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Bacterial/archaeal/organellar polyadenylation

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Abstract

Although the first poly(A) polymerase (PAP) was discovered in *Escherichia coli* in 1962, the study of polyadenylation in bacteria was largely ignored for the next 30 years. However, with the identification of the structural gene for *E. coli* PAP I in 1992, it became possible to analyze polyadenylation using both biochemical and genetic approaches. Subsequently, it has been shown that polyadenylation plays a multifunctional role in prokaryotic RNA metabolism. While the bulk of our current understanding of prokaryotic polyadenylation comes from studies on *E. coli*, recent experiments with *Cyanobacteria*, organelles and *Archaea*, although limited, have widened our view on the diversity, complexity, and universality of the polyadenylation process.

For example, the identification of polynucleotide phosphorylase (PNPase), a reversible phosphorolytic enzyme that is highly conserved in bacteria, as an additional PAP in *E. coli* caught everyone by surprise. In fact, PNPase has now been shown to be the source of post-transcriptional RNA modifications in a wide range of cells of prokaryotic origin including those that lack a eubacterial PAP homologue. Accordingly, the past few years have witnessed increased interest in the mechanism and role of post-transcriptional modifications in all species of prokaryotic origin. However, the fact that many of the poly(A) tails are very short and unstable as well as the presence of polynucleotide tails has posed significant technical challenges to the scientific community trying to unravel the mystery of polyadenylation in prokaryotes. This review discusses the current state of knowledge regarding polyadenylation and its functions in bacteria, organelles and *Archaea*.

Keywords

poly(A) polymerase; polynucleotide phosphorylase; Hfq; RNA degradation

Polyadenylation is a post-transcriptional event that involves the addition of untemplated adenosine residues to the 3’ ends of RNA substrates. The first bacterial poly(A) polymerase (PAP) was identified almost 50 years ago in *Escherichia coli* [1,2]. A PAP was also identified in eukaryotic cells at about the same time [3,4]. However, polyadenylation in bacteria was virtually ignored for next 30 years, in part because eukaryotic poly(A) tails were relatively long, nearly uniform in length, and were found on almost all mRNAs. Furthermore, even though poly(A) tails were detected in *E. coli* and *Bacillus subtilis* [5–9], the overall low abundance of polyadenylated transcripts and the apparent lack of evidence for a physiological role led to the belief that polyadenylation was only important in higher organisms (See definition of polyadenylation in the glossary of Lewin, *Genes I* through *Genes VIII*).

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PAP I, encoded by the *pcnB* gene, and polynucleotide phosphorylase (PNPase), a 3'→ 5' exonuclease encoded by the *pnp* gene, are responsible for the post-transcriptional addition of 3' tails to transcripts in exponentially growing *E. coli* [10,11]. Interestingly, *in vivo* PAP I synthesized tails exclusively contain A residues, while PNPase synthesized tails are primarily heteropolymeric (the tails contain all four nucleotides but are ~50% A) [10,12]. It has now been shown that polyadenylation in many prokaryotic organisms, *Archaea*, and organelles of prokaryotic origin lacking a PAP I protein is carried out by PNPase. In fact, PNPase functioning as a polymerase is probably much more prevalent than previously thought [13–16].

Organelles such as mitochondria and chloroplasts are thought to have originated from ancient invasions and successful symbiosis of eukaryotic hosts by eubacteria more than a billion years ago [17,18]. Analysis of the genomic organization of these organelles indicates that while mitochondria evolved from α-proteobacterium, chloroplasts originated from a cyanobacterial ancestor. During the evolutionary development that shaped organelle biogenesis, both chloroplasts and mitochondria lost many of their eubacterial characteristics and acquired many host-derived properties. However, for the most part, their post-transcriptional activities still mimic bacterial systems with only a few significant differences. Recently, post-transcriptional modifications have also been reported in hyperthermophilic and methanogenic *Archaea* [15,16,19].

In prokaryotes, the exact method of substrate selection by either PAP or PNPase is still not clear. However, it would appear that any transcript that is a substrate for the exonucleolytic activity of PNPase can probably also be modified by the addition of polynucleotide tails [20]. Furthermore, the observation that Hfq, an abundant RNA binding protein, modulates poly(A) levels in *E. coli* [12,21] has raised the interesting question of whether PAP I acts independently *in vivo* as suggested by *in vitro* experiments [22]. The reduced ability of the PAP I protein to add poly(A) tails at the 3' termini of mRNAs containing Rho-independent transcription terminators in Hfq mutants coupled with the increase in the biosynthetic activity of PNPase has suggested that the regulation of polyadenylation involves a multiprotein complex [12]. The distinct difference in the polyadenylation pattern of transcripts with and without Rho-independent transcription terminators also suggests the presence of a discrete polyadenylation signal in *E. coli* [20].

While many transcripts decay rapidly following polyadenylation, recent studies indicate that its major role involves quality control for transcriptional or processing errors [23]. In addition, polyadenylation in *E. coli* has been implicated as a sensing mechanism for adjusting the levels of ribonucleases such as RNase E and PNPase [24]. Although the studies on *E. coli* transcripts have led the way towards a better understanding of the molecular mechanism and role of prokaryotic polyadenylation, the detection of poly(A) tails in all three domains of life has established their universal presence. In this review, we describe how information gained from experiments over the past decade has expanded our knowledge of the role played by polyadenylation in the post-transcriptional regulation of prokaryotic, archaeal and organellar gene expression. Readers are also encouraged to consult other comprehensive reviews that have been published recently on the subject [25–27].

I. NATURE OF 3'-TAILS: POLY(A), POLY(U), AND POLYNUCLEOTIDE TAILS

The initial discovery of poly(A) tails, comprised of multiple untemplated adenosine residues, at the 3' ends of RNA substrates dates back to early 1960's. With the development of new detection techniques and analytical procedures, scientists have identified post-transcriptionally added 3' tails that contain combinations of all four nucleotides and are present in many different organisms (Table 1). For example, many 3' tails are A-rich

polynucleotide tails (the tails contain all four nucleotides but generally are ~50% A) [10,15,28–32] and some are A/U tails (contain a few U residues besides A) [33]. For sake of clarity, in this article we refer poly(A) tails as homopolymeric adenosine tails and all other kind as polynucleotide tails (Fig. 1). It should also be noted that polyuridylated [poly(U)] tails have recently been reported in both human and *Chlamydomonas* mitochondrial RNA [34–36].

II. PROTEINS INVOLVED IN POLYADENYLATION

Bioinformatic studies have shown that eubacterial PAPs, which belong to a superfamily of nucleotidyl transferases [37,38], are present in only a limited number of bacteria and plant organelles and are absent in *Archaea* and eukaryotes. In fact, it is believed that eubacterial PAPs have evolved fairly recently such that they do not exist in many bacterial species [39]. In the absence of a PAP I-like activity, post-transcriptional modification of RNA species is carried out by other enzymes such as PNPase and the exosome [13,40]. Recent data suggests that bacterial PAPs have evolved from the CCA-adding enzymes (tRNA nucleotidyltransferases) as evident from the presence of comparable structural elements within these proteins [41,42].

a. Bacteria

PAP I (ATP:polyribonucleotide adenylyl transferase) I is the major polyadenylating enzyme in *E. coli*. It catalyzes the template independent addition of AMP moieties to 3’-hydroxyl termini of RNA substrates using ATP as a substrate (Fig. 1). Although PAP I can inefficiently add the other three nucleotides *in vitro* [43,44], *in vivo* the enzyme only synthesizes homopolymeric poly(A) tails [10]. The structural gene encoding PAP I (*pcnB*, plasmid copy number) was first identified based on N-terminal sequencing of the purified PAP I protein from *E. coli* [11]. While deletion of *pcnB* has only a moderate effect on growth rate [11,45,46], overproduction of PAP I is highly toxic [11,47].

PAP I accounts for ~90% of the poly(A) tails in *E. coli*, which are on average 15–30 nt long [10,12,20,48]. However, a limited number of the poly(A) tails can be longer than 30 nt, while the bulk of the tails are less than 10 nt in length (Fig. 2). Since deletion of the *pcnB* gene did not abolish all the polyadenylating activity in cell extracts (Fig. 2), it was speculated that *E. coli* contained a second PAP (PAP II). The *f310* gene, encoding a 36 KDa protein, was initially identified as the putative PAP II [49], even though the protein had no sequence similarity to the nucleotidyl transferase superfamily of proteins [50]. Further investigation of F310, however, failed to support its involvement in polyadenylation [51]. Specifically, a *pcnB f310* double mutant was still viable and overexpression of F310 had no significant effect on poly(A) levels or tail length either *in vivo* or *in vitro* [51]. Furthermore, cells containing increased levels of F310 did not show a detectable change in the copy number of a ColE1 plasmid [51].

