The Poly(A) Tail of mRNAs: Bodyguard in Eukaryotes, Scavenger in Bacteria

Minireview

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In eukaryotes, poly(A) tails usually act as stabilizers of intact mRNAs, whereas in *E. coli* they serve to accelerate the destruction of fragments. The mechanisms underlying these contrasting effects of the same RNA modification are discussed.

Four decades ago, closely related enzymes that synthesize polyadenylate from ATP (poly(A) polymerases, or PAPs) were discovered in both higher organisms and *Escherichia coli* (for a comprehensive review on polyadenylation, including historical aspects, see Edmonds, 2002). Soon afterwards, PAP-mediated polyadenylation of 3′ mRNA ends was recognized as a nearly universal feature of eukaryotic mRNAs. In contrast, despite pioneering reports on *E. coli*, *Bacillus subtilis*, and the archaea, mRNA polyadenylation in bacteria was long regarded as anecdotal (Sarkar, 1997). This was due to the fact that, unlike in eukaryotic cells, only a small fraction of mRNAs is measurably polyadenylated at a given time in wild-type *E. coli* cells. We now know that this scarcity does not mean that polyadenylation is rare, but simply that the relative activities of PAP and of exonucleases that remove poly(A) tails in the cell are such that poly(A) tail length is kept to a minimum. Whereas the existence of bacterial polyadenylation is no longer disputed, the metabolism of poly(A) tails and, even more so, their biological role—particularly their impact on mRNA stability—appears at almost complete variance in eubacteria as compared to eukaryotes. Focusing on *E. coli*, which has been most studied in this respect, we discuss these differences in relation to the mechanisms of gene expression in eubacteria and in eukaryotes (for a related discussion with a different scope, see Carpousis et al., 1999).

Polyadenylation of eukaryotic mRNA initially occurs in the nucleus, where it is tightly coupled to primary transcript cleavage and transcription termination (see Proudfoot et al., 2002, for review). Normally, this initial polyadenylation step is the only such event that occurs during the life span of the mRNA. Deadenylation usually occurs in the cytoplasm, and it is irreversible (Figure 1A). Interestingly, despite the variety of 3′→5′ exonucleases present in the cytoplasm of eukaryotic cells (cf. the yeast exosome), deadenylation appears to be carried out by

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dedicated exonucleases (Tucker et al., 2001). In contrast, synthesis and degradation of poly(A) take place in the same compartment in *E. coli*, due to the absence of a nuclear membrane. Moreover, polyadenylation can occur not only on mature mRNAs, but also on fragments resulting from endo- or exonucleolytic degradation, and even on stable RNAs or their precursors. In fact, any accessible 3′ end seems to be a potential target for polyadenylation in *E. coli* (Figure 1B; Haugel-Nielsen et al., 1996). This difference can be ascribed to the fact that, in contrast to eukaryotic PAP that acts within the transcription termination complex (Figure 1A), bacterial PAP operates on its own or within complexes involved in mRNA decay (see below). Finally, no strictly poly(A)-specific exonuclease is known in *E. coli*. Rather, the 3′→5′ exoribonucleases, RNase II and polynucleotide phosphorylase (PNPase), which are involved in the degradation of the mRNA body, also appear to be capable of removing the poly(A) tails.

A closer look at available data somewhat attenuates this contrasting picture, however. During the early development of many metazoan embryos, dormant mRNAs of maternal origin are polyadenylated in the cytoplasm, and recently one specific case of cytoplasmic polyadenylation has been documented in yeast as well (see Saitoh et al., 2002, and references therein). Therefore, eukaryotic mRNAs are eventually deadenylated and readenylated within the same cellular compartment, like bacterial mRNAs. Second, even nuclear polyadenylation can take place independently of cleavage, also similar to the situation in prokaryotes. For example, extended mRNAs that result from terminator readthrough can be trimmed back by the exosome and then polyadenylated de novo in yeast (Torchet et al., 2002). Third, the *E. coli* enzymes RNase II and PNPase may not be truly equivalent in the degradation of poly(A) tails or the bodies of mRNAs. Although RNase II activity surpasses PNPase activity in the cell, it is more sensitive to RNA structure. Therefore, structureless poly(A) tails have a greater chance of being removed by RNase II, whereas the more structured internal regions of mRNAs are primarily degraded by PNPase. In this respect, RNase II and PNPase would be equivalent to the eukaryotic poly-(A)-specific exonucleases and the exosome, respectively. Interestingly, PNPase is regarded as structurally equivalent to the exosome core (Symmons et al., 2002). How far do these formal resemblances between poly(A) metabolism in eukaryotes and eubacteria extend to poly(A) function, particularly when it comes to mRNA decay?

