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Surprises at the 3′ End of Prokaryotic RNA

Minireview

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The addition of multiple adenosine residues to the 3′ end of eukaryotic cell transcripts has a key role in generating functional mRNA and in regulating the decay of this RNA (for recent reviews, see Sachs and Wahle, 1993; Baker, 1993). While adenylation of RNA also has been observed in prokaryotic cells, until recently there has been no understanding of its biological role. Now, however, the results of recent investigations indicate that adenylation at the 3′ terminus of a variety of bacterial transcripts has major effects on transcript stability. As in eukaryotes, the events set in motion by the adenylation of bacterial RNA can influence decay at both ends of transcripts.

The isolation of a poly(A) polymerase from Escherichia coli (August et al., 1962) preceded by almost a decade the discovery that mRNA in eukaryotic cells contains poly(A) tails. However, the lack of success of early efforts to detect adenylated RNA in bacteria and the obligate association of adenylation in eukaryotes with the 3′ end processing of RNA polymerase II transcripts (reviewed in Sachs and Wahle, 1993; Baker, 1993, and references therein) have led to the view that poly(A) tails are a special feature of eukaryotic mRNA. Remarkably, the notion that polyadenylation of RNA does not occur to any meaningful extent in prokaryotes has persisted despite multiple reports over a 20 year period of the existence of adenylated RNA in E. coli and other bacterial species (e.g., Nakazato et al., 1975; Srinivasan et al., 1975; Cao and Sarkar, 1992a, and references therein). Consequently, little attention was given to identifying a function for RNA polyadenylation in bacteria.

In 1992, it was reported that the E. coli *pcnB* (plasmid copy number) gene, which had been identified initially by the ability of *pcnB* mutations to decrease greatly the copy number of ColE1-type plasmids (Lopilato et al., 1986), encodes a poly(A) polymerase (Cao and Sarkar, 1992b). Concurrently, studies of the mechanism responsible for the effects of *pcnB* mutations on plasmid DNA replication revealed that E. coli cells mutated in *pcnB* show a dramatic decrease in the rate of degradation of RNA I, the antisense repressor of replication of ColE1-type plasmids (Xu et al., 1993; He et al., 1993). Furthermore, RNA I isolated from $pcnB^+$ cells, but not from *pcnB* mutants, was found to be adenylated at its native 3′ terminus, and also at internally located sites exposed by partial degradation of the transcript (Xu et al., 1993).

Decay of RNA I (Figure 1), which has a cloverleaf structure and strongly resembles tRNA, is initiated by the single strand–specific endonuclease, ribonuclease E (RNase E; Tomcsanyi and Apirion, 1985; Lin-Chao and Cohen, 1991), which also initiates the processing of 9S ribosomal RNA (rRNA) and other transcripts in E. coli (for review, see Melefors and von Gabain, 1993). However, while 9S rRNA and RNA I are cleaved by RNase E at approximately the same rate in vitro (Lin-Chao et al., 1994), decay of the resulting cleavage products in vivo is markedly different: the polyadenylated products generated by RNase E cleavage of RNA I show the rapid decay typical of bacterial mRNA (Xu et al., 1993), rather than the extreme stability characteristic of the cleavage products of 9S rRNA and other highly structured bacterial transcripts (King and Schlessenger, 1987). However, in *pcnB* mutants, RNA I lacks poly(A) tails, and its decay intermediates exhibit the slow degradation characteristic of tRNA; polyadenylation thus appears to cause tRNA-like RNA I molecules to undergo mRNA-like decay.

The absence of a functional *pcnB* gene product in E. coli cells can also retard decay of mRNAs encoded by the *lpp* (lipoprotein), *ompA* (outer membrane protein A), *trxA* (thioredoxin), and *rpsO* (ribosomal protein S15) genes (O'Hara et al., 1995; Hajnsdorf et al., 1995). Moreover, as has been observed for RNA I, *pncB*-dependent poly(A) tails have been identified on *rpsO* mRNA (Hajnsdorf et al., 1995) and in total RNA isolated from E. coli (O'Hara et al., 1995). Thus, the addition of multiple adenosine residues to the 3′ ends of RNA transcripts by the *pcnB*-encoded poly(A) polymerase appears to occur broadly in E. coli, leading to accelerated degradation of the polyadenylated RNA.

