Review Letter

NON-POLYADENYLATED mRNAs FROM EUKARYOTES

P. K. KATINAKIS, A. SLATER and R. H. BURDON
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

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1. Introduction

The investigation of eukaryotic mRNA has focussed on molecules that possess a 3′-terminal poly(A) segment [1–3] since they can be isolated easily by virtue of their high affinity for oligo(dT)–cellulose [4,5], poly(U)–Sepharose [6,7] or Millipore filters [8]. The possible functions of the poly(A) tracts in terms of nuclear and cytoplasmic events have been extensively reviewed [2,3,10–13]. Recently, however, a considerable body of evidence has accumulated suggesting the presence of mRNA molecules lacking poly(A) (poly(A)$^-$ mRNA) as judged by their failure to bind to Millipore filters [8], oligo(dT)–cellulose [14–16] or poly(U)–Sepharose [6,17]. The purpose of this short review is to assess present knowledge of these poly(A)$^-$ mRNAs.

2. Detection of poly(A)$^-$ mRNAs

During the early 1970s it was assumed that most of the eukaryotic mRNA molecules, with the exception of histone mRNA, possess a poly(A) segment attached at the 3′-end [2,3,6,18–20]. In these studies, however, little attention was given to a substantial proportion (20–35%) of the rapidly labelled polysome-associated messenger-like RNA which appears to lack poly(A).

A more detailed study was carried out by Penman and colleagues, who reported that about 30% and 45%, respectively, of HeLa [15] and Aedes [16] cell mRNA lack poly(A), when the cells were labelled in the presence of rRNA synthesis inhibitors such as actinomycin D [21] or fluorouridine [22]. The relative amounts of labelled poly(A)$^+$ and poly(A)$^-$ mRNAs in eukaryotic cells might not reflect the real situation, since it has been reported that actinomycin D at low levels may inhibit either the transport of some mRNAs [23] or the synthesis of some hnRNA species [24]. However, Greenberg [25,26], using Cs_2SO_4 density gradients isolated mRNP particles from L-cell polysomes and found that about 30% of the labelled mRNA lacked poly(A) regardless of the presence or absence of actinomycin D during the short labelling period. Also, about 50% of the labelled mRNA from mouse kidney, purified by benzoylated-cellulose (BC-cellulose) chromatography, was found to lack poly(A), regardless of the use of actinomycin D during labelling [27]. The sea urchin system is a better system for the study of labelled mRNA, since rRNA is not synthesised at all during the early stages of development [28]. Nemer et al. [29] reported that about 40% of the rapidly labelled mRNA from sea urchin embryos (excluding histone mRNA) lacks poly(A). In all the above studies the poly(A)$^-$ mRNAs were shown to be functionally associated with polysomes as judged by their release from polysomes by EDTA treatment. Using the same criteria poly(A)$^-$ mRNAs have been also detected in mouse brain [30], BHK-21 cells [31], Friend cells [32] and plant cells [17].

The techniques of Cs_2SO_4 density gradient centrifugation [25,26] and BC-cellulose chromatography [27,30,33] are useful for the isolation of the total mass of mRNA independent of the occurrence of poly(A). The former method relies on the existence of proteins which remain tightly and specifically bound to mRNA [34,35]. Thus, mRNP particles band at a lower density than rRNP particles in Cs_2SO_4 buoyant density gradients [25,26]. On the other hand, although BC-cellulose appears to be effective for the separation of mRNA from rRNA the physical basis of this separation is not fully understood [30,33]. The use of BC-cellulose has made possible a quantitative estimation (on a mass

basis) of the steady-state total polysomal mRNA (poly(A)$^+$ and poly(A)$^-$). In this way it was found that about 70% and 40% of the mouse kidney [27] and brain [30] mRNA, respectively, lack poly(A).