Pathways of mRNA decay differ sharply in eukaryotes and in *E. coli*. In yeast and in higher organisms, the degradation of cytoplasmic mRNAs is normally initiated by poly(A) shortening (for a review on eukaryotic mRNA decay, see Wilusz et al., 2001). This initial step is also usually rate limiting, i.e., the life span of most mRNAs is determined by the time required to deadenylate them (see Tucker et al., 2001, for supporting arguments). Therefore, the poly(A) tail behaves as a stabilizing element. After its removal, the deadenylated mRNA is rap-
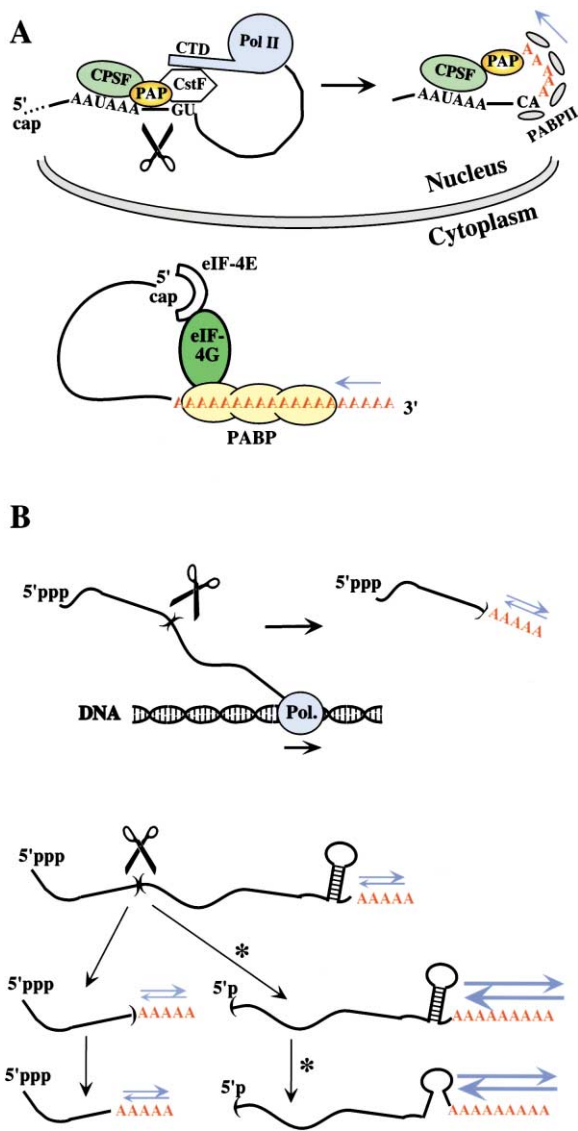
Figure 1. Comparison of mRNA Decay and Poly(A) Metabolism in Eukaryotes and in *E. coli*

(A) In eukaryotes the synthesis of poly(A) tails and their removal from mature mRNAs (blue arrows) occur in different compartments. Synthesis in the nucleus is coupled to primary transcript cleavage and transcription termination. The mammalian factors CPSF and CstF, which recognize a consensus hexamer and a GU-rich motif located upstream and downstream of the cleavage site, respectively, are shown interacting with PAP and with the terminating RNA polymerase (Pol II). Factors CFI and CFII, which also participate in cleavage, are omitted for clarity. The cleaved transcript undergoes PAP-mediated poly(A) synthesis, assisted by Poly(A) Binding Protein II (adapted from Proudfoot et al., 2002). In the cytoplasm, the poly(A) tail interacts with the 5′ end of mRNA via eIF-4G, which binds both the Poly(A) Binding Protein (PABP) and the cap binding factor eIF-4E. The initial and rate-limiting step in mRNA decay is usually the removal of the poly(A) tail by poly(A)-specific nucleases (blue arrow). (B) *E. coli* lacks a nuclear membrane, so poly(A) synthesis and degradation occur in the same compartment (opposed blue arrows). Top: mRNAs may start decaying before RNA polymerase (Pol.) has reached the end of the gene. Decay is usually initiated by the endonuclease RNase E (scissors; the cleavage site is figured by back-to-back parentheses). Since translation occurs on nascent transcripts in *E. coli*, the mRNA remains functional in this case. Bottom: Even when full-length mRNA is produced, the first attack is usually

idly decapped and then degraded by the 5′-3′ exonuclease Xrn1p (in yeast); alternatively (and less frequently), it can enter a 3′→5′ degradation pathway mediated by the cytoplasmic exosome. In contrast, no 5′→3′ exonuclease exists in *E. coli*, and the action of 3′→5′ exonucleases involved in mRNA decay (presumably mostly PNPase) is usually confined to the scavenging of fragments. Indeed, the initial attack on most intact mRNAs is mediated by endonucleases, generally RNase E (for reviews on bacterial mRNA decay, see Coburn and Mackie, 1999; Grunberg-Manago, 1999). As for poly(A) tails, there is ample evidence that, in sharp contrast with the eukaryotic situation, they *destabilize* RNA by facilitating exonucleolytic attack. In vitro and in vivo data suggest that deadenylation is rapid compared to the exonucleolytic degradation of the RNA body (especially if this RNA is structured) and that deadenylation is eventually reversible: poly(A) tails can be repetitively degraded and resynthesized by RNase II/PNPase and PAP, respectively. Thereby, poly(A) tails would provide a "toehold" from which PNPase can reiterate its attacks on the body of the RNA until the structures eventually breathe and can be invaded. This model, which contrasts with the situation in eukaryotes where poly(A) removal is slow and irreversible, nicely explains why, paradoxically, RNase II has been found to behave as an mRNA stabilizing factor; indeed, it would remove poly(A) tails without being able to degrade the rest of the RNA efficiently. One may wonder why poly(A) tails appear more labile than the rest of the mRNA in *E. coli*, whereas the reverse holds in eukaryotes. A reasonable guess is that the structureless poly(A) tails are intrinsically very sensitive to exonucleases but that in vivo they are protected by proteins. Indeed, the Poly(A) Binding Protein (PABP; Figure 1A) inhibits deadenylation in mammalian cell-free assays. Although poly(A) binding proteins exist in *E. coli* as well and although they can protect poly(A) tails against PNPase in vitro (Feng et al., 2001), they may be unable to exert a significant protection in vivo because poly(A) tails never grow long enough to enable them to bind.

Thus, the contrasting impact of polyadenylation on mRNA stability in bacteria as compared to eukaryotes presumably reflects the absence of PABPs on poly(A) tails, plus the fact that deadenylation is readily reversible. As noted above, another distinctive feature of bacteria is the fact that polyadenylation usually affects the degradation of *fragments* only, because the initial attack on most intact mRNAs is endonucleolytic. Studies on the model molecule RNA I suggest that this temporal hierarchy reflect the activation of exonucleolytic degradation by prior endonucleolytic cleavage. The decay of this 108 nt regulatory RNA resembles that of a typical

endonucleolytic. In the top and bottom cases, fragments are scavenged by 3′→5′ exonucleases. Successive cycles of PAP-mediated poly(A) synthesis and PNPase-mediated poly(A) degradation assist this scavenging, particularly when the fragment contains secondary structures (bottom, pathway marked with asterisk). The PAP/PNPase pathway appears to be much more efficient with fragments bearing a 5′ monophosphate end (enlarged blue arrows, pathway marked with asterisk), explaining in part the temporal hierarchy between endo- and exonucleolytic attacks (see text).

mRNA; in particular, it is initiated by an RNase E cleavage 5 nt from the 5′ end. Although this modest truncation is unlikely to affect RNA structure (it does not abrogate biological activity), it is enough to activate very fast decay via the PAP-PNPase pathway (Xu and Cohen, 1995). Other striking examples of ordered endo- and exonucleolytic attack exist (for review, see Régnier and Marujo, 2002). The molecular basis for this ordered action may reside in the unusual properties of RNase E itself. First, this enzyme associates with PNPase in a "degradosome" that also notably comprises an RNA helicase, RhlB. As for PAP, even though it does not copurify with the degradosome, it can interact with RNase E and RhlB in vitro (Raynal and Carpousis, 1999). Second, despite the fact that it is an endonuclease, RNase E preferentially cleaves substrates bearing accessible 5′ monophosphate (5′p) extremities, indicating that it binds specifically to such 5′ ends (Coburn and Mackie, 1999). Conceivably then, by tethering the degradosome to the newly formed 5′p end, an initial RNase E cleavage would bring PNPase and/or PAP into the vicinity of the 3′ end of the same fragment, favoring polyadenylation-degradation cycles (enlarged blue arrows in Figure 1B, pathway marked with asterisk). In this way, the exonucleolytic pathway would be activated by prior endonucleolytic cleavage. Alternatively, this activation may not involve the degradosome. There is in vitro evidence that purified PAP and PNPase, like RNase E, are more active on substrates bearing a 5′p rather than a 5′ppp extremity (Feng and Cohen, 2000; Xu and Cohen, 1995). Although the reason for this preference is unknown, it may contribute to the activation of the PAP-PNPase pathway following RNase E cleavage.

Although the ordered action of endo- and exonucleases is presumably the rule in *E. coli*, it may not be absolute; indeed, PAP seems to play a role in the degradation of some intact mRNAs as well (see Mohanty and Kushner, 1999, and references therein). Moreover, precursors of misfolded "stable" RNAs can also be targets for degradation by the PAP/PNPase-mediated decay; thus, this pathway participates in RNA quality control (Li et al., 2002). Interestingly, another RNA quality control mechanism recently identified in eukaryotes bears some resemblance to PAP/PNPase-mediated decay. Polyadenylated mRNAs lacking a termination codon ("nonstop" mRNAs) are rapidly degraded by the exosome in the yeast cytoplasm (van Hoof et al., 2002). This situation resembles the PNPase/PAP-mediated decay in the sense that the poly(A) tail fails to protect the nonstop mRNA and that it is presumably degraded by the same machinery (i.e., the exosome) as the body of the mRNA. The analogy has its limit, however. It is not known whether deadenylation occurs faster than the degradation of the mRNA body in this case, nor whether it can assist this degradation. Moreover, the poly(A) tail does not appear to act by itself as a toehold for the exosome. Rather, it is the presence of a ribosome stalled at the poly(A) 3′ end that is thought to mediate exosome recruitment, a unique feature of this mechanism.

Future work will show whether the present ideas reflect a more general picture. It is known that in yeast, efficient mRNA degradation occurs not only in the cytoplasm but also in the nucleus; however, the impact of poly(A) tails on nuclear degradation is still unclear. Our knowledge of poly(A) function in bacteria other than *E. coli* is also limited. Interestingly, however, there is evidence that in chloroplasts and mitochondria, poly(A) tails destabilize the mRNAs to which they are appended, as they do in *E. coli*.

The polyadenylation of mRNA 3′ ends constitutes an ancient trait as attested by its occurrence throughout the living world, as well as by the conservation of PAP sequences (Carpousis et al., 1999). In addition to its role in mRNA turnover, polyadenylation plays other essential roles in eukaryotic gene expression that have no equivalent in bacteria, e.g., in transport of mRNAs from the nucleus to the cytoplasm or in translation. The contrasting impact of polyadenylation on mRNA decay in eukaryotes and in bacteria is just another illustration of the remarkable functional versatility of this apparently simple, universal modification of RNA.

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