation assays were performed as described (19). The reaction (100 μl) contained exonuclease assay buffer (20 mM Tris-HCl (pH 7.5), 1 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, and 10 mM K<sub>2</sub>HPO<sub>4</sub>), 1 mM ATP, and 80 units of RNasin (Promega) and was incubated with enzyme at 37 °C. Unless otherwise stated, 0.18 μg of His-tagged PNase or 0.66 μg of the degradosome was added to each reaction. Aliquots were taken at appropriate time points, loaded onto a 4 or 6% sequencing gel, and electrophoresed at 60 watts. The gel was dried on Whatman No. 3MM paper, and radioactivity in each band was quantitated with a PhosphorImager (Fig. 2).

**RESULTS**

**Effects of 3' Adenylation on the Stability of Structured RNA in Vitro**—To examine the influence of polyadenylation on RNA degradation in vitro, various polyadenylated and non-polyadenylated mRNAs were incubated with the degradosome in the presence or absence of 1 mM ATP. As the primary substrate a segment of malE-malF mRNA (19, 20) was used (named stem). The 3'-end of this RNA corresponded to the last nucleotide of the stable stem-loop structure of the malE-malF intergenic region (Fig. 1A), such that the 3'-end was fully incorporated into the secondary structure. Derivatives of this mRNA were generated with an additional 15 adenosine residues (stemA<sub>15</sub>) or a poly(A) tail ("stem-poly(A)" containing 200–300 adenosine residues), providing a single-stranded 3'-tail.

**Cloning of the Gene Encoding Polyadenylate Phosphatase—**The gene encoding PNase was amplified by PCR from E. coli HB101 genomic DNA using Vent polymerase (New England Biolabs Inc.). Oligonucleotide primers 5'-ATATTAATACGACTCACTATAGGGCCCGCAGATGTCCGCTTTC-3' and 5'-CCCCCAAGCTTCTCGCCCTGTCGACGTCAC-G3' were based on the lpp sequence (GenBank™ accession number U18996) and amplified the ppq gene flanked by Ndel and HindIII restriction sites (boldface) to facilitate subsequent cloning. After amplification, gel purification, and cleavage with HindIII, the 2254-bp PCR product was cloned into pBluescript II KS(+) digested with SmaI and HindIII. The resultant plasmid, pPNPHIS, contained the ppq gene under the control of the T7 promoter, allowing inducible overexpression of the PNase derivative with a C-terminal hexahistidine tag in E. coli strain BL21.

**Purification of His-tagged PNase—**His-tagged PNase was purified by metal-chelate affinity chromatography (24) as described previously for His-tagged polyphosphate kinase (20) with the following modifications. Buffer A for resuspending the cell pellet and for washing steps consisted of 0.1 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (pH 7.8), 10% glycerol, 1% Tween, 1 mM NaCl, and 5 mM imidazole. The protein was eluted in steps using 5 ml of buffer B (0.1 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (pH 7.8), 10% glycerol, and 1% Tween) containing 30, 50, 80, 120, and 500 mM imidazole, respectively. His-tagged PNase was purified to near homogeneity as judged by Coomassie Blue staining (see Fig. 4A). The fractions containing the protein peak were pooled and stored in 50% glycerol (final concentration) at −20 °C. Protein concentrations were determined as described (25) with bovine serum albumin as standard.

**FIG. 1.** RNA secondary structures. A, sequence of the malE-malF stem-loop structure. In all experiments described here, a derivative was used with an A → C change, as indicated, to correct the mismatch at the base of the stem and to ensure that no single-stranded "tail" is exposed. B, terminator of the lpp mRNA (23).

In all reactions, the oligo(A) tail (when present) was rapidly removed to generate a stable intermediate with its 3'-end close to the base of the stem-loop structure (Fig. 2 (22). The curves are biphasic (Fig. 2B) because, for a small proportion of the
RNA starting material, it appears the degradosome falls off the RNA at the base of the stem-loop (having removed the poly(A) tail), and this species is then unable to be degraded further (see below). In the absence of ATP this intermediate RNA species was only poorly degraded by the degradosome, irrespective of the starting RNA from which it was generated, because ATP is required for the RhlB helicase to unwind the secondary structure (19). In the presence of ATP, however, this intermediate was rapidly degraded, but only for the RNA molecules that initially had a single-stranded A15 or poly(A) tail at their 3'-ends (stemA15 or stem-poly(A); △, ▲) downstream of the stem-loop structure were incubated with the degradosome in the absence (open symbols) or presence (closed symbols) of ATP.