3. The distinction between poly(A)$^+$ and poly(A)$^-$ mRNA species

Poly(A)$^+$ mRNA can be defined as the fraction of polysomal mRNA species which is selected by affinity chromatography on oligo(dT)–cellulose, poly(U)–Sepharose or Millipore filters. However, Millipore filters are only capable of retaining poly(A)$^+$ mRNAs which contain poly(A) tracts larger than 50 nucleotides [36,38]. Oligo(dT)–cellulose will retain poly(A)$^+$ mRNAs which contain poly(A) tracts larger than 20 nucleotides long [36,37], while poly(U)–Sepharose will retain poly(A)$^+$ mRNAs with a poly(A) sequence larger than about 10–15 nucleotides long [39,40]. Therefore, the proportion of mRNA in the poly(A)$^+$ and poly(A)$^-$ mRNA fractions will vary with the technique used, since it has been found that poly(A)$^+$ mRNA species have a somewhat heterogeneous poly(A) size distribution of between 20 and 250 nucleotides long [3,9,36–38]. For instance, a significant proportion of the β-actin mRNA fails to bind to oligo(dT)–cellulose but some of this non-bound material does bind to poly(U)–Sepharose [41]. However, a large proportion does not bind and this represents a poly(A)$^-$ mRNA class [41]. It might therefore be expected that some, or even all, of the poly(A)$^-$ mRNAs will contain small oligo(A) segments (less than 10 nucleotides) which do not bind to poly(U)–Sepharose under the conditions used. Recently, Levenson and Marcu [42] and Morrison et al. [38] have reported that using oligo(dT)–cellulose at 4°C (as opposed to 20°C) it was possible to isolate poly(A)$^+$ mRNA from *Xenopus* and mouse neuroblastoma which has a poly(A) tract larger than 8–10 nucleotides, but again about 32% of the mRNA translatable activity was found in the poly(A)$^-$ mRNA fraction [38]. Also, the work of Brandhorst et al. [43] suggested that more than 85% of the sea urchin poly(A)$^-$ mRNAs lack detectable poly(A) tracts larger than 8 nucleotides long. On the other hand, Van Ness et al. [30] reported that only one oligo(A) tract of about 20 nucleotides long per 100 poly(A)$^-$ mRNA molecules from mouse brain was observed, implying that most of the poly(A)$^-$ mRNAs do not contain oligo(A) tracts. Furthermore, Milcarek [44] reported that HeLa cell poly(A)$^-$ mRNAs contain an oligo(A) tract (6–8 nucleotides long) which is internally located rather than at the 3′-end, which may account for the 4% of hybridizable poly([^{3}H]U) material detected in an earlier study [15].

Therefore, it appears that the vast majority of the poly(A)$^-$ mRNA defined above does not contain detectable poly(A) tracts. Hence, it might be expected that the poly(A)$^-$ mRNA would have unique properties distinct from those of poly(A)$^+$ mRNA.

4. Properties of poly(A)$^-$ mRNA species

The relative amounts of rapidly labelled polysomal poly(A)$^+$ and poly(A)$^-$ mRNAs appear to vary widely (20–90%) being dependent on the cell type examined [15,16,25,27,45–47], the stage of development [48], the stage of proliferation [49], or even the cytoplasmic location [50].

Polysomal rapidly labelled poly(A)$^+$ mRNA species from sea urchin embryo appears to be more fully loaded on ribosomes than the non-histone poly(A)$^-$ mRNAs [51]. On the other hand, trout testis polysomes appear to be proportionally more loaded with poly(A)$^-$ protamine mRNA compared to poly(A)$^+$ protamine mRNA [52]. However, the results of Brandhorst et al. [43] and Dworkin et al. [53] suggest that both sea urchin embryo poly(A)$^+$ and poly(A)$^-$ mRNAs initiate almost equally with reticulocyte ribosomes. Further, it has been suggested that the variations in proportions of rapidly labelled polysomal sea urchin poly(A)$^-$ and poly(A)$^+$ mRNAs during development may be due to differences in the relative rates of initiation, with the poly(A)$^+$ mRNA being loaded at a higher rate compared with poly(A)$^-$ mRNAs [54].

Although the 3′-ends of polysomal poly(A)$^+$ and poly(A)$^-$ mRNAs from see urchin embryo appear to be different, it seems that the 5′-terminal structure and extent of 'capping' are similar in both mRNA classes [55,56]. Evidence for possible existence of the 'cap' structure in poly(A)$^-$ mRNA from the HeLa cells [57] and L-cells [58], has also been reported.