The enzyme polynucleotide phosphorylase (PNPase), which has been found in most prokaryotic and eukaryotic cells where it has been looked for, can, depending on the experimental conditions used, add or remove nucleotides from the 3′ ends of RNA molecules (for review, see Littauer and Soreq, 1982). The length and number of poly(A) tails on RNA I are increased in E. coli cells mutated in *pnp*, the gene encoding PNPase (Xu and Cohen, 1995). Similarly, *lpp* mRNA (Cao and Sarkar, 1992a) and total RNA (O'Hara et al., 1995) are increased in length in *pnp* mutants defective also in *rnb*, which encodes RNase II, the only other 3′ to 5′ exonuclease known to attack mRNA molecules in E. coli (for review, see Shen and Schlessinger, 1982); these findings suggest that the 3′ adenosine residues added by poly(A) polymerase are removed by 3′ to 5′ exonucleolytic digestion. But how does the posttranscriptional addition of adenosines at the 3′ terminus of RNA promote decay of the upstream primary transcript?

While current undertanding of this process is limited, recent work indicates that polyadenylation may facilitate access to the primary transcript sequence by one or more 3′ to 5′ exonucleases. Whereas polyadenylated RNA I decay intermediates are degraded rapidly in wild-type bacteria, their half-lives increased more than 10-fold in *pnp* mutant cells, implying that the mRNA-like decay properties conferred upon RNA I by posttranscriptional polyadenylation are dependent significantly on PNPase (Xu and Cohen, 1995). Direct support for this notion is provided by evidence that poly(A) tails increase the rate of PNPase digestion of RNA I in vitro, and also increase exposure to