**Effect of Alternative Tails on the Degradation of Structured RNA**—Since addition of a 3'-poly(A) tail stimulated degradation of the stem-loop structure by the degradosome, we assessed whether there was any specificity for the composition of the 3'-tail. The rate of degradation of RNAs with a 3'-tail of 15 guanosines (“stemG15”) or 15 uridines (stemU15) was compared with that of the stem, stemA15, and stemN15 substrates. The stemN15 substrate incorporated a 3'-tail consisting of the 15 nucleotides normally 3'-of the stem-loop in the malE-malF mRNA. Assays were in the presence of 1 mM ATP. The stemN15 RNA was degraded as rapidly as the stemA15 RNA (Fig. 5). In contrast, the stemG15 and stemU15 RNAs were degraded slowly, at a rate similar to that of the stem RNA. Furthermore, and in contrast to the stemA15, stemN15, and stemU15 RNA substrates, the oligo(G) tail of the stemG15 RNA was removed poorly by the degradosome (Fig. 6). Incubation of RNA substrates with purified His-tagged PNPase showed that the purified enzyme was also unable to degrade the oligo(G) tail (Fig. 6), demonstrating that the oligo(G) tail is not accessible to either enzyme.

**Degradation of Native malE-malF RNA Intermediates in Vitro**—In vivo, transcription of the malE-malF mRNA terminates several hundred base pairs downstream of the large intergenic stem-loop. A stable RNA molecule with the stem-loop near its 3'-end is then generated by processive exoribonucleolytic degradation from the 3'-end (22, 26). To mimic the in vivo situation in vitro, stemN15 RNA was incubated with His-tagged PNPase for 15 min; the reaction was stopped; and the resulting RNA was purified. Fig. 7B shows that all the initial
substrate was converted to the stable intermediate, designated "stem(N15)*." Aliquots of stem(N15)* RNA were then polyadenylated and used as substrate for the degradosome in the presence of ATP. Although the residual 3′-tail on stem(N15)*, compared with stem RNA, permitted slightly faster degradation of the stem-loop structure, the degradosome degraded the polyadenylated RNA significantly faster than the non-polyadenylated precursor (Fig. 7, A and C). Similar results were obtained when the stem(N15)* RNA was generated using the degradosome (in the absence of ATP) rather than His-tagged PNPase (data not shown). Thus, polyadenylation enhances the rate of degradation of intermediates equivalent to those generated in vivo.

FIG. 3. Rates of degradation of malE-malF RNA with different length adenylated tails 3′ to the stem-loop structure. A, example autoradiograph showing a time course of RNA degradation assayed in the presence of ATP. B, graph showing the rates of RNA degradation, derived by phosphoimaging of gels similar to the gel in A. As the "fuzzy" 0-min band was difficult to accurately quantitate, the 1-min part was designated the 100% value. The RNA substrates used have no 3′-tail (stem (●)) or have 3′-A tails of different lengths (stemA5 (●), stemA10 (■), stemA15 (▲), and stem-poly(A) (▲)).

FIG. 4. Purified C-terminally His-tagged PNPase is active. A, Coomassie blue-stained protein gel loaded with 1.2 μg of purified His-tagged PNPase (Pnp-His6; arrow). B and C, autoradiographs of 6% gels of RNA degradation assays as a function of time (in minutes). B, 8 ng of purified His-tagged PNPase was incubated with malE-malF mRNA (19) to demonstrate that the enzyme is active. Because this study was designed to show that the His-tagged PNPase was active, only a small amount of enzyme was used. Under these conditions, the enzyme paused at small stem-loop structures (as does native PNPase) (22) and finally stalled at the large stem-loop, generating a stable degradation intermediate (arrow). C, His-tagged PNPase did not degrade the stem RNA (left panel). Only the A tail of the stemA15 RNA substrate (right panel) was degraded by His-tagged PNPase, again generating a stable intermediate (arrow). The intermediate migrated slightly slower than the stem RNA, indicating that PNPase does not remove the entire 3′-tail, but leaves a few nucleotides (nt) of the base of the stem-loop structure (as shown previously (22)).
them from the substrate RNA following gel electrophoresis. These shorter RNAs (stemA15 and stemN15) ended with an A15 or N15 tail 3′ to the stem-loop structures, respectively. In this assay, the concentration of enzyme was reduced to amounts equimolar to the substrate RNA. The competitor RNAs were also added in equimolar amounts. The rate of degradation of the N15 tail of stemN15 in the presence of either competitor (band a going to band b) calculated from the data shown in Fig. 8 give the time, in which 50% of the full-length stemN15 substrate is shortened to the base of the stem-loop structure (td50), as 0.5 min for the stemN15 substrate alone. Addition of either of the competing substrates (stemN15 or stemA15) resulted in a similar increase in td50 to about 2 min. The removal of the 3′-tails from stemN15 and stemA15 can also be seen to occur at a similar rate (generated band d and f, respectively). These data demonstrate that, as long as the 3′-tail is single-stranded and accessible to the enzyme (unlike the G15 tail; see above), it does not matter whether the 3′-tail is poly(A) or is a generic sequence.