The metabolic behaviour of sea urchin embryo poly(A)$^+$ and poly(A)$^-$ mRNAs appears to be similar

as judged by their similar rates of entry and decay in the cytoplasm [51]. This situation also appears to hold for HeLa poly(A)$^-$ and poly(A)$^+$ mRNAs [15]. However, it has been reported that poly(A)$^-$ mRNA from mouse sarcoma enters the cytoplasm faster than poly(A)$^+$ mRNA [59]. Also a subclass of BHK-21 cell [31] and Friend cell [32] poly(A)$^-$ mRNAs, having an affinity for poly(A)–Sepharose (poly(A)$^-$u$^+$mRNA) exits the nuclei much faster than poly(A)$^+$ mRNA species.

Furthermore, the base composition of non-histone poly(A)$^-$ mRNAs from sea urchin embryo [29] and spinach chloroplasts [60] seems to be distinct from that of poly(A)$^+$ mRNAs, having an unusually high proportion (about 34%) of uridylate residues. High levels (about 31%) of uridylate residues have also been reported for the poly(A)$^-$u$^+$mRNAs from BHK-21 [31] and Friend [32] cells.

5. Functional integrity of poly(A)$^-$ mRNAs

That the cytoplasmic poly(A)$^-$ mRNA may function as a messenger was first suggested by the work of Lodish et al. [61], who reported that poly(A)$^+$ and poly(A)$^-$ mRNAs from slime moulds direct the synthesis of polypeptides in a wheat germ cell-free protein synthesising system. Upon analysis of the products on one dimensional SDS–polyacrylamide gel electrophoresis both types of mRNA directed the synthesis of actin and other polypeptides with similar electrophoretic mobilities. Similar results have also been reported by other workers, who found that polysomal poly(A)$^+$ and poly(A)$^-$ mRNA from mouse sarcoma ascites [62,63], chicken muscle cells [64,65], sea urchin embryos [66,67], *Xenopus* ovaries [66,68] and plant cells [17,69], directed the synthesis of polypeptides with similar mobilities. The extent of these similarities was examined in more detail using the more sensitive technique of two-dimensional gel electrophoresis [70]. Kaufmann et al. [71] have shown that when the protein products encoded in vitro by HeLa cell poly(A)$^+$ and poly(A)$^-$ mRNAs were analyzed by two-dimensional gel electrophoresis, three classes of polypeptides were identified:

(i) A class of about 10 polypeptides was detected among only the poly(A)$^-$ mRNA products;
(ii) A class of about 40 polypeptides was produced only by poly(A)$^+$ mRNA;
(iii) A class of about 10 proteins was coded for by both poly(A)$^+$ and poly(A)$^-$ mRNAs.

This situation appears to hold for the protein products directed by both mouse brain poly(A)$^+$ and poly(A)$^-$ mRNAs [72]. Also, the results of Brandhorst et al. [43] revealed a similar situation for the proteins encoded in vitro by sea urchin embryo poly(A)$^+$ and poly(A)$^-$ mRNAs. In this latter study, the vast majority of in vitro products coded by both poly(A)$^+$ and poly(A)$^-$ mRNA were identical to proteins labelled in vivo, demonstrating that the poly(A)$^-$ mRNAs are functional in vivo.