Subsequently, it was noted that *E. coli* contained three additional proteins that belonged to the same nucleotidyl transferase superfamily as PAP I [50]. These are PNPase encoded by *pnp*, RNase PH encoded by *rph*, and tRNA nucleotidyl transferase encoded by *cca*. While PNPase, as well as its homologue RNase PH, are both reversible enzymes that can either degrade RNA by using inorganic phosphate or synthesize RNA by using NDPs as precursors (Fig. 1) [52,53], the high intracellular levels of inorganic phosphate (>10 mM) [54] led to the assumption that this class of enzyme functioned exclusively *in vivo* as an exoribonuclease. However, Mohanty and Kushner [10] demonstrated that PNPase was, in fact, the second *E. coli* PAP, which adds A rich polynucleotide tails to the 3’ ends of RNA (Fig. 1). Not only did the identification of PNPase as the second PAP in *E. coli* significantly alter more than 50 years of thinking about how PNPase works *in vivo*, but it also provided

the much needed basis for identification of post-transcriptional modifications in organisms and organelles lacking an eubacterial PAP (see below).

Unfortunately, current techniques do not allow researchers to accurately measure the length or amount of either poly(A) or polynucleotide tails. Poly(A) sizing assays (Fig. 2) [55], which provide the most accurate status regarding the size of poly(A) tails in the cell, are not helpful for estimating the length of polynucleotide tails since RNase A and RNase T1 cleave after C/U and G residues, respectively. Furthermore, traditionally researchers have used oligo(dT)-dependent detection [55] for identifying polynucleotide tails in all organisms [10,12,19,20,28–30,56,57]. This approach, however, probably does not reflect the accurate length of such tails. With this caveat, the average length of polynucleotide tails have been reported to be in the 100's of nucleotides compared to poly(A) tails, which are relatively short except for *Trypanosomes* (Table 2A–B).

While post-transcriptional addition of 3' tails has now been reported in a large number of bacterial species, many of the proteins responsible for these activities have yet to be identified (Table 1). Both poly(A) and polynucleotide tails, with a mean size of ~40 nt, have been reported in the gram positive bacterium *B. subtilis* [29]. Although the presence of two PAP activities was reported in a *pnp* mutant of *B. subtilis*, their identities are still unknown. A protein encoded by *papS*, with 18.9% identity to *E. coli* PAP I, was predicted to have PAP activity in *B. subtilis* [58]. However, careful characterization of the protein *in vitro* revealed that it had tRNA nucleotidyl transferase activity and the gene was renamed *cca* [59]. Furthermore, the fact that the absence of PNPase did not change the polyadenylation profile significantly indicated its limited role in the post-transcriptional modifications observed in *B. subtilis* [29].

Similarly, a putative *E. coli* PAP I homologue in *S. coelicolor* with 36% amino acid sequence identity [56] was also identified to be a tRNA nucleotidyl transferase [14]. However, cloning and sequencing data strongly suggest that a PNPase homologue plays an important role in generating the predominately polynucleotide tails *in vivo* [28,60,61].

b. Organelles

Although no *E. coli* PAP I homologues have been detected in the chloroplast genome, the identification of PNPase as the second PAP in *E. coli* [10] led to the demonstration that its homologue was the only enzyme responsible for polyadenylation in the spinach chloroplast [13], producing predominately polynucleotide tails [30,57]. Subsequently, polyadenylation in *Cyanobacteria*, the ancestor of chloroplasts, was also shown to be carried out by PNPase [31]. Interestingly, the 3'-tails in the chloroplasts of *Chlamydomonas* are nearly homopolymeric (>98% A) [62], even though recent data suggests that PNPase may be the only polyadenylating enzyme present [35]. The nature of 3' tails in *Arabidopsis* chloroplasts is still unknown [35,63]. Moreover, an increase in PNPase levels directly correlated with a decrease in the level of polyadenylation, suggesting a degradative role rather than synthetic role for the enzyme [63]. A more recent study, however, suggests that the poly(A) tails may be synthesized by a nuclear encoded eubacterial PAP homologue targeted to the chloroplast and mitochondria [35].

The nature of post-transcriptional modifications in mitochondria varies significantly among organisms [64] (Table 1). Plant mitochondrial RNA contains mostly poly(A) tails [65–67], although some polynucleotide tails have been reported [65]. However, no PAP-like enzymes have been identified yet. Furthermore, mtPNPase most likely plays a major role as a degradative enzyme [66,67]. Poly(A) tails of 40–60 nt in length have been detected in mammalian mitochondrial mRNAs [68–70], but the occasional incorporation of other

nucleotides has also been reported [68,71]. The presence of mostly homopolymeric tails suggests their synthesis by a specific PAP.

While a rat liver mitochondria specific PAP has been reported [72], its identity is still a mystery. In contrast, two groups have independently identified a human mitochondrial poly(A) polymerase (hmtPAP), which directly affects the length of poly(A) tails in mitochondria [68,69]. However, silencing of hmtPAP only reduced poly(A) tail length from ~50 nt to ~8 nt. Recent data suggest that more than one polyadenylating enzyme is responsible for polyadenylation in human mitochondria [36,68]. PNPase is probably not one of them because of the presence of predominantly homopolymeric tails and the localization of PNPase in the intermembrane space whereas RNA polyadenylation occurs in the matrix [68,69,73].

The mitochondria of *Trypanosome brucei*, one of the earliest branching eukaryotes, contain poly(A) tails that can broadly be divided into two classes, short (~20–25 nt) and long (~120–250 nt) tails [74]. While short tails are adenosine homopolymers, the long tails contain many uridine residues [75]. KPAP1 (kinetoplast poly(A) polymerase 1) was recently identified as the *T. brucei* mitochondrial PAP responsible for synthesizing both the short and long tails [75]. Interestingly, the enzyme is also essential for parasite viability and mitochondrial function.

Yeast mitochondria lack both PAP and PNPase homologues. However, poly(A) tails up to approximately 8 nt in length have been detected in *Saccharomyces cerevisiae* mitochondrial RNA [76], although no specific RNAs containing poly(A) tails have been identified. Instead, an A/U-rich dodecamer sequence that is encoded in the yeast mitochondrial genome and is attached to the 3’ ends of mRNAs, providing protection from exonucleolytic degradation has been identified [64,77,78].

c. Archaea

Neither eubacterial PAP or PNPase homologues have been identified in any class of *Archaea*. However, an archaeal exosome, which bears both structural and functional similarities to prokaryotic PNPases, has been found in both hyperthermophiles and some methanogens, but not in halophiles (Table 1). The archaeal exosome is a nine subunit complex containing three copies of proteins Rrp41 and Rrp423, which are homologuges of RNase PH and another protein containing a KH/S1 RNA binding domain [79]. The crystral structure of the exosome assembly has a ring structure that is very similar to bacterial PNPases [40,80]. Thus, not surprisingly, polynucleotide tails have been detected in hyperthermophiles and methanogens containing exosomal assemblies [15,19].

III. REGULATION OF POLYADENYLATION

It has been estimated that as much as 15–25% of total RNA is polyadenylated in *B. subtilis* [8]. In contrast, less than 2% of total RNA in *E. coli* is estimated to be polyadenylated [5–7], even though recent genome-wide analysis suggests that polyadenylation of *E. coli* transcripts occurs more frequently than previously envisioned [20]. A comparison of the oligo(dT)-dependent cDNA transcriptome between the wild type and Δ*pcnB* strains indicated that the majority of transcripts (~90%) undergo some degree of polyadenylation either as full-length transcripts or decay intermediates during exponential growth [20]. However, the most important question, namely what fraction of each full-length transcript gets polyadenylated still remains unanswered.

The limited data available indicate that the level of polyadenylation of specific transcripts actually varies significantly and is independent of both transcript size and overall abundance

(Table 3). For example, while both 16S and 23S rRNAs transcripts are highly abundant, only 0.6% of 16S rRNA transcripts are polyadenylated compared to 10% for the 23S rRNA [47]. Similarly, the percentage of polyadenylated transcripts among specific mRNAs varies from as low as 0.4 % to as high as 10% in *E. coli* (Table 3). Similar observations have been made for plant mitochondrial mRNAs [65].

The precise reason for the significantly lower levels and limited length of prokaryotic poly(A) tails compared to their eukaryotic counterparts is not clear. It is possible that the tails are added only to a limited number of transcripts in response to specific needs such as for RNA surveillance and/or processing pathways [23,81]. However, there are also multiple levels of regulation both before and after the synthesis of poly(A) tails in *E. coli*, which are discussed in the following sections.

a. Low intracellular levels of PAP I

It is estimated that there are only 32–50 molecules of PAP I in *E. coli* [12]. One of the reasons for such a low PAP I level may be related to the toxicity, which is rapid and irreversible, when excess PAP I is synthesized in the cell [47]. Even though the exact reason for the toxicity is not clearly understood at this time, macroarray analysis ruled out the possibility of rapid turnover of one or more mRNAs essential for cell viability during increased polyadenylation (Mohanty & Kushner, unpublished results). Since PAP I uses ATP as substrate, it is possible that rapid ATP depletion and/or excess NDP accumulation may cause the toxicity. However, this hypothesis is unlikely since bacteria growing in a rich medium should be able to quickly replenish ATP levels. Another possibility is the polyadenylation of essential RNAs, which are not usually substrates for PAP I in a wild type cell, such as the mature CCA termini of tRNAs. Addition of even a single A residue will render a mature, uncharged tRNA non-functional, leading to a shutdown of protein synthesis. In fact, the mature 3’ termini of tRNAs in wild type strains do not seem to be polyadenylated [82,83]. Similarly, excessive polyadenylation of small regulatory RNAs (sRNAs) might lead to a change in conformation thereby altering their functionality.