Degradation of RNA Containing the lpp Terminator Stem-loop—To demonstrate the general relevance of polyadenylation, the degradation of a primary transcript ending in a native terminator stem-loop was characterized. The lpp terminator (Fig. 1B) was selected because the lpp mRNA is relatively stable and polyadenylation has been reported in vivo (6, 27). The native lpp terminator was used to replace the large stem-loop of the malE-malF RNA, and the resulting mRNA (lpp-stem) and its polyadenylated derivative were used as substrates for the degradosome and for His-tagged PNPase. Equimolar amounts of PNPase were used, whether His-tagged or in the degradosome (0.18 mg of purified His-tagged PNPase is equivalent to 0.66 mg of degradosome containing 28% PNPase (19)). In contrast to the malE-malF stem-loop, ATP was not required for degradation of the lpp stem-loop by the degradosome, presumably because the lower thermodynamic stability of the stem-loop is such that the RhlB helicase is not essential for unwinding (Fig. 9). Therefore, addition of ATP promoted the degradation only slightly. Nevertheless, the presence of a 3′-poly(A) tail still markedly stimulated degradation of the RNA (whether or not ATP was present) both by the degradosome and by purified PNPase (Fig. 9).

DISCUSSION

Polyadenylation of bacterial mRNA is increasingly recognized as a potentially important destabilizing signal for the RNA degradation machinery. RNA molecules or fragments generated by endoribonuclease cleavage are degraded by one of
the 3' → 5' exoribonucleases: PNPase or RNase II. These processive exoribonucleases are stalled by stem-loop structures; temporarily at smaller stem-loop structures, but more stable stem-loops (e.g., the malE-malF intergenic stem-loop structure) (19, 22) or complex secondary structures (3'9-end of the S20 mRNA) (14) act as a very effective block to these enzymes. In the cell, however, a proportion of PNPase is part of a multiprotein complex, the degradosome (16–19). The ATP-dependent helicase activity of RhlB in the degradosome strongly promotes the degradation of structured RNA by unwinding sequences and offering them as single-stranded substrates to PNPase (19). Thus, the degradosome appears to be fully equipped to degrade highly structured RNA molecules (19). Since polyadenylation is important for destabilizing structured RNA molecules (9, 11, 13), we set out to address the apparent paradox of how polyadenylation might stimulate the activity of the degradosome.

The malE-malF intergenic region forms a very stable stem-loop structure, and its degradation does not require RNase E cleavage (19).² The degradosome and purified PNPase are stalled by the stem-loop structure to give a very stable intermediate in vivo and in vitro (19, 22, 26–29). A derivative of the malE-malF message with its 3' terminus incorporated into the stem-loop structure was degraded very poorly by the degradosome in vitro, even when RhlB was active. In contrast, 3'-adenylated derivatives of the RNA were degraded rapidly. This demonstrates that the ability of the degradosome can be limited by the absence of a single-stranded 3'-RNA end. Addition of an oligo(A) tail efficiently destabilized the message in vitro, even when as few as 5 nucleotides were added. Fewer than 5 adenosine residues did not seem to be recognized (data not shown). Although it has been suggested that as few as 2 adenosine residues might target RNA for degradation (12), these studies were carried out in vivo, and nucleases other than the degradosome might be involved. Similarly, Coburn and Mackie (14) have indicated that RNA with 6–10 unpaired 3' residues is stable; these were not adenosines, and the experiments were carried out with purified nucleases, not the degradosome. Although the model malE-malF transcript studied initially is not normally generated in vivo, we also showed that polyadenylation stimulated degradation of a malE-malF intermediate equivalent to that generated in vivo and of the lpp 3'-stem-loop generated in vivo by transcription termination. Thus, polyadenylation appears to stimulate degradation by the degradosome of any structured RNA generated as a termination product or degradation intermediate where the 3' end has a limited single-stranded tail. Since an N₁₅ generic single-stranded sequence of the stem-loop was as effective as the A₁₅ sequence and since A₁₅ and N₁₅ sequences competed equally well for

²G. S. C. Dance and C. F. Higgins, unpublished data.
degradation, the destabilizing effect appears to be due to the single-stranded nature of the 3'-end rather than to a high affinity for poly(A) per se.