The cell-free protein synthesising systems almost certainly only allow the detection of the most 'abundant' mRNAs [64]. Therefore, it is likely that the polypeptides observed in the above studies are coded for by abundant mRNAs. Since a number of these polypeptides are coded in vitro by both poly(A)$^+$ and poly(A)$^-$ mRNAs, it is likely that some of the abundant mRNA sequences may be present in adenylated and non-adenylated forms. Indeed, Kaufmann et al. [71] have shown that a fraction (10%) of cDNA representing abundant HeLa poly(A)$^+$ mRNAs is also present in poly(A)$^-$ mRNAs at a relatively high concentration. In addition to these studies of unidentified proteins, there are a number of well characterized proteins which appear to be coded by mRNAs present in both poly(A)$^+$ and poly(A)$^-$ form (e.g., protamine [73,74], histone [42,65,66,68], casein [75,76], ovalbumin [77,78], β-actin [41], albumin [79], human β-globin [80]). The existence of proteins encoded only by poly(A)$^-$ mRNA in vitro, suggests that there are some poly(A)$^-$ mRNA sequences which are present in high concentration but are not present in abundant poly(A)$^+$ mRNA. Indeed a subclass of HeLa cell poly(A)$^-$ mRNA sequences has been shown by hybridization studies to exist in high concentration which is not present in the abundant class of poly(A)$^+$ mRNA [44]. These results are compatible with the results of Katinakis and Burdon [32] who reported that a sub-class of Friend cell poly(A)$^-$ mRNA (poly(A)$^-$u$^+$RNA) appears to direct the synthesis of some proteins which are distinct from those directed by poly(A)$^+$ mRNA. On the other hand, a number of proteins are encoded only by poly(A)$^+$ mRNA in vitro [43,71,72] and some well characterized proteins (e.g., γ-actin [41], cellulase [82], leghaemoglobin [81], sheep and mouse α- and β-globin [83]), are known to be encoded only by poly(A)$^+$ mRNA.

6. Sequence similarities between poly(A)$^-$ and poly(A)$^+$ mRNAs

Although a proportion of the 'abundant' poly(A)$^+$ and poly(A)$^-$ mRNAs appears to contain similar sequences, the low abundance high sequence complexity class of both poly(A)$^+$ and poly(A)$^-$ mRNAs appears to contain distinct sequences. Grady et al. [84] prepared DNA sequence probes complementary to the cellular RNA of mouse liver and cultured polyoma transformed mouse cells (PyAC/N). This was achieved by hybridizing highly labelled single-copy mouse liver or PyAC/N cell DNA to total cellular RNA from both cell types and isolating the hybrids. Using the DNA from these hybrids (expressed DNA) they examined the sequence complexities of polysomal poly(A)$^+$ and poly(A)$^-$ mRNAs. This method is particularly useful because it can detect the low abundance high complexity class of mRNA sequences (rare mRNAs) [85]. They found that about 40% of the mRNA sequence complexity resides in poly(A)$^-$ mRNA in both cell types examined. Saturation hybridization experiments show that these poly(A)$^+$ and poly(A)$^-$ mRNAs share very little or no sequence homology [84]. In a similar study, Chikaraishi [72] and Van Ness et al. [30] using highly labelled mouse brain unique DNA sequences have shown that polysomal poly(A)$^-$ and poly(A)$^+$ mRNAs from mouse brain contain a non-overlapping set of sequences, with poly(A)$^-$ mRNA representing about 50% of the mRNA sequence complexity. By combining the results of Nemer et al. [29], Galau et al. [86,87] and McColl and Aronson [88] a much higher value (about 90%) of the sea urchin mRNA sequence complexity appears to reside in the poly(A)$^-$ mRNA fraction. However, it is important to note that this situation does not hold in every cell type examined so far. Plant [89] and yeast [85] polysomal poly(A)$^+$ and poly(A)$^-$ mRNAs appear to share the same sequences using a similar experimental approach to that described above.

7. Why poly(A)$^+$ and poly(A)$^-$ mRNAs?

The biological significance of the coexistence of poly(A)$^+$ and poly(A)$^-$ mRNAs is still obscure. Nevertheless, a close examination of the data presented by Ruderman and Pardue [66] and James and Tata [90] suggest that the role of 'abundant' poly(A)$^-$ mRNAs is most significant during the early developmental stages.

Ruderman and Pardue [66] have analyzed the pattern of labelled polypeptides (by one-dimensional SDS gel electrophoresis) synthesised when poly(A)$^+$ and poly(A)$^-$ mRNAs prepared from the various developmental stages of sea urchin embryos were translated in a wheat germ cell-free protein synthesising system. Their data suggest that although sea urchin egg poly(A)$^-$ mRNA species encode a wide variety of non-histone proteins in vitro, fewer such non-histone products are detected in the translational products of the morula and gastrula stages of development. The same situation is observed during the embryogenesis of *Artemia salina*, when again the number of in vitro non-histone products encoded by poly(A)$^-$ mRNA decreases during development [90].

