

## REVIEW ARTICLE

# Polyadenylation of mRNA in bacteria

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### Overview

The presence of polyadenylate sequences at the 3'-end of mRNA was first observed in eukaryotic cells about 25 years ago (Darnell *et al.*, 1971; Edmonds *et al.*, 1971; Lee *et al.*, 1971) and is now recognized as a characteristic feature of eukaryotic mRNAs other than those encoding the histones (Marzluff & Pandey, 1988). Until recently, it was generally accepted that the 3'-polyadenylation of mRNA is a eukaryotic feature and does not occur to a significant extent in prokaryotes. This view persisted in spite of numerous reports on bacterial polyriboadenylate [poly(A)] RNA over almost a 20-year period. There were several reasons for this scepticism: (i) the lack of success of early efforts to detect poly(A) RNA in bacterial cells (Edmonds *et al.*, 1971; Perry *et al.*, 1972), (ii) the difficulty of studying a labile segment of the already very short-lived bacterial mRNA, especially at a time when the methodology available for studying mRNA was still quite primitive, (iii) the relatively short length of the bacterial poly(A) and the apparently limited extent of mRNA polyadenylation, which cast doubt on its physiological significance, and (iv) the difficulty of rationalizing a role for the 3'-terminal modification of bacterial mRNA in organisms where transcription and translation are usually closely coupled. This review presents the accumulated evidence for the extensive 3'-polyadenylation of bacterial mRNA and for a complex enzymic machinery that determines the level of mRNA polyadenylation. The functional role of bacterial polyadenylation remains unclear and may include the modulation of mRNA stability. Other possibilities, such as a role in translation analogous to that recently proposed for eukaryotic mRNAs (Sachs, 1990; Sachs & Deardorff, 1992) have not yet been explored.

### Detection of poly(A) RNA in bacteria

As early as 1975, three separate groups of researchers described low levels of poly(A) RNA in bacteria. Examining the RNA from the Gram-negative bacterium *Caulobacter crescentus* by poly(U) filter binding, Newton

and his co-workers (Ohta *et al.*, 1975) reported that 7% of rapidly turning-over mRNA contained poly(A) tracts ranging from 15 to 50 residues in length. Almost simultaneously, Edmonds and co-workers (Nakazato *et al.*, 1975) described the occurrence of low levels (0.2%) of poly(A) RNA in pulse-labelled RNA of *Escherichia coli*, with 3'-terminal poly(A) tracts 25–50 residues long. In the same year, Srinivasan *et al.* (1975), using *E. coli* grown in low-phosphate medium, extracted pulse-labelled RNA [in the presence of high salt to inhibit poly(A)-degrading enzymes] and showed as much as 15% of pulse-labelled RNA to be polyadenylated. Although the studies from these three laboratories were biochemically convincing, they had little impact on the prevailing view that polyadenylation of bacterial mRNA was of no physiological significance.

Yet at the same time, an indication that bacterial RNA polyadenylation is physiologically significant emerged from studies on RNA synthesis in permeabilized cells of *Bacillus brevis*, where the degradation of newly synthesized RNA is primarily due to the action of a 3'-exonuclease that is inhibited by guanosine 3',5'-monophosphate (Paulus & Sarkar, 1974; Sarkar & Paulus, 1975). This 3'-exonuclease acts much more slowly on poly(A) than on other polynucleotides, suggesting that the stability of bacterial mRNA might be modulated by polyadenylation. To explore this possibility, a one-step procedure for the isolation of bacterial poly(A) RNA was developed, which involved lysis of pulse-labelled cells and immediate digestion by proteinase K in the presence of SDS, 1,10-phenanthroline and heparin, to degrade or inhibit 3'-exonucleases (Gopalakrishna *et al.*, 1981). Pulse-labelled RNA isolated by this procedure from *B. brevis* (Sarkar *et al.*, 1978; Gopalakrishna & Sarkar, 1982a), as well as from *Bacillus subtilis* and *E. coli* B (Gopalakrishna *et al.*, 1981), had a relatively high degree of polyadenylation (15–40%). It seems that earlier reports of very low levels of poly(A) RNA (0.1–0.3%) in the budding bacterium *Hypomicrobium* sp. B522 (Kaur & Jayaraman, 1979), and the absence of poly(A) RNA from vegetative cells of *B. subtilis* (Schultz *et al.*, 1978) and *Bacillus polymyxa* (Kaur & Jayaraman, 1979), could be attributed to the use of