Interestingly, a 3'-oligo(G) tail did not stimulate degradation of RNA in vitro (Fig. 5). Furthermore, neither the degradosome nor purified PNPase could even shorten the G15 tail, suggesting that the sequence is inaccessible to these enzymes. G-rich sequences are able to form intramolecular or intermolecular G quartets (30, 31), which are very resistant to nucleases (32) and which presumably render the 3'-end inaccessible to enzymatic attack.

A 3'-U15 tail also failed to destabilize the RNA. There is no known structure an oligo(U) tail might adopt, although in contrast to the G15 tail, the U15 tail was shortened by the degradosome and by PNPase in vitro (Fig. 6), showing that it remains accessible. More important, every RNA generated from a gene with a Rho-independent terminator ends in a 3'-oligo(U) tail. Thus, apart from the terminator stem-loop itself, this oligo(U) sequence of the terminator may contribute significantly to the stability of such mRNAs, protecting against 3'→5' exoribonucleolytic attack.

Recently, a purified N-terminal fragment of RNase E was reported to remove 3'-poly(A) and 3'-poly(U) tails (1). However, several lines of evidence argue against the possibility that RNase E, rather than PNPase, is responsible for the exoribonucleolytic activities of the degradosome observed here. First, the exoribonucleolytic activity of the degradosome containing a temperature-sensitive RNase E is still efficient at the nonpermissive temperature (19). Since both the endoribonucleolytic and poly(A) tail-shortening activities of RNase E are in the same N-terminal region of the polypeptide (1), the poly(A) shortening should also be inactive at the nonpermissive temperature. Second, purified PNPase gave the same pattern of 3'-poly(A) tail shortening as the degradosome. Third, characterization of the released nucleotides by thin-layer chromatography allowed us to distinguish which enzyme is involved. Thus, the reported exoribonucleolytic activity reported for a fragment of RNase E is unlikely to be significant in RNA degradation by the degradosome.

In conclusion, we have shown that polyadenylation can play an important role in the degradation of mRNA molecules that have 3'-terminal secondary structures, providing a single-stranded toehold recognized by the degradosome. 3'-Structured RNAs are normally generated by Rho-independent termination, by endonuclease cleavage, or by stalling of 3'→5' exonucleases at an internal secondary structure. Poly(A) polymerase can potentially pin a label “to be degraded” to structured

\[ \text{Fig. 8. Relative affinities of PNPase for 3'-N}_{15} \text{ or 3'-A}_{15} \text{ tails. An autoradiograph showing the processing of RNA with a 3'-N}_{15} \text{ tail (stemN}_{15} \text{) by His-tagged PNPase (100 fmol). Upper panel, 100 fmol of stemN}_{15} \text{ (band a) as the only substrate was processed to a stable intermediate (band b). Middle and lower panels, 100 fmol of 5'-truncated malE-malF RNAs were added as competitors to the stemN}_{15} \text{ RNA. Bands c and e indicate the full-length stemN}_{15} \text{ and stemA}_{15} \text{ RNAs, respectively, and bands d and f indicate the stable intermediates generated.} \]

\[ \text{Fig. 9. Degradation of RNA with the 3'-lpp terminator stem-loop and its 3'-polyadenylated derivative. A, autoradiograph showing RNA degradation as a function of time. The polyadenylated and degradation intermediate RNAs are indicated as schematic symbols. B, graphs showing the rates of RNA degradation derived from the autoradiograph in A. Left panel, the substrate RNA containing the lpp terminator at the 3'-end (lpp-stem; □, ○) and its polyadenylated derivative (lpp-stem-poly(A); ▦, □) were incubated with the degradosome in the presence (closed symbols) or absence (open symbols) of ATP. Right panel, the substrate RNAs were incubated with His-tagged PNPase (lpp-stem ( Vinci ) and lpp-stem-poly(A) ( △)).} \]

3 C. Burns and C. F. Higgins, unpublished data.
RNA generated by any of these processes that is otherwise inaccessible to ribonucleases. Thus, “initiation of degradation” of structured RNA by polyadenylation may play an important role not only in the degradation of functional RNA molecules with inaccessible (structured) 3’-ends, but also in recycling the small structured RNA degradation intermediates generated during cellular RNA turnover.

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