This apparent prevalence of 'abundant' poly(A)$^-$ mRNAs in cell types which are not highly differentiated may be due to the requirement of the cells to respond rapidly to both internal and external changes. A very rapid response of these cells may well involve poly(A)$^-$ mRNA, since the very act of polyadenylation in animal cells takes about 5–120 min [91–96].

Furthermore, a rapid response of these cells may require the ability to rapidly degrade particular mRNAs. It is known that, in general, polyadenylation increases the stability of certain mRNAs [97]. Therefore, it is possible that some poly(A)$^-$ mRNAs are unstable and could be removed rapidly from the cytoplasm in response to an internal or external stimulus. Moreover, it is possible that a given cell type within a differentiated tissue (e.g., brain, liver) may require poly(A)$^-$ mRNAs which could be degraded very rapidly. Indeed, in general, the changes which occur within a cell during the cell cycle might involve poly(A)$^-$ mRNAs. For example, poly(A)$^-$ histone mRNA in mammalian cells is translated only during S-phase of the cell cycle, and disappears rapidly at the end of S-phase [98–101].

That poly(A)$^-$ mRNAs may also play some role in cell proliferation is suggested by comparing the results from a number of independent studies. Grady et al. [102] reported that the nucleotide sequence complexity of total polysomal mRNA from subconfluent mouse fibroblast cells in culture is greater than that found in confluent cells, while Williams and Penman [103] reported that the sequence complexities of mouse fibroblast cells poly(A)$^+$ mRNA examined in both growth states are very similar. Also the results

of Burdon et al. [31] and Chermovskaya et al. [49] suggest that there are differences in the proportion of rapidly labelled poly(A)$^-$ mRNAs dependent on the growth state of the cells.

Additionally, a comparison of different studies suggests that viral transformation may result in a change in the population of poly(A)$^-$ mRNAs. For example, Grady et al. [102] showed that the sequence complexity of total polysomal mRNA in mouse fibroblast cells increased after transformation with polyoma virus. On the other hand, the sequences of poly(A)$^+$ mRNA from human fibroblasts and the same cell transformed with SV-40 virus appear to have a high degree of homology [104]. Therefore, it is possible that the difference in the sequence complexity of total polysomal mRNA between confluent and subconfluent cells and normal and transformed cells could be due to differences in the poly(A)$^-$ mRNA sequences.

8. Poly(A)$^+$ and poly(A)$^-$ mRNAs coding for the same functional protein

A number of specific mRNAs appear to be 'bimorphic', i.e., there are two forms of mRNA (poly(A)$^-$ and poly(A)$^+$) coding for the same functional protein [73–80].

It is of interest to ask whether this bimorphism is due to transcription of separate genes which have different properties in terms of polyadenylation of the transcription. Alternatively, some of the transcripts of one gene which are polyadenylated in the normal fashion could be more susceptible to reduction and loss of the poly(A) segment than others. Evidence supporting the former possibility came from studies with histone genes, 'early' and 'late', which code for mRNAs with different primary structure and size [105–108]. These results could be compared with the results of Ruderman and Pardue [66], who showed that about 50% of the translatable activity of histone mRNAs from sea urchin embryos is found in the poly(A)$^-$ mRNA fraction, whilst about 30% of the translatable activity of histone mRNA from the morula is found in poly(A)$^-$ mRNA and only about 10% is found in the histone poly(A)$^-$ mRNA fraction in gastrulae. These results taken together raise the question of whether the histone mRNAs transcribed from the 'early' genes are non-polyadenylated whilst the mRNAs transcribed from 'late' genes are polyadenylated. Additionally, since 20% of ovalbumin mRNA activity is found in the poly(A)$^-$mRNA fraction [77,78] and it has been reported that there are three ovalbumin-like genes [109] it is a matter of speculation as to whether the poly(A)$^+$ and poly(A)$^-$ ovalbumin mRNAs derive from different genes. However, the possibility that some poly(A)$^+$ and poly(A)$^-$ mRNAs derive from the same transcriptional unit can not be excluded . There is evidence that a number of proteins are synthesised by mRNAs containing different lengths of poly(A) tracts which range between 10–150 nucleotides long [36–38, 80]. As mentioned