Thus it may not be surprising that PAP I levels in wild-type *E. coli* appear to be kept very low by a combination of factors. Changes in the *in vivo* PAP I level as a function of growth rate have been reported [84]. Furthermore, while *pcnB* transcription does not appear to be autoregulated, the steady-state level of the transcript is low despite having a moderately strong promoter [47,85]. PAP I levels are also downregulated by the presence of a weak non-canonical translation initiation codon [11,85].

Besides transcriptional and translational control, PAP I activity may also be controlled through specific localization or modification(s) of the protein. The recent demonstration that PAP I is either membrane localized or cytosolic based on growth phase is one such example [86,87]. However, it is possible that such localization may be indirect through a loose association with RNase E, which is also membrane localized [88]. Furthermore, a preliminary study indicates that PAP I may be phosphorylated in *E. coli* [87], a modification that helps regulate human PAP activity [89,90]. While not much is known about the conditions regulating the addition of polynucleotide tails by PNPase, a recent study suggests that the intracellular ATP concentration may play a role in the process [91].

b. Substrate selection

One of the biggest mysteries in prokaryotic polyadenylation is the nature of substrate selection by the various polyadenylating enzymes. In *E. coli*, the low level of PAP I is most probably one of the major factors in substrate selection, since transient overproduction of PAP I significantly increased the number of polyadenylated transcripts [20,47]. *In vitro* data

suggest that PAP I selects its substrates based on structural features without help from any ancillary protein(s). Thus, it was reported that a substrate with single-stranded segment at either 5' or 3' end, along with monophosphorylation at an unpaired 5' terminus, becomes highly susceptible to 3' polyadenylation [22]. However, this work has never been reproduced. In fact, PAP I has been reported to have physical interactions both *in vivo* and *in vitro* with Hfq, PNPase, RNase E and the DEAD-box RNA helicase encoded by *rhlB* [12,92]. In fact, the interactions between PAP I, Hfq and PNPase have been proposed to play an important role in deciding between PAP I and PNPase as the polymerizing enzyme (see below).

While it has long been presumed that 3' polyadenylation is restricted only to mRNAs, another unexpected feature of PAP I and PNPase in prokaryotes appears to be their ability to polyadenylate almost any RNA species, including rRNAs and tRNAs and sRNAs (Table 4). What is also interesting is that the tails found on tRNAs and sRNAs tend to be very short (1–8 nt) (Table 2B) [47,83,93,94], while those on rRNAs resemble the ones found on mRNAs (Table 2B) [12,82,83]. Unfortunately, the abundance of very short poly(A) tails (less than 12 nts) (Fig. 2) has made it technically extremely difficult to accurately assess the true state of polyadenylation in a bacterium such as *E. coli*.

c. Polyadenylation signals

Although it is clear that PAP I adds poly(A) tails and PNPase adds polynucleotide tails to the 3'-ends of a transcripts in *E. coli* [10], the absence of 100% polyadenylation for any given transcript in the bacterium suggests a regulatory mechanism for substrate selection by both enzymes. *In vitro* most RNA molecules can be polyadenylated by *E. coli* PAP I [43,44]. *In vivo*, however, RNA breakdown products generated by endoribonucleolytic cleavages are considered the most favored substrates for 3'-tailing by either enzyme facilitating in their rapid exonucleolytic degradation in both bacteria [95–97] and organelles [57,65,66].

Are there any features of a prokaryotic RNA molecule that could serve as a polyadenylation signal? Clearly, there are no sequence specific motifs such as the AAUAA signal found in eukaryotic pre-mRNAs. However, in bacteria and organelles many transcripts are terminated by Rho-independent transcription terminators, which form stem-loop structures (Fig. 3). Recent data suggest that 3' ends of the Rho-independent transcription terminators of *lpp*, *rpsO* and *ompA* mRNAs are preferred substrates for polyadenylation by PAP I [10,12,20,98]. Interestingly, all of these terminators have a 3–4 nt 3' extensions as opposed to no 3' extension in the *rplY* Rho-independent transcription terminator where no poly(A) tails were detected [20]. These results are consistent with the *in vitro* data showing inhibition of PAP I activity by Rho-independent transcription terminators that have no 3' single-stranded extension [43]. Conversely, a 2–6 nt single-stranded region is sufficient to be recognized as a PAP I substrate [43].

Furthermore, a recent genome-wide analysis of the polyadenylated transcripts in *E. coli* revealed that ~72% of the ORFs showing high levels of polyadenylation were associated with either monocistronic or polycistronic mRNAs containing a Rho-independent transcription terminator [20]. In contrast, transcripts terminated in a Rho-dependent fashion tended to contain only polynucleotide tails, generated by PNPase, which were located throughout the coding sequences [12,20] (Table 5). These observations led to the suggestion that Rho-independent transcription terminators may serve as polyadenylation signals in *E. coli* [12,20].

In contrast, decay intermediates of transcripts terminated with Rho-independent terminators (*lpp*, *ompA* and *rpsO*) contained more polynucleotide tails than poly(A) tails [12,20]. Of

particular significance is the fact that polynucleotide tails are generally found closer to the 5' termini of transcripts suggesting that they may arise after PNPase stalls at secondary structures while degrading an RNA molecule and, in the presence of high concentrations of NDPs, biosynthetically adds untemplated nucleotides onto the substrate that it had been degrading [10] (Fig. 4).

d. Potential polyadenylation complexes

While *in vitro* data suggests that PAP I selects its substrate independent of any ancillary proteins, it has been shown both *in vivo* and *in vitro* that both the poly(A) tail length and total polyadenylation levels are modulated by the RNA binding protein Hfq [12,21]. It has been suggested that Hfq regulates polyadenylation by changing PAP I from a distributive to a processive enzyme [21,26,99]. Interestingly, increasing Hfq levels *in vivo* did not change the polyadenylation level [12] similar to what was observed *in vitro* [21]. In addition, PAP I can easily overcome the absence of Hfq *in vivo* when overproduced (Mohanty and Kushner, unpublished results). Furthermore, the absence of Hfq did not alter PAP I protein levels but augmented synthesis of polynucleotide tails suggesting increased PNPase activity [12].

Thus, the role of Hfq as a facilitator in RNA polyadenylation by PAP I has been proposed. In this model (Fig. 4), it is hypothesized that Hfq primarily helps PAP I to compete with the more abundant PNPase to find its substrates and to control the biosynthetic activities of PNPase through protein-protein interactions [12]. This process most likely determines which enzyme synthesizes a 3' tail. Initial support for this model was obtained from the demonstration of RNA-independent protein-protein interactions among PAP I, PNPase and Hfq using immunoprecipitation and co-purification experiments [12,100]. However, the exact nature of the complex(es) is still unknown. It is also possible that more proteins are involved in forming an *in vivo* polyadenylation complex.

e. Role of ribonucleases

The net rate of poly(A) synthesis *in vivo* is determined by a combination of the rate of 3'-tail synthesis by polyadenylating enzymes versus degradation by ribonucleases (Fig. 5). Both RNase II and PNPase are considered the major exoribonucleases that degrade poly(A) tails in *E. coli* [101–105]. Furthermore, recent data also suggest that poly(A) tails stimulate RNA degradation by RNase R [106,107].

PNPase catalyzes both processive 3'→ 5' phosphorolytic degradation in presence of inorganic phosphate (Pi) and 5'→ 3' polymerization in presence of NDPs, of an RNA substrate (Fig. 1). In contrast, RNase II and RNase R, which belong to the same RNB exoribonuclease family, degrade an RNA substrate hydrolytically in the 3'→ 5' direction. With the exception of *Mycoplasma, Trypanosomes*, yeast and *Archaea*, all bacteria and organelles contain a PNPase homologue [27]. Some hyperthermophiles and methanogenic *Archaea* do not have a direct PNPase homologue, but instead contain an multiprotein exosome complex that is structurally similar to PNPase [27]. Homologues of the RNB exoribonuclease family are found in all bacteria, organelles and in some archaeal species with the exception of methanogens that do not contain an exosome [27].

Both PNPase and RNase II stall when they encounter a G/C rich secondary structure (Fig. 5A). While PNPase dissociates relatively rapidly, RNase II does not. Thus one can get multiple rounds of polyadenylation/deadenylation in the case of PNPase but not RNase II (Fig. 5A). In fact, it has been shown that RNase II protects mRNAs from exonucleolytic degradation by either blocking the 3' ends or generating short single-stranded extensions that are not substrates for either PNPase or RNase R [108–110]. This role of RNase II can also effectively block or significantly slow down polyadenylating enzymes resulting in very

short tails [111]. Interestingly, RNase II seems to be involved in modulating the level of poly(A) tails associated with 23S rRNA, the major polyadenylated species in *E. coli* (Table 3), whereas PNPase is more effective with 16S rRNA and mRNAs [101]. In contrast, if RNase R binds to a substrate containing a poly(A) tail, it can degrade through secondary structures effectively reducing the level of that transcript.

In vitro data suggest that the endoribonuclease RNase E can also act as a poly(A) nuclease to degrade the poly(A) tails [112], although its mode of action is still controversial [113]. However, RNase E does seem to contribute indirectly to increased levels of polyadenylation *in vivo* by generating new 3' termini, through endonucleolytic cleavages, which can serve as substrates for the polyadenylating enzymes [101]. In fact, recent *in vitro* data suggest that polyadenylation of the *cspA* mRNA by PAP I enhanced its degradation by the RNase E-based degradosome [114]. Thus, it is possible that RNase E can use poly(A) tails or A/U rich polynucleotide tails to bind a potential substrate as part of the multiprotein complex, called the degradosome [115], leading to faster RNA decay (Fig. 5B). Since it has been demonstrated that many mRNAs decay more rapidly in the presence of increased polyadenylation [47], efficient degradation by the RNase E-based degradosome could account for why only a small percentage of *E. coli* RNA appears to be polyadenylated at any given time.

Although no poly(A) binding proteins have yet been identified *in vivo* in prokaryotic cells, Hfq, CspE and ribosomal protein S1 have been shown to bind and protect poly(A) tails from ribonucleases *in vitro* [114,116,117]. While it is possible that these proteins can serve an identical function *in vivo* (Fig. 5C), similar to eukaryotic poly(A) binding proteins [118], experimental confirmation is still awaited. It has been suggested that binding of Hfq to poly(A) tails protects them from nucleolytic degradation [21,26,116]. However, the fact that there was ~60% reduction in the total poly(A) level along with significant reduction in the poly(A) tail length in an *hfq* mutant missing all the major ribonucleases compared to the control strain suggests a more complicated role for Hfq [12,99].

IV. ROLE OF POLY(A) AND POLYNUCLEOTIDE TAILS

The exact role(s) of poly(A) and polynucleotide tails in prokaryotic RNA metabolism are still not clear. However, although the specific function of tails in different prokaryotic entities may differ to some extent, the current consensus is that polyadenylation functions in the regulation of RNA stability and quality control [26,27,48,64,119].

It is interesting to note that the majority of the post-transcriptionally added tails in bacteria, organelles, and *Archaea* are polynucleotide in composition. However, it is currently not clear whether these polynucleotide tails play an identical role to poly(A) tails. Using the RNA Star program [120], we analyzed polynucleotide tails sequenced from bacteria, organelles, and *Archaea* for predicted secondary structures (Fig. 6). Surprisingly, polynucleotide tails are structurally almost indistinguishable from homopolymeric poly(A) tails. In fact, a 15 nt polynucleotide tail was as effective as a 15 nt poly(A) tail for degrading a transcript by PNPase *in vitro* [121]. However, if polynucleotide tails affect RNA degradation *in vivo* their role seems to be limited to breakdown products only, since the half-lives of full-length transcripts in an *hfq* mutant which contains mostly polynucleotide tails, were identical to a Δ*pcnB* mutant [12]. This result is consistent with the observation that polynucleotide tails are added mainly to breakdown products, whereas poly(A) tails are added to both breakdown and full-length transcripts.

a. RNA stability

The reduction in plasmid copy number of ColE1 plasmids in a *pcnB* mutant of *E. coli* [122] was not fully understood until it was shown that polyadenylation helped regulate plasmid copy number by controlling the stability of an untranslated RNA (RNA I) [94,123]. With the identification of *pcnB* as the structural gene for PAP I [11], multiple reports demonstrated that 3' polyadenylation led to decreased stability of variety of mRNAs and sRNAs [48,93,121,124–130]. A direct correlation between increased polyadenylation and decreased mRNA stability was subsequently established [47].

Surprisingly, the effect of 3' polyadenylation on the stability of specific full-length mRNAs in a *pcnB* single mutant are minimal when ribonucleases such as RNase E and PNPase are present in the cell [48]. In many cases, such as the *rpsO* and *rpsT* mRNAs, the differences in half-lives between wild type and *pcnB* strains are so subtle that different laboratories have published contradictory reports [47,125,126]. Nevertheless, when one or more of the ribonucleases along with PAP I are missing, many transcripts are significantly stabilized [48]. These results indicate that the presence of poly(A) tails is not required for the initiation of decay but rather that it facilitates the degradation process, possibly identifying a target (Fig. 5). In the absence of poly(A) tail, a ribonuclease such as RNase E still can degrade the transcript, although less efficiently [114].

Interestingly, increased global polyadenylation by transient overexpression of *pcnB* also led to the stabilization of some transcripts [20,47]. The most notable among these were the *pnp* (PNPase) and *rne* (RNase E) mRNAs, transcripts that encode enzymes involved in mRNA decay [47]. The stabilization of these transcripts also resulted in higher protein levels that were directly related to the autoregulation of both transcripts, leading to increase RNA degradative capacity [24,47]. While the mRNAs of other ribonucleases were not affected [24], these findings suggested possible regulatory controls balancing polyadenylation and induced mRNA decay. Thus, the increased polyadenylation of transcripts in *E. coli* serves as a sensing mechanism to facilitate intracellular adjustments in the levels of both RNase E and PNPase [24].

While the role of poly(A) tails in rRNA and tRNA processing and degradation is still unknown [83], they probably have a destabilizing effect on sRNAs similar to what is observed with mRNAs. In addition to RNA I, the antisense RNAs CopA, which controls the replication frequency of plasmid R1, and Sok, which inhibits translation of *hok* mRNA of plasmid R1 that mediate plasmid stabilization, are also stabilized by the absence of PAP I [124,129]. The small RNA GlmY has been shown to be polyadenylated by PAP I, which decreases it stability, leading to activation of *glmS* mRNA translation [93]. Another small RNA, MicA which is required for the accurate expression of outer membrane proteins, is also stabilized in the absence of polyadenylation by PAP I [130].

The destabilizing effects of poly(A) tails in *E. coli* on various transcripts have led to the assumption that they have similar effects in all bacteria with similar polyadenylation profiles. Detection of poly(A) and polynucleotide tails associated with decay intermediates of *B. subtilis* and *Streptomyces* sp. has provided some initial support for a comparable role for polyadenylation in other species [29,60]. However, this hypothesis still awaits direct confirmation.

Poly(A)-mediated RNA degradation in plant and algae chloroplasts and mitochondria was found to be very similar to that of bacteria [131], although its effect varied on different types of RNA species [62,132]. The polyadenylated transcripts in plant mitochondria are degraded mainly through 3'→ 5' exoribonucleolytic activity [57,65,67,133–135]. Endonucleolytically cleaved mRNA decay intermediates containing 3' polynucleotide tails have been identified

in both the spinach and *Chlamydomonas* chloroplasts [30,57,62], which may be degraded through exoribonucleolytic activity.

No specific role for polyadenylation in yeast mitochondrial RNA metabolism has been identified [64]. The exact function of polyadenylation in mammalian mitochondrial mRNA stability is also still unclear [71]. Long homopolymeric poly(A) tails in human mitochondria provide stability to mRNAs, while deadenylation by PNPase destabilizes the transcripts [69]. Current *in vitro* data suggest that short poly(A) tails may affect mRNA stability in *T. brucei* depending on the editing status of individual transcripts [136,137].

b. Quality control

Not long ago it was believed that only ribonucleases were required for RNA degradation. However, *E. coli* exoribonucleases, such as PNPase, RNase II and RNase R, require 3' single-stranded regions for initial binding to the RNA substrates. For example, a minimum 10–11 nt single-stranded region is required for PNPase, RNase II, and RNase R to bind to a substrate and initiate degradation [102,104,138]. Thus, in most cases the unstructured nature of poly(A) or polynucleotide tails (Fig. 6) has been hypothesized to provide the required toe-hold for exoribonucleases like RNase II and PNPase, which are particularly inhibited by secondary structures [121,127,139–143]. Accumulation of mRNA breakdown products of the *lpp, rpsO, ompA* and *rpsT* mRNAs in a *pcnB* mutant provides further support for this notion [48,96,97,125,144]. While some full-length mRNAs decay exclusively through the actions of 3'→ 5' exoribonucleases like PNPase and RNase II [108], the majority of the transcripts are believed to be degraded via initial endonucleolytic cleavages by either RNase E, its homologue RNase G or RNase Z, followed by 3'→ 5' exonucleolytic decay [145,146].

Mapping of polyadenylation sites by cDNA cloning and sequencing of a variety of transcripts has indicated that poly(A) and polynucleotide tails are frequently attached to RNA breakdown products, which are most probably generated by endonucleolytic cleavages [10,12,19,29,30,57,97,147]. Thus, it is generally believed that prokaryotic polyadenylation is a scavenging mechanism that helps recycle breakdown products, which can form highly structured molecules [81,97,110,148]. RNA degradation by RNase R, which is not inhibited by secondary structures, is also stimulated by the presence of poly(A) tails [106,107], since this enzyme requires single-stranded regions of 10–12 nucleotides to bind [149]. More recently, polyadenylation has been shown to be required in PNPase-mediated degradation of defective tRNAs in *E. coli* [23].

c. Translation and Editing

Unlike what is observed in eukaryotes, polyadenylation of bacterial RNAs probably has no significant effect on translation [150]. Although absence of polyadenylation leads to increased half-lives of many transcripts in bacteria [12,47,48,126], it is still unclear if the stabilized transcripts contribute to increased protein synthesis. However, a recent report suggests that the *glmS* mRNA in *E. coli*, which is highly susceptible to poly(A)-dependent degradation, overproduces glucosamine-6-phosphate synthase in a PAP I deficient strain [151]. Surprisingly, PAP I has been implicated in increasing σ^S protein levels indirectly by affecting the global regulator RssB, which helps control the levels of σ^S dependent transcripts [86,152]. A recent study suggests that polyadenylation also has a minor effect on the processing of $tRNA^{Leu5}$ in *E. coli* [83].

Polyadenylation does play an important role in the translation of both *Trypanososme* and mammalian mitochondrial mRNAs. Most protein coding transcripts in *T. brucei* mitochondria undergo massive post-transcriptional editing via the insertion or deletion of U residues [153]. The presence of short or long tails appears to correlate with the editing status

of the mRNA. Thus pre-edited forms contain only short tails whereas never-edited and edited forms contain both short and long tails [74,154,155]. It was recently shown that short poly(A) tails are required and sufficient to maintain the steady-state level of partially edited, fully edited and never edited mRNAs, which were extended with long poly(A) tails containing many uridines [75].

In mammalian mitochondria, the polyadenylation of mRNAs is required to create UAA stop codons to be functional that are not encoded in mtDNA. In some cases, polyadenylation is also required for the tRNA maturation by editing of its 3' terminus [64,156,157].

VI. CONCLUSIONS

Over the past 15 years considerable progress has been made in unraveling the mysteries of polyadenylation in bacteria and organelles. However, it is still unclear what constitutes the polyadenylation complex in *E. coli*, let alone bacteria such as *B. subtilis* which does not have a PAP-like protein and still contains poly(A) tails in the absence of PNPase. Another issue that needs further study relates to the nature of substrate selection and how PAP enzymes compete with PNPase for 3' termini, particularly in *E. coli* where there is at least 20-fold excess of PNPase. It is also not clear what is the function of the polynucleotide tails that are added by PNPase in *E. coli* and the large number of other bacterial species.

Perhaps most importantly, the biological significance of polyadenylation in prokaryotes is still not really understood. In contrast, the polyadenylation of eukaryotic mRNAs has long been viewed as a stabilizing element that facilitates localization and improves the translation. However, recent studies showing the polyadenylation induced decay of non-functional RNAs in eukaryotes [158–160] has prompted many to believe that the major role of this ancient trait is quality control where a living cell tags its aberrant and unused transcripts for degradation. While RNA surveillance may only be the common function performed by polyadenylation, it is premature at this time to consider RNA surveillance as its primary function in bacteria, particularly since there is so little PAP I protein found in *E. coli*. Finally, it should be noted that the study of polyadenylation in prokaryotes is still significantly limited by technical issues relating to the ability to easily identify RNA species with poly(A) tails less than 10 nt in length.

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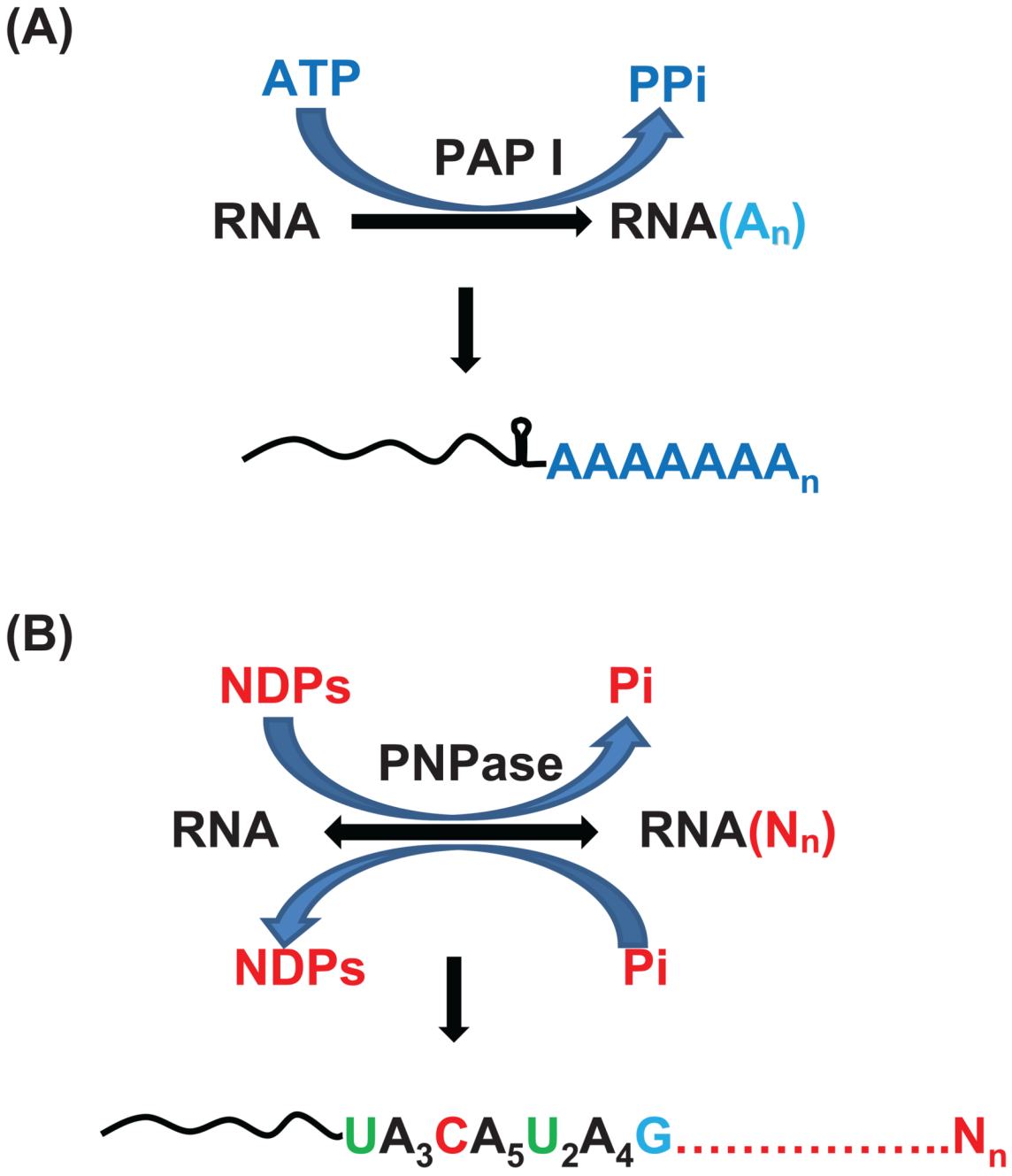

Fig. 1.

Addition of (A) poly(A) tails by PAP I and (B) polynucleotide tails by PNPase in *E. coli*. Since the equilibrium constant of the PNPase catalyzed reaction is close to one, the enzyme can work either degradatively or biosynthetically depending upon the availability of inorganic phosphate (Pi). High NDP and low Pi concentrations favor the biosynthetic reaction which generates untemplated polynucleotide tails. Low NDP and high Pi concentrations favor the exoribonucleolytic degradation of transcripts. N: any nucleotide.

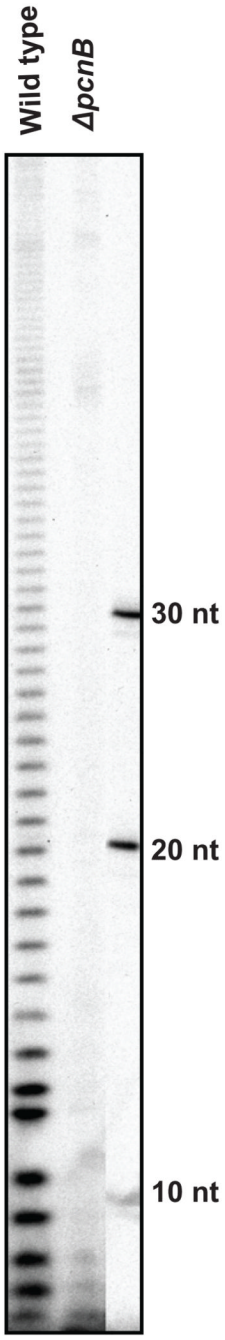

Fig. 2.
Poly(A) tail profiles in wild type and *pcnB* deletion strains of *E. coli*. Total RNA was processed for the poly(A) sizing assay as described by Mohanty *et al.* [55].

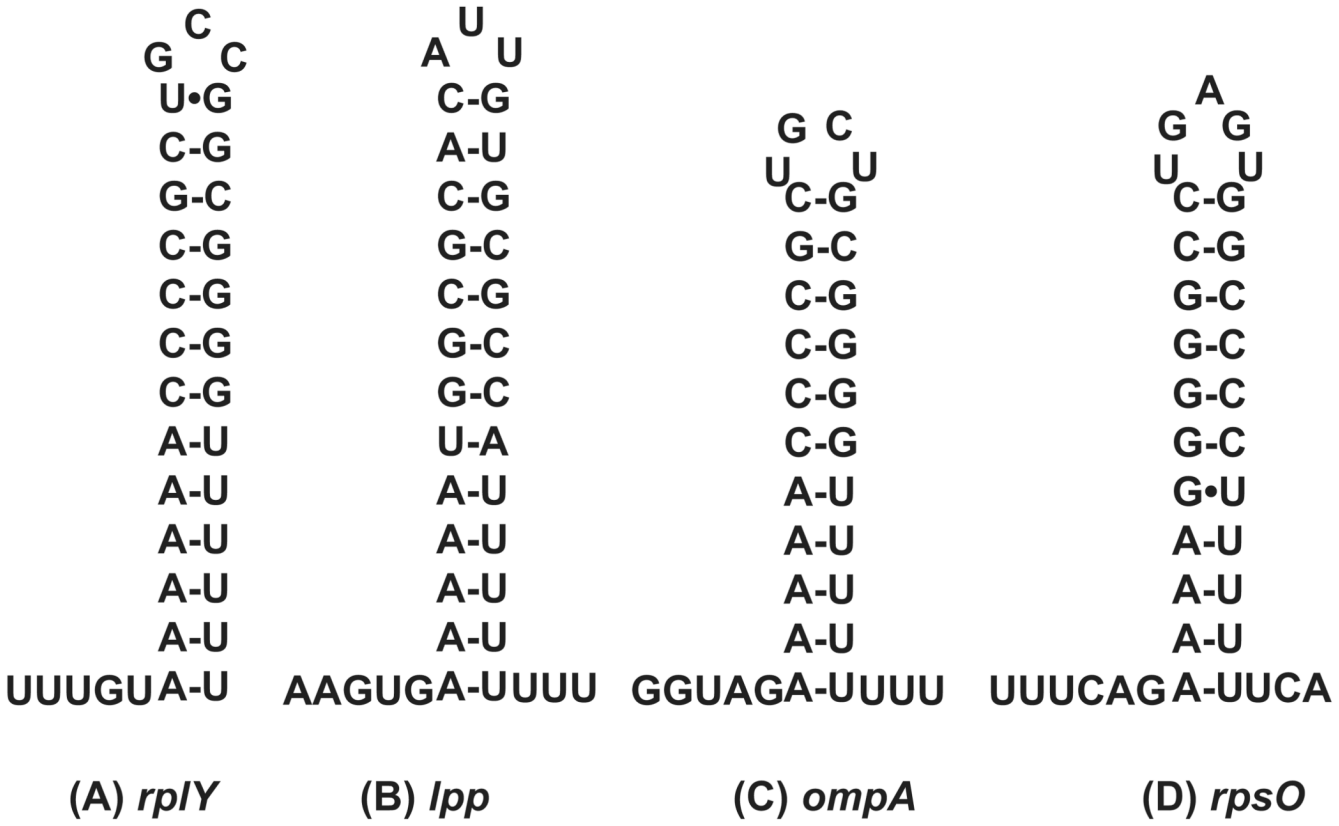

Fig. 3.
Predicted secondary structures of various Rho-independent transcription terminators in *E. coli*. Sequencing of cDNAs copies of these four transcripts has confirmed the nature of each single-stranded 3' extension [12,20,47].

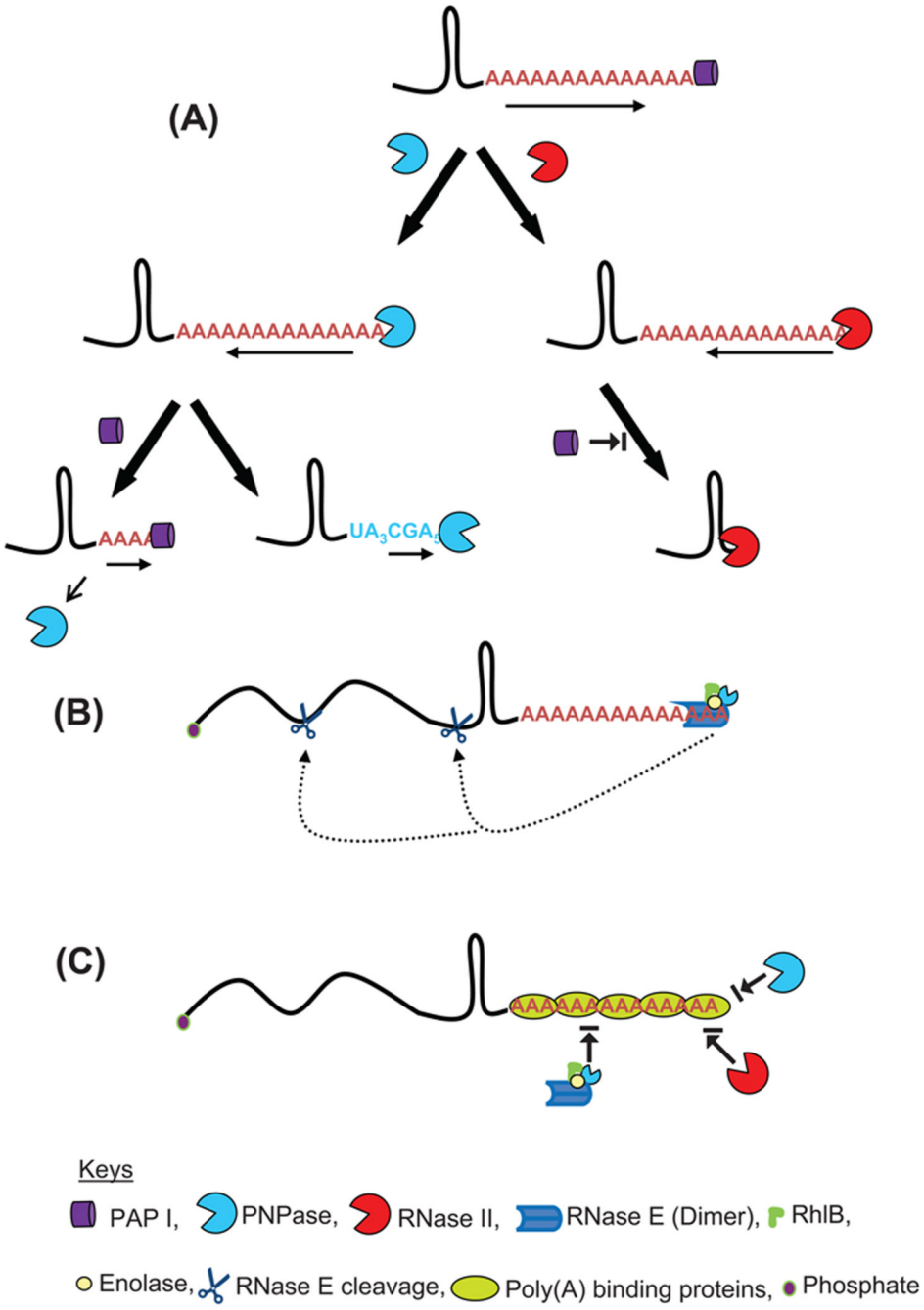

Fig. 4.

Hfq mediated polyadenylation by PAP I in *E. coli*. Recent studies suggest that Rho-independent transcription terminators in *E. coli* transcripts may serve as polyadenylation signals [12,20]. Hfq has been shown to preferentially bind to the base of A/U rich region of the terminator [12]. It has been hypothesized that Hfq in its hexameric form interacts with PAP I and PNPase to form a polyadenylation complex [12], which binds to the base of the stem-loop associated with the Rho-independent transcription terminator. Consequently, some or all of the A/U base pairs of the stem loop melt permitting PAP I to bind to the resulting single-stranded 3' end and add poly(A) tails processively. The interaction is believed to help PAP I to compete the vast excess of 3' → 5' exoribonucleases in order to

find its substrate and to also suppress PNPase's biosynthetic activity [12]. Terminal 5' triphosphates can be converted to 5' phosphomonoesters by RppH [161], a requirement for polyadenylation *in vitro* [22]. However, this requirement has not been demonstrated *in vivo*. An endoribonuclease such as RNase E may access the transcript from the 5' end at the same time. The 5' phosphorylation status, which can affect RNase E activity, probably varies for individual transcripts [162–164]. In addition, it is also possible that RNase E can access the substrate as part of the degradosome by binding to the poly(A) tail (Fig. 5B).

Once PAP I dissociates, along with Hfq, PNPase can degrade the poly(A) tail in the 3'→5' direction. Endonucleolytically derived decay intermediates are mostly degraded by 3'→5' exoribonucleases such as PNPase, RNase II, and RNase R. However, some of the decay intermediates contain strong G/C rich secondary structures forcing PNPase to stall and possibly switch to a biosynthetic mode, thereby generating unstructured polynucleotide tails (Fig. 6). These tails either change the conformation of the substrate or provide necessary single-stranded region for either PNPase, RNase II, or RNase R to bind and complete the degradation process.

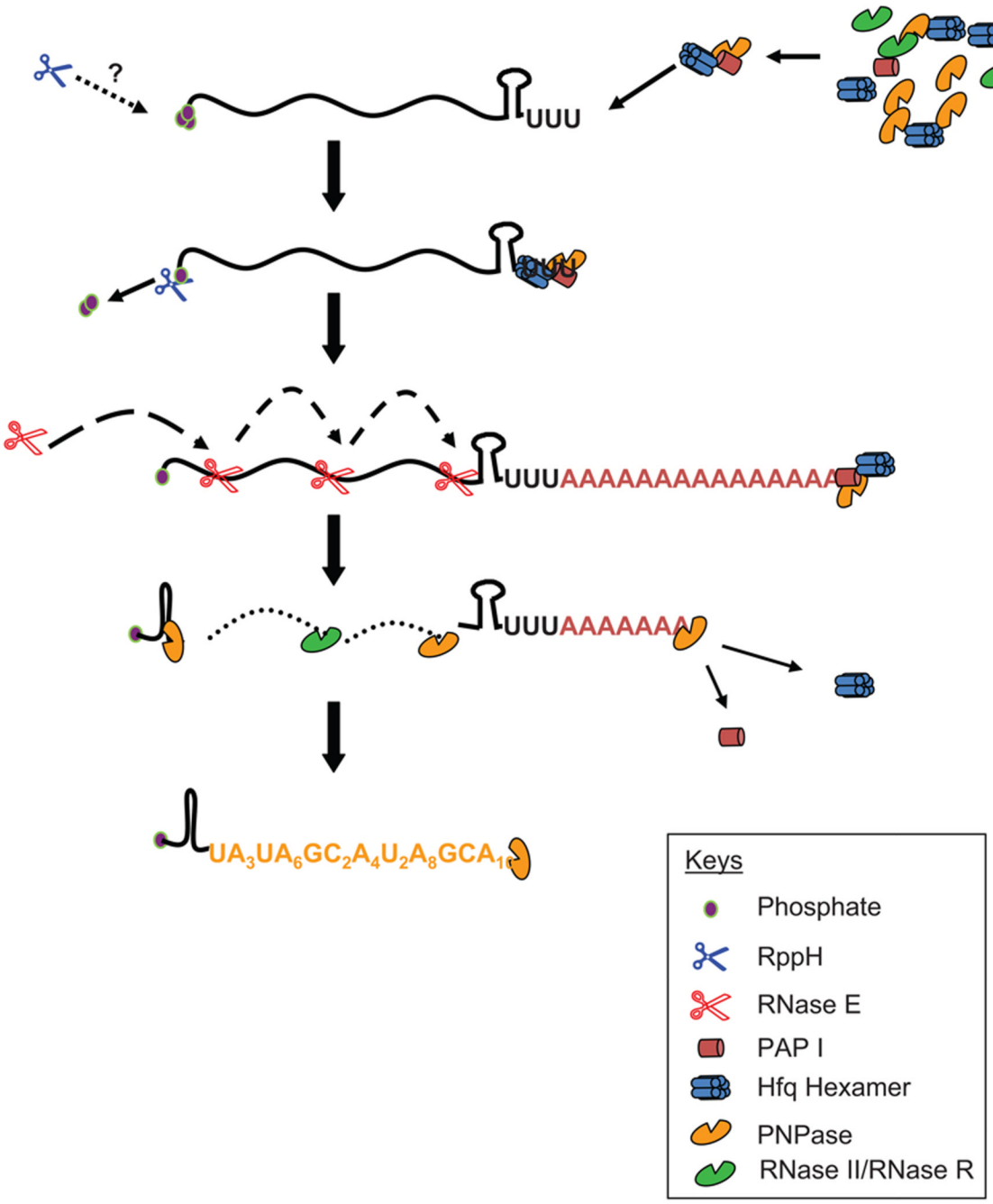

Fig. 5.

Polyadenylation assisted RNA decay in *E. coli.* (A) Addition of poly(A) tails by PAP I to the 3' ends of an RNA substrate provides the single stranded binding site for both PNPase and RNase II that initiate the degradation. While PNPase catalyzes both 3'→ 5' phosphorolytic degradation in presence of in organic phosphate and 5'→ 3' polymerization in presence of NDPs, RNase II can only degrade RNA hydrolytically in the 3'→ 5' direction. Both the ribonucleases pause upon encountering a G/C rich secondary structure. PNPase either dissociates relatively quickly or reverses its activity to polymerize polynucleotide tails. Dissociation of PNPase may initiate multiple rounds of polymerization

by PAP I. In contrast, RNase II remains bound to the base of the secondary structure thereby effectively blocking the binding of either PAP I or PNPase.

(B) RNase E alone or as part of the multiprotein complex called the degradosome can bind A/U rich poly(A) and polynucleotide tails to initiate degradation of a potential substrate through endonucleolytic cleavage. A full-length polyadenylated RNA substrate may be degraded very fast [114] by direct or internal entry [164] resulting in very few steady-state polyadenylated RNA species. This type of RNase E entry to an RNA substrate has yet to be experimentally demonstrated.

(C) Potential poly(A) binding proteins can block RNA decay in *E. coli*. Proteins such as CspE, Hfq and ribosomal protein S1 could bind to poly(A) or polynucleotide tails blocking endonucleolytic access by the RNase E-based degradosome through its PNPase moiety or direct exonucleolytic degradation by exoribonucleases such as RNase II, RNase R, and PNPase.

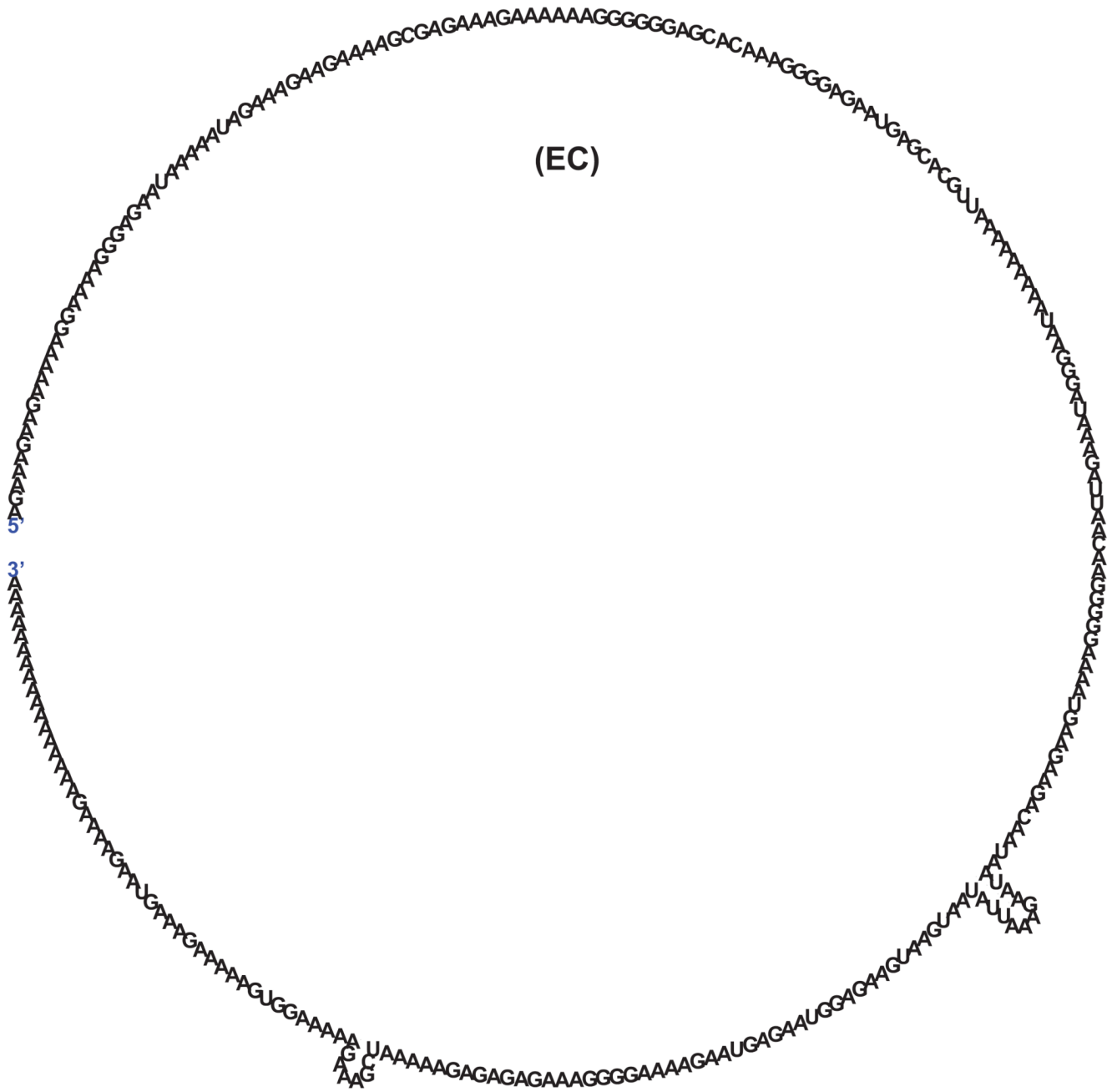

Fig. 6.

Secondary structure of a polynucleotide tail that was cloned and sequenced from a *pcnB* transcript of *E. coli*, (257 nt, −3.0 KCal) [20]. Similar analysis of polynucleotide tails derived from a *pnp* transcript of *S. antibioticus*, (116 nt, −0.6 KCal) [165], an *rpsD* transcript of *B. subtilis* (56 nt, −4.2 KCal) [29], a *psbA* transcript from spinach chloroplast (177 nt, −2.4 KCal) [30], an *rbcL* transcript from *Synechocystis* (172 nt, −1.9 KCal), [31], and an exosome complex exonuclease 2 transcript from *M. kandleri* (124 nt, −2.5 KCal), [15] yielded identical results (data not shown). The secondary structures and energy level (total energy for all the stems in a structure) were obtained by using RNA STAR program [120].

Table 1

Nature and source of 3' tails in bacteria, *Archaea* and organelles

Bacteria	Nature of tails	Protein/Gene	Mol. Wt (KDa)	Crystal Structure	Reference
E. coli	Poly(A) Polynucleotide	PAP I/*pcnB* PNPase/*pnp*	53.9 77.1	ND Yes	[12,20,47] [105]
Bacillus subtilis	Poly(A) Polynucleotide	ND PNPase/*pnpA*,	- 77.5	- ND	[29]
Streptomyces coelicolor	Poly(A) Polynucleotide	ND ND	- -	- -	[28,56]
Pseudomonas aeruginosa	Unknown	ND	-	-	[166]
Caulobacter cresentus	Poly(A)	ND	-	-	[167]
Rhodospirillum rubrum	Poly(A)	ND	-	-	[168]
Mycobacterium	Unknown	ND	-	-	[169]
Cyanobacteria	Polynucleotide	PNPase/*Sll1043*	-	-	[31]
Organelles					
Spinach Chloroplasts	Polynucleotide	PNPase/ND	~100	ND2	[13,30,57]
Algae Chloroplasts	Polynucleotide (>98% A)	ND			[62]
Arabidopsis Chloroplasts	Unkown	ND			[35,63]
Plant Mitochondria	Poly(A) Polynucleotide	ND ND			[65,67]
Mammalian Mitochondria	Poly(A)	hmtPAP/*hmtPAP*	65–68	ND	[68,69]
Trypanosomes Mitochondria	Poly(A) Polynucleotide (A/U)	KPAP1/ND	ND	ND	[74]
Yeast Mitochondria	Unknown	ND			[76]
Archaea					
Hyperthermophiles	Polynucleotide	Exosome*	~250	Yes	[19,80]

Bacteria	Nature of tails	Protein/Gene	Mol. Wt (KDa)	Crystal Structure	Reference
Methanogens (with exosome)	Polynucleotide	Exosome*	~250	Yes	[15] [80]
Methanogens (no exosome)	None				[15]
Halophile	None				[19]

* The archaeal exosome is a nine subunit complex. Each subunit is composed of two proteins Rrp41 and Rrp42 (homologous to RNase PH) and another protein containing a KH/S1 domain [80].

ND: Not determined

Table 2

(A) Average length of poly(A) tails reported in bacteria and organelles

Bacteria/Organelles	Length (nt)	References
E. coli	1–50	[12,47,48]
B. subtilis	1–40	[29]
Plant Chloroplasts	Unknown	[35,63]
Plant mitochondria	5–36	[65–67]
Mammalian Mitochondria	40–75	[68–70]
Trypanosome Mitochondria	20–25 120–250	[74]
Yeast Mitochondria	1–8	[76]

(B) Actual length of poly(A) tails reported on specific transcripts in wild type prokaryotes and organelles

Bacteria/ Organelles	Transcripts	Length (nt)*	References
E. coli	5S rRNA	1	[170]
	16S rRNA	15–18	[47]
	23S rRNA	17–18	[47]
	GlmY	1–8	[93]
	lpp	16–28	[12,47]
	rmf	1–5	[171]
	RNA I	3	[94]
	rpsO	1–20	[172]; Mohanty & Kushner, Unpublished results
	ompA	17–31	[20]
	cysT	1	[82]
	hisR	1–3	[82]
	leuU	1–3	[82]
	leuX	1–5	[83]
B. subtilis	*rnpB*	2–14	[29]
	cry1Aa	2–7	[29]
	23S rRNA	2–7	[29]
	$tRNA^{Cys\text{-}LeuU}$	1–2	[29]
Streptomyces	*leuA*	17	[60]
	16S rRNA	12–15	[28]
	23S rRNA	9–16	[28]
Plant Mitochondria	*cox2*	14–36	[65]
	atp9	5–17	[67]
	18S rRNA	4–6	[66]
Human Mitochondria			
	ND3	18–58	[68]
	ATP618	13–62	[68]
	COX III	10–56	[68]

(B) Actual length of poly(A) tails reported on specific transcripts in wild type prokaryotes and organelles			
Bacteria/ Organelles	**Transcripts**	**Length (nt)***	**References**
	CYTB	29–36	[68]
	12S rRNA	1–2	[68]

* The length of poly(A) tails reported were measured by various techniques. In our experience, each technique has specific limits on the types and length of poly(A) tails can be detected.

Table 3

Percentage of specific transcripts polyadenylated in *E. coli*

Transcripts	% polyadenylated	Reference
23S rRNA[a]	10 ± 2	[47]
16S rRNA[a]	0.6 ± 0.2	[47]
lpp[a]	0.43–0.74 ± 0.02	[20]
ompA[a]	1.3 ± 0.1	[20]
rpsO	10	[172]

[a]Full-length transcripts

Table 4

Identified transcripts with poly(A) or polynucleotide tails in bacteria, organelles and *Archaea*

Group	Species	RNA Type	Transcripts	Reference
Bacteria	*E. coli*	mRNA	*lpp, rpsO, ompA, secG, rmf, pcnB, trxA*	[10,12,20,47,126,171]
		rRNA	16S rRNA, 23S rRNA	[47]
		nc RNA	6S RNA, 4.5S RNA, RNA I, SoK, SraK, SraL, GlmY, SsrA, RnpB	[94,124,173–175]
		tRNA	*cysT, hisR, leuX, trpT, leuU, tyrT, tyrV*	[82,83,175]
	B. subtilis	mRNA	*rnpB, rpsD, cry1Aa*	[29]
		rRNA	23S rRNA	[29]
		tRNA	$tRNA^{Cys\text{-}LeuU}$	[29]
	Streptomyces	mRNA	*redD, actII-orf4, pnp, clpP, leuA*	[28,60]
		rRNA	16S rRNA, 23S rRNA	[60]
	Synechocystis	mRNA	*rbcL*	[31]
		rRNA	23S rRNA	[31]
		tRNA	$tRNA^{Fmet}$	[31]
Chloroplast	Spinach	mRNA	*psbA, petD*	[30,176]
	Algae	mRNA	*cox1, atpB, petD*	[35,62]
		rRNA	5S rRNA	[62]
		tRNA	$tRNA^{Arg}$, $tRNA^{Glu}$	[62]
	Plant	mRNA	*psbA, rbcL, rps14*	[63]
Mitochondria	Plant	mRNA rRNA	*cox2, atp9* 18S	[65–67]
	Mammalian	mRNA	*co1,co2,co3,atp6, ND3*	[69]
Archaea	*M. kandleri*	mRNA	Exosome complex exonuclease 2	[15]
		rRNA	16S rRNA	[15]
	S. solfataricus	mRNA	NADH dehydrogenase subunit H	[19]
		rRNA	16S rRNA	[19]

Table 5

Relationship between transcription terminators and the nature of the added tails in *E. coli*.

Transcript	Terminator[1]	% of Transcripts with		Reference
		Poly(A) tail	Polynucleotide tail	
lpp	RI	>70	<30	[10,12,47]
rpsO	RI	>73	<27	[10]
ompA	RI	>77	<23	[20]
trxA	RD	0	100	[12]
pcnB folK[2]	RD	0	100	[20]

[1] Each transcript contains either a Rho-independent (RI) or Rho-dependent (RD) transcription terminator.

[2] In the *pcnB folK* operon, the *pcnB* coding sequence overlaps the downstream *folK* gene. The dicistronic transcript is terminated in a Rho-dependent fashion.