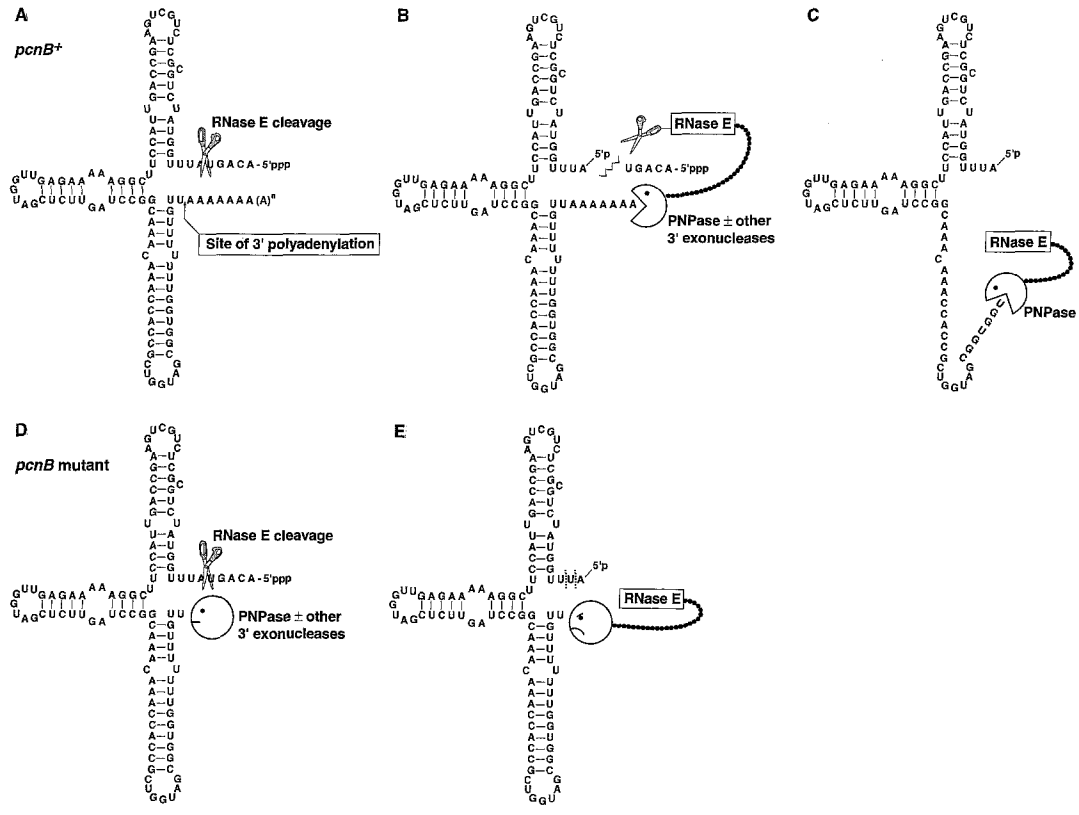

Figure 1. Proposed Model Showing Steps in the In Vivo Decay of RNA I

RNA I is the antisense RNA repressor of replication of ColE1-type plasmids. Ribonuclease E (RNase E) cleaves RNA I endonucleolytically, removing five nucleotides from the 5′ end of the primary transcript (A), and producing a 5′ monophosphate terminus on the cleavage product (B; see Tomcsanyi and Apirion, 1985). In the presence of the *pcnB* gene product, which encodes a poly(A) polymerase (Cao and Sarkar, 1992b), RNA I undergoes 3′ polyadenylation (Xu et al., 1993); the resulting poly(A) tails facilitate exonucleolytic attack at the 3′ end of the RNase E–generated decay intermediate by polynucleotide phosphorylase (PNPase; Xu and Cohen, 1995), which is complexed with RNase E in E. coli cells (Carpousis et al., 1994; Py et al., 1994), and by other 3′ exonucleases. PNPase then proceeds in a highly processive mode (Nossal and Singer, 1968) in the 3′ to 5′ direction through hairpin loop structures that would otherwise impede its digestion of RNA I (C). In *pcnB* mutant cells, RNA I molecules lack poly(A) tails and have dramatically increased stability in vivo (D); however, an additional one or two nucleotides are removed from the RNase E–generated 5′ ends by an undefined mechanism (E; Xu et al., 1993).

digestion; in the presence of poly (A) tails, greater than 80% of substrate sequences are digested by PNPase to acid solubility, whereas only 30% become acid soluble when the 3′ end is not adenylated (Xu and Cohen, 1995). Thus, adenylation appears to convert to PNPase susceptibility a population of molecules that is otherwise resistant to digestion by this enzyme. Interestingly, E. coli tRNA, which does not contain poly(A) tails (Deutscher, 1990), also includes a large population of molecules in a conformation that is resistant to PNPase in vitro (Thang et al., 1971).

Regions of secondary structure are known to impede 3′ to 5′ decay of RNA and specifically to slow the transit of PNPase (for review, see Higgins, 1993, and references therein), and poly(A) tails may provide a "toehold" for PNPase, which is a highly processive enzyme (Nossal and Singer, 1968)—enabling PNPase to begin its phosphorolysis at some distance 3′ to the stem-loop structure formed at the RNA I 3′ end (Figure 1), and consequently to proceed more rapidly through this and other regions of secondary structure. In vivo, digestion of primary transcript sequences by PNPase may be aided by poly(A) binding proteins analogous to one that recruits a poly(A)-dependent nuclease to the polyadenosine tails of mRNA molecules in yeast (Sachs and Deardorff, 1992), or may be impeded further by proteins that interact with the stem loops (e.g., Pepe et al., 1994). However, it still remains to be determined whether the effects of 3′ polyadenylation on digestion by PNPase are controlled by such events or are instead mediated by an alternative mechanism such as alteration of the global (secondary and/or tertiary) conformation of RNA I by poly(A) tails.