isolation procedures which promoted the degradation of poly(A) RNA by cellular nucleases. Indeed, later studies using the RNA isolation method of Gopalakrishna *et al.* (1981) revealed high levels of poly(A) RNA (16%) in the archaeon *Methanococcus vannielii* (Brown & Reeve, 1985) and even higher levels (20–50%) in the protein-secreting *B. brevis* 47 (Hussain *et al.*, 1982). The proportion of poly(A) RNA in total unlabelled RNA at steady state was estimated in *B. subtilis* by the formation of ribonuclease-resistant hybrids with [<sup>3</sup>H]poly(U) as 2–3% of the total cellular RNA, which corresponds to about 50% of the steady-state level of mRNA (Gopalakrishna & Sarkar, 1982a).

### Characterization of poly(A) RNA from bacteria

In most of the studies cited above, isolation of poly(A) RNA was achieved by affinity chromatography of RNA on oligo(deoxythymidylate) [oligo(dT)]-cellulose or poly(U)-Sepharose (Srinivasan *et al.*, 1975; Sarkar *et al.*, 1978; Hussain *et al.*, 1982; Gopalakrishna & Sarkar, 1982a; Brown & Reeve, 1985). These studies also showed that poly(A) is associated with pulse-labelled RNA, presumably mRNA, whereas long-term-labelled cellular RNA contains a very low level of poly(A) RNA. Susceptibility of the poly(A) tracts to preferential degradation by snake-venom phosphodiesterase indicated that they are primarily located near the 3'-ends of the RNA molecules (Sarkar *et al.*, 1978; Hussain *et al.*, 1982; Gopalakrishna & Sarkar, 1982a; Majumdar & McFadden, 1984). The mean length of the poly(A) tracts, isolated by digesting poly(A) RNA with ribonucleases A and T1, was estimated from their electrophoretic mobility in polyacrylamide gels and the adenosine:AMP ratio after alkaline hydrolysis, and was found to vary widely amongst bacteria: 13–17 nucleotides in *Caulobacter crescentus* (Ohta *et al.*, 1978), 10–50 nucleotides in *E. coli* (Nakazato *et al.*, 1975), 60 nucleotides in *B. brevis* (Sarkar *et al.*, 1978), 49 nucleotides in *B. subtilis* (Gopalakrishna & Sarkar, 1982a), 10 nucleotides in the archeon *M. vannielii* (Brown & Reeve, 1985), and 80 nucleotides in the photosynthetic bacterium *Rhodospirillum rubrum* (Majumdar & McFadden, 1984). The bacterial poly(A) tracts are thus considerably shorter than the poly(A) tracts associated with mRNA in higher eukaryotes but are in approximately the same size range as those from yeast (McLaughlin *et al.*, 1973).

Sedimentation analysis and polyacrylamide gel analysis of pulse-labelled poly(A) RNA showed a polydisperse pattern ranging from 4S to 16S in *E. coli* (Nakazato *et al.*, 1975) and *C. crescentus* (Ohta *et al.*, 1975). The weight mean sedimentation coefficient of *B. brevis* poly(A) RNA was 12.5S, corresponding to a mean length of 800–900 nucleotides (Sarkar *et al.*, 1978). This is a typical size range for mRNA; indeed, a significant amount of poly(A) RNA in *C. crescentus* (Ohta *et al.*, 1978) and *B. brevis* (Käuffer *et al.*, 1981) was found to be associated with polysomes. Further evidence that poly(A) RNA is mainly mRNA comes from measuring its ability to serve as a template for *in vitro* protein synthesis. Poly(A) RNA from *B. subtilis*

was 20-fold more effective than non-poly(A) RNA in stimulating the incorporation of radioactive amino acids in a cell-free system derived from *E. coli* MZ9 (Gopalakrishna & Sarkar, 1982a). Using a cell-free protein synthesis from wheat germ, Majumdar & McFadden (1984) observed an even more impressive stimulation of amino acid incorporation by poly(A) RNA from *R. rubrum* (220-fold compared to non-poly(A) RNA) and were able to show by the use of specific antibodies that one of the major products was ribulosebisphosphate carboxylase/oxygenase. Additional evidence for translational activity of poly(A) RNA came from the high translational activity of poly(A)-RNA-containing polysomes from *B. brevis* in the presence of soluble factors (Käuffer *et al.*, 1981).

In view of the evidence that poly(A) RNA is primarily mRNA, it was of interest to determine whether polyadenylated mRNA functioned only in certain physiological contexts. To address the possibility of a specific function during developmental transitions, the levels of poly(A) RNA were compared during vegetative growth and sporulation of *Bacillus* species, but no significant differences in the poly(A) RNA content in pulse-labelled RNA were found either with *B. brevis* (Gopalakrishna *et al.*, 1981) or with *B. subtilis* (Gopalakrishna & Sarkar, 1982a). Earlier reports that poly(A) RNA was present only in sporulating cells of *B. polymyxa* (Kaur & Jayaraman, 1979) and *B. subtilis* (Kerjan & Szulmajster, 1980) have not been confirmed. Similarly, poly(A) RNA was found to be present at all stages of growth and development of *C. crescentus* (Ohta *et al.*, 1978).

The question of whether poly(A) RNA consists of a restricted subpopulation of mRNA molecules or whether it comprises most mRNA sequences was addressed by comparing sequence complexity of poly(A) RNA and non-poly(A) RNA by hybridization to Southern blots of *EcoRI*-digested *B. subtilis* DNA. Complex hybridization patterns were observed with both types of RNA, but no bands could be identified as unique to non-poly(A), consistent with the idea that poly(A) RNA comprises most mRNA species (Gopalakrishna & Sarkar, 1982a). Independent confirmation that a broad range of bacterial mRNAs is polyadenylated was obtained by the oligo(dT)-primed synthesis of complementary DNA (cDNA) by reverse transcriptase. With purified poly(A) RNA from *B. subtilis* as template, the mean chain-length of the cDNA was 400 nucleotides, with a range from 230 to 800 (Gopalakrishna & Sarkar, 1982b). Moreover, the cDNA thus synthesized could serve as template for double-stranded cDNA synthesis, making possible its insertion into plasmids such as pBR322 and subsequent cloning in *E. coli*, to yield a cDNA library (Karnik *et al.*, 1986). Six clones from the first such cDNA library (400 clones) were sequenced and found to carry at the 3'-end of the sense strand runs of deoxyadenylate residues ranging from 4 to 19, preceded by at least one translation termination codon in one of the possible reading frames. It should be noted that the length of the poly(dA) tracts in the cDNA does not necessarily reflect the poly(A) length in the corresponding mRNA, but rather that of the oligo(dT)

primers used in cDNA synthesis, and may have been shortened as a result of the extensive S1 nuclease treatment that was needed to allow the cloning of double-stranded cDNA. The demonstration that cDNA libraries can be constructed from bacterial mRNA (Karnik *et al.*, 1986) provides a valuable tool for future studies of bacterial transcription patterns.

### Identification of specific polyadenylated mRNAs in *E. coli*

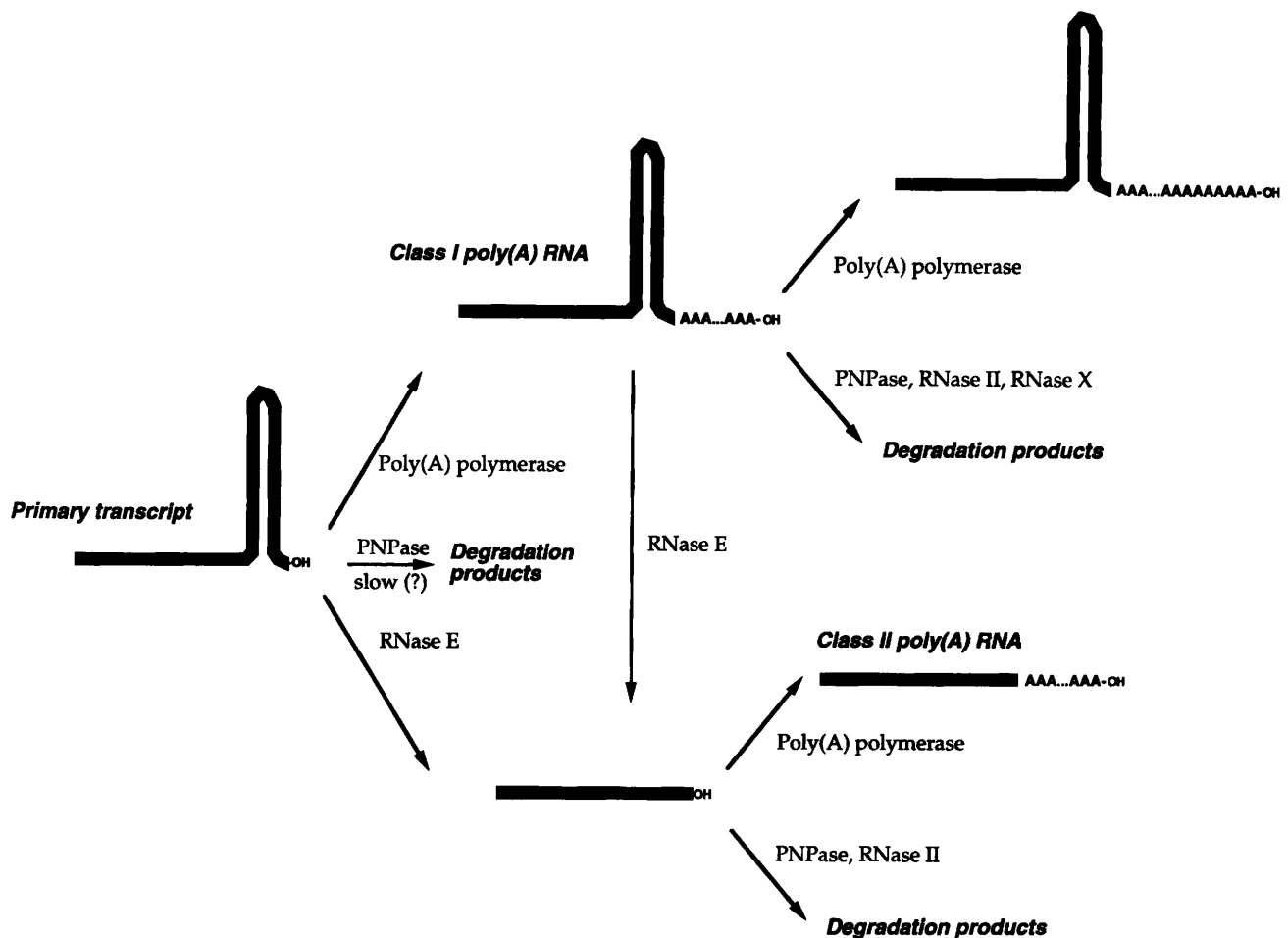
One of the reasons for the relatively slow acceptance of the notion that polyadenylation of bacterial mRNA is a process of physiological significance may have been the fact that many of the earlier studies on poly(A) RNA were done in bacterial species that were biochemically and genetically less well-characterized than *E. coli*. There was therefore an important need to initiate studies of mRNA polyadenylation in *E. coli* on the molecular level. For this purpose, we chose two of the biochemically and genetically best-characterized *E. coli* mRNAs as subjects for the study of mRNA polyadenylation: the outer-membrane lipoprotein mRNA (Taljanidisz *et al.*, 1987), encoded by *lpp*, and the mRNA for the  $\alpha$ -subunit of tryptophan synthase (Karnik *et al.*, 1987), encoded by *trpA*. *lpp* is expressed constitutively to produce the most abundant and one of the most stable mRNAs in *E. coli* and its product is a structural protein that is secreted through the cytoplasmic membrane. *trpA*, on the other hand, is part of a polycistronic biosynthetic operon under repressor and transcription attenuation control and has the short half-life typical of most *E. coli* mRNAs. These specific mRNAs, expressed from plasmids, were characterized by (i) their ability to bind to oligo(dT)-cellulose and the loss of that ability by treatment with RNase H and oligo(dT), (ii) the isolation and sizing of the poly(A) tracts after digestion with RNase A and  $T_1$ , and (iii) their ability to serve as templates for the oligo(dT)-dependent synthesis of cDNA by reverse transcriptase. Parallel studies were carried out on newly synthesized mRNA identified by *in vivo* pulse-labelling, on the steady state mRNA population, isolated by hybrid selection with specific probes, followed by 3'-end labelling, and on RNA synthesized in permeable cells, described in the next section. The results obtained with *lpp* mRNA (Taljanidisz *et al.*, 1987) and with *trpA* mRNA (Karnik *et al.*, 1987) are summarized in Table 1.