The discovery that 3′ adenylation is a major factor in the regulation of RNA decay in bacteria is only one of the recent surprises involving the 3′ ends of RNA molecules of prokaryotes. The absence of *pcnB*-mediated 3′ polyadenylation appears to trigger the removal of one or two nucleotides from the 5′ end of RNA I (Xu et al., 1993). Additionally, whereas polyadenylation itself does not directly affect endonucleolytic cleavage by RNase E near the 5′ end of RNA I, mutation of *pnp* can decrease RNase E cleavage, in addition to reducing the rate of exonucleolytic decay

from the 3′ end (Xu and Cohen, 1995). Conversely, RNase E cleavage near the 5′ end of RNA I promotes 3′ to 5′ decay by PNPase (Xu and Cohen, 1995). These observations suggest that the ribonucleolytic actions of RNase E and PNPase, which recently have been found in the same multiprotein complex in E. coli cells (Carpousis et al., 1994; Py et al., 1994), may be functionally coordinated. The conformation of RNA I, which based on tRNA structural analyses seems likely to bring the two ends of the RNA I molecule into juxtaposition, provides a possible framework for the coordinated action of RNase E and PNPase on this substrate. Proximity of the ends of folded RNA I molecules may also account for the observation that the extent of phosphorylation at the 5′ terminus can affect the rate of PNPase digestion at the 3′ end of RNA I in vitro (Xu and Cohen, 1995).

While RNase II has been implicated in the removal of poly(A) tails from transcripts (Cao and Sarkar, 1992a), this enzyme appears unable to proceed through primary transcript sequences at a sufficent rate to compensate fully for the absence of PNPase: the decay of adenylated RNase E cleavage products of RNA I is slowed to a fraction of its normal rate in cells that are mutated in *pnp* but still contain a functional *rnb* gene. In fact, there is evidence that RNase II may impede 3′ to 5′ exonucleolytic digestion by PNPase by binding to terminal hairpin loop structures (Hajnsdorf et al., 1994; Pepe et al., 1994). Other, still unidentified 3′ to 5′ exonucleases may also be affected by *pcnB*-mediated 3′ adenylation, since adenylated RNA is degraded faster than nonadenylated RNA in *pnp* mutants (Xu and Cohen, 1995) and in *pnp rnb* double mutants (Hajnsdorf et al., 1995; O'Hara et al., 1995).

It is well recognized that 3′ end processing of tRNA is crucial to the attachment of amino acids, and thus to tRNA function in both bacteria and eukarytotes (for reviews, see Deutscher, 1990; Soll, 1993). The work summarized here indicates that events occurring at the 3′ end can affect the biological activity of other RNAs also. While 3′ polyadenylation appears to govern principally 3′ to 5′ exonucleolytic decay, the effects of poly(A) tails on events occurring at the 5′ end of transcripts are reminiscent of interactions between 3′ adenosine residues and upstream sequences reported for eukaryotic mRNA (see Sachs and Deardorff, 1992; Decker and Parker, 1994). Although currently it is not known whether specific sequences trigger the addition of poly(A) tails to prokaryotic transcripts, as they do in eukaryotic cells, the basic mechanisms for the polyadenylation of prokaryotic and eukaryotic transcripts appear to be different: there is no indication that endonucleolytic cleavage of the primary transcript, which creates the substrate for 3′ polyadenylation of eukaryotic mRNA, is required for addition of poly(A) tails to bacterial RNA.

To understand the apparently multifaceted role of processing at the 3′ end of RNA made in prokaryotic cells, it will be important to learn the effects of *pcnB*-mediated mRNA stabilization on the synthesis of protein encoded by the stabilized mRNA, and whether poly(A) tails themselves can influence translation of bacterial mRNA, as occurs in eukaryotes. Moreover, while there presently is no evidence that the 3′ ends of bacterial transcripts can affect RNA transport, as they have been observed to do in eukaryotic cells, the ability of the 3′ exonuclease PNPase to interact with RNase E (Carpousis et al., 1994; Py et al., 1994), together with indications that the RNase E protein may include domains having contractile, structural, and/or transport functions (Casaregola et al., 1992; McDowall et al., 1993), raises the possibility that the 3′ ends of prokaryotic RNA molecules may be the source of still other biological surprises.

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