Taking advantage of the elevated levels of poly(A) RNA in *E. coli pnp rnb* mutant strains (see below), DNA complementary to polyadenylated lipoprotein mRNA, encoded by the *lpp* gene, was successfully synthesized using avian myeloblastosis virus reverse transcriptase and an oligo(dT)-containing primer. By amplifying the cDNA by PCR and appropriate oligonucleotide primers, it was possible to clone the DNA complementary to the 3'-end of *lpp* mRNA in pUC18 and to determine its sequence (Cao & Sarkar, 1992a). Four clones containing the C-terminal coding region of *lpp* mRNA were obtained and were found to contain poly(A) tracts at either of two sites in the 3'-terminal untranslated region of the transcript, suggesting that there are at least two classes of polyadenylated transcripts (Fig. 1). In class I transcripts, the polyadenylate tract is attached at the putative  $\rho$ -independent transcription termination site of *lpp* mRNA (Nakamura *et al.*, 1980), whereas in class II transcripts, the polyadenylate moiety is attached to the residue just preceding the terminal stem-loop of the primary transcript (Nakamura *et al.*, 1980), thus replacing the  $\rho$ -independent transcription terminator (Cao & Sarkar, 1992a). The possibility that class II polyadenylated cDNA are an artefact produced by oligo(dT) priming at the A-rich region at the base of the terminal stem-loop is ruled out by the observation that the length of many of the cloned poly(A) tracts exceed the length of the oligo(dT) primers (Cao & Sarkar, 1992a, 1993). An important implication of this work, which represented the first molecular description of a bacterial poly(A) RNA, is that mRNA polyadenylation is not necessarily coupled directly to transcription termination, but may occur post-transcriptionally as one of a complex set of mRNA-processing events, which also include endonucleolytic and exonucleolytic cleavage reactions (Cao & Sarkar, 1992a). More recently, the site of polyadenylation of another *E. coli* mRNA, *rpsO* mRNA encoding ribosomal protein S15, was elucidated using a similar approach, except that the *E. coli* strain used was also deficient in the endonuclease RNase E, encoded by *rne* (Hajnsdorf *et al.*, 1995). Of 33 cDNA clones analysed, 32 were of class I (Hajnsdorf *et al.*, 1995). The absence of class II poly(A) RNAs in this case is not surprising since the *E. coli* strain that served as the source of mRNA was deficient in RNase E, the enzyme thought to be responsible for endonucleolytic cleavage just upstream of the stem-loop of the  $\rho$ -independent terminator structure. In contrast, Hajnsdorf *et al.* (1995)

**Table 1.** Degree of polyadenylation of *E. coli trpA* mRNA and *lpp* mRNA

Type of RNA analysed	<i>trpA</i> mRNA		<i>lpp</i> mRNA	
	Percentage polyadenylated	Length of poly(A) tract (nt)	Percentage polyadenylated	Length of poly(A) tract (nt)
Steady state	36	9	47	9
Newly synthesized	48	15–20	42	10–15
<i>In vitro</i> synthesized	45	14	50	15





**Fig. 2.** Postulated steps in the processing of primary transcripts produced by rho-independent transcription termination. RNase X refers to unknown 3'-exonucleases. See text for discussion.

the degradation of newly synthesized RNA in permeabilized cells of *B. brevis* (Sarkar & Paulus, 1975), and that polyadenylated mRNA is relatively resistant to its action (Gopalakrishna & Sarkar, 1981), suggests that the role of mRNA polyadenylation in *B. brevis* may primarily be protection against exonucleolytic degradation. Similar experiments have not yet been carried out in *B. subtilis*. Important questions that need to be addressed concern the role of the 3',5'-GMP-sensitive exonuclease in mRNA degradation in *B. subtilis* and the identity of its natural allosteric ligand, 3',5'-GMP not being found in *Bacillus* species at appreciable concentrations (Sarkar & Paulus, 1975).

### Synthesis and polyadenylation of mRNA in permeabilized *E. coli* cells

Cells of *E. coli* made permeable to nucleotides and other small molecules by treatment with toluene are capable of synthesizing poly(A) RNA from nucleoside triphosphate precursors, with 10–15% of the newly synthesized RNA being polyadenylated (Gopalakrishna & Sarkar, 1983). The poly(A) tracts associated with the newly synthesized

RNA were similar in their general characteristics to those described earlier after pulse-labelling of growing *E. coli* cells (Nakazato *et al.*, 1975). Moreover, it was possible to effect the synthesis of *lpp* mRNA (Taljanidisz *et al.*, 1987) and *trpA* mRNA (Karnik *et al.*, 1987) by using permeable cells of *E. coli* transformed with a plasmid carrying the appropriate gene. Such permeable cell systems may be useful as the first step in defining the enzymology of mRNA polyadenylation, because they allow the study of polyadenylation of pre-existing mRNAs by carrying out the synthesis in the presence of the RNA polymerase inhibitors rifampicin or streptolydigin (G.-j. Cao & N. Sarkar, unpublished results). A cell-free system for specific polyadenylation of mRNA has not yet been developed.

### PAPs from bacteria

As early as 1962, an enzyme that polymerizes adenylate residues on an RNA primer, PAP, was discovered and partially purified from *E. coli* (August *et al.*, 1962). A number of other purification procedures which led to PAP preparation with different properties were described

subsequently (Schäfer *et al.*, 1972; Sippel, 1973; Ramanarayanan & Srinivasan, 1976). The recent progress in the characterization of bacterial poly(A) RNA again focused attention on PAP as the source of the poly(A) moieties at the 3'-end of *E. coli* mRNA. To evaluate the role of polyadenylation in bacteria, it was important to identify the gene encoding PAP. To this end, the major PAP (PAP I) of *E. coli* was purified and its 25 residue N-terminal amino acid sequence was used to design primers for the amplification of the corresponding coding region by PCR from an *E. coli* DNA template (Cao & Sarkar, 1992b). A 74 bp DNA segment was obtained that matched a region in the *pcnB* locus of *E. coli*, a gene that had originally been identified as controlling plasmid copy-number (Lopilato *et al.*, 1986), and was subsequently cloned and sequenced (Liu & Parkinson, 1989). Confirmation that the *pcnB* locus encodes PAP was provided by the observation that a bacterial strain transformed with an inducible expression vector carrying *pcnB* as a translational fusion produced 300-fold elevated levels of PAP upon induction (Cao & Sarkar, 1992b).

Sequence comparison of the deduced PcnB product with other proteins reveals a region with high similarity to a segment of *E. coli* tRNA nucleotidyltransferase (Masters *et al.*, 1990) that may be an RNA binding site. The properties of purified *E. coli* PAP are similar to those reported for the eukaryotic enzyme involved in the polyadenylation of mRNA (Gershon *et al.*, 1991; Lingner *et al.*, 1991; Raabe *et al.*, 1991; Wahle, 1991; Wahle *et al.*, 1991): both enzymes are monomeric, have a strong tendency to aggregate under certain ionic conditions, have a pH optimum near pH 8, require Mg<sup>2+</sup> or Mn<sup>2+</sup> for activity, and are relatively unspecific with respect to the primer for poly(A) synthesis. On the other hand, in spite of the many similarities in the properties of the PAPs from prokaryotes and eukaryotes, there is no obvious sequence similarity between the *E. coli* PAP and the corresponding enzymes from calf thymus (Raabe *et al.*, 1991), vaccinia virus (Gershon *et al.*, 1991) and yeast (Lingner *et al.*, 1991).

The *pcnB* gene encoding PAP is not essential for *E. coli* survival; indeed, mutants deleted for *pcnB* show no consistent growth disadvantages (Liu & Parkinson, 1989; Masters *et al.*, 1993). Although the polyadenylation of *rpsO* mRNA was completely suppressed in *pcnB* mutants (Hajnsdorf *et al.*, 1995), the mean level of mRNA polyadenylation was reduced by less than 50% (Kalapos *et al.*, 1994). This suggests the presence in *E. coli* of multiple PAPs with some functional overlap, a situation similar to that of the 3'-exonucleases (Kelly & Deutscher, 1992; Reuven & Deutscher, 1993a). *E. coli* deficient in both RNase II and PNPase are non-viable (Donovan & Kushner, 1986) and it is possible that, analogously, *E. coli* deficient for a second poly(A)-polymerizing activity would suffer a growth disadvantage or even fail to survive. Indeed, a PAP activity that differs from the *pcnB*-encoded enzyme, was discovered in the course of studies of terminal adenylatetransferase activities in *E. coli* strains with a disrupted tRNA nucleotidyltransferase gene (Reuven & Deutscher, 1993b). In a systematic search for

additional PAPs in *E. coli* strains with a deletion of the *pcnB* locus (Liu & Parkinson, 1991), a second PAP (PAP II) was identified and purified about 1000-fold (Kalapos *et al.*, 1994). While PAP I encoded by the *pcnB* gene has a  $M_r$  of 53 000, PAP II is a smaller protein with a native  $M_r$  of ~ 35 000; however, the properties of the two enzymes are similar, except that PAP II is more thermolabile and sensitive to salt concentrations (Kalapos *et al.*, 1994). The gene for PAP II has recently been cloned and over-expressed as inclusion bodies, which on renaturation exhibit PAP activity (Cao *et al.*, 1996). The relative contributions of PAP I and PAP II to mRNA metabolism of wild-type *E. coli* is of great interest and should be possible to define when deletion mutants of the gene encoding PAP II become available. Preliminary studies suggested that *E. coli* mutants lacking the genes encoding PAP I and PAP II are non-viable (Cao & Sarkar, 1995).

Although PAPs have not been studied in other bacterial species, genes with significant homology to *E. coli pcnB* have been found in *B. subtilis* (A. V. Sorokin & others, GenBank accession no. L47709), *Mycobacterium leprae* (E. DeRossi & others, GenBank accession no. L39923) and *Haemophilus influenzae* (Fleischmann *et al.*, 1995). It is interesting that the genome of the parasitic bacterium *Mycoplasma genitalium* (Fraser *et al.*, 1995) lacks coding sequences homologous to PAPs or any of the known *E. coli* mRNA-degrading enzymes.

Very little is known about the mechanism of polyadenylation of bacterial mRNA. There is no evidence for a consensus sequence that serves as a polyadenylation signal. How bacterial PAPs achieve selectivity for mRNA while avoiding polyadenylation of rRNAs and tRNAs, how polyadenylation is coordinated with transcription termination, and how the desired length of poly(A) tail is achieved are all questions that have not yet been addressed.

### Possible roles of mRNA polyadenylation

The role of mRNA polyadenylation has been difficult to define in molecular terms even in the eukaryotic field where this process has been known for 25 years. In bacteria, functional analysis is often facilitated by study of the phenotypic consequences of mutations affecting a process. Identification of *pcnB* as the gene encoding PAP I has helped to define its function (Cao & Sarkar, 1992b). *pcnB* mutations were originally identified as host mutations that reduce the copy-number of ColE1-type plasmids (Lopilato *et al.*, 1986), and thus polyadenylation by PAP I clearly has a role in regulating plasmid copy-number. Copy-number-control of ColE1 plasmids involves an antisense RNA (RNA I), which, by annealing to a primer RNA, prevents its use as a replication primer. An unrelated group of plasmids, IncFII, in which copy-number is also regulated, albeit differently, by an antisense RNA, also exhibits reduced copy-number in *pcnB* mutants (Masters *et al.*, 1993). In the normal ColE1 replication cycle, RNA I is polyadenylated (Xu *et al.*, 1993; Xu & Cohen, 1995) and, after processing by RNase E, is rapidly degraded, with a half-life typical of mRNAs (He *et al.*,

1993; Xu *et al.*, 1993; Xu & Cohen, 1995). In *pcnB* mutants, the lack of polyadenylation leads to stabilization of RNA I after processing by RNase E, thus causing repression of plasmid replication (He *et al.*, 1993; Xu *et al.*, 1993; Xu & Cohen, 1995). The degradation of RNA I is also much reduced in *pnp* mutants, suggesting that PNPase has a major role in the degradation of polyadenylated processed RNA I (Cohen, 1995; Xu & Cohen, 1995). These studies indicate that one function of RNA polyadenylation may be to facilitate the rapid degradation of RNA molecules that terminate in a stem-loop structure by 3'-exonucleases such as PNPase (Cohen, 1995), which are known to be impeded by stem-loop structures (Guarneros & Portier, 1990; McLaren *et al.*, 1991).

Does PAP I have a role only in plasmid replication or also in host mRNA degradation? There is some evidence that poly(A) tracts do indeed destabilize certain types of mRNA. This includes the observation by O'Hara *et al.* (1995) that the half-lives of *lpp*, *trx A* and *omp A* mRNAs are significantly increased by deletion of *pcnB* when studied in a *pnp rnb rne* background. Similarly, *rpsO* mRNA is stabilized by *pcnB* deletions in a *pnp rnb rne* background (Hajnsdorf *et al.*, 1995). In both cases, mRNA stabilization was associated with a reduced level of polyadenylation. It should be noted that these results were obtained in the absence of RNase E and that the 3'-ends of most mRNAs therefore consist of the stem-loop structures of the  $\rho$ -independent transcription terminators, as shown in the top portion of Fig. 2. Since exonucleolytic degradation of RNA by PNPase and RNase II (Guarneros & Portier, 1990; McLaren *et al.*, 1991; Coburn & Mackie, 1996) is strongly impeded by terminal stem-loop structures, polyadenylation of primary transcripts resulting from  $\rho$ -independent transcription termination would provide a 'toe-hold' for launching the processive exonucleolytic degradation by these enzymes (Cohen, 1995; Coburn & Mackie, 1996) (Fig. 2).

However, the picture of RNA degradation at the 3'-ends of mRNA is far from complete. It must be noted that the effects of *pcnB* deletions described in the preceding paragraph were observed only in *rne* mutants and not in a wild-type background and that mRNA destabilization by polyadenylation was much less pronounced (O'Hara *et al.*, 1995) or absent (Hajnsdorf *et al.*, 1995) in a wild-type background. Moreover, the observation that the absence of RNase II can stabilize transcripts (Hajnsdorf *et al.*, 1994; Pepe *et al.*, 1994; Py *et al.*, 1996), the discovery of specific complexes involving RNase E and PNPase (Carpousis *et al.*, 1994; Py *et al.*, 1994), as well as the fact that mRNA degradation occurs efficiently even in the absence of RNase E, PNPase and RNase II (Hajnsdorf *et al.*, 1995; O'Hara *et al.*, 1995) suggests that additional factors must be taken into consideration before the roles of PAPs and ribonucleases in mRNA degradation can be fully understood.

Another major uncertainty concerns the relative roles of the two PAPs found in *E. coli* (Kalapos *et al.*, 1994). The fact that deletion of the gene for PAP I, *pcnB*, is not lethal (Liu & Parkinson, 1989; Masters *et al.*, 1993) suggests

that, if polyadenylation is an important function, there must be some functional overlap between PAP I and PAP II. However, it is clear that some functions are unique to PAP I, such as polyadenylation of the antisense RNAs involved in the control of plasmid replication (He *et al.*, 1993; Xu *et al.*, 1993; Xu & Cohen, 1995) and the destabilization of *rpsO* mRNA (Hajnsdorf *et al.*, 1995). It is possible that the two *E. coli* PAPs specialize in the polyadenylation of different classes of mRNA, perhaps with some overlap in specificity, or that the enzymes differ in their preference for poly(A) chain initiation and elongation. The examination of the phenotype of *E. coli* mutants with defects in either or both of the PAP genes should help in resolving these uncertainties.

### Concluding remarks

In spite of its initial neglect, the field of bacterial mRNA polyadenylation is making rapid progress and is beginning to uncover aspects of bacterial RNA metabolism that had not been anticipated. The recent definition of bacterial mRNA polyadenylation on the molecular and genetic levels summarized in this review provided the impetus for the recent advances in the field by demonstrating that the evolution of mRNA polyadenylation predates the emergence of eukaryotes and is therefore a respectable subject for investigation. Although the study of bacterial mRNA polyadenylation is still in its infancy, the possibility of genetic analysis promises rapid advances, and it is not unreasonable to anticipate that the role of mRNA polyadenylation in bacteria will have become clear long before the enormous complexities of eukaryotic poly(A) RNA have been sorted out. Indeed it is quite possible that the lessons learned from the study of bacterial RNA polyadenylation may pave the way for future advances in the eukaryotic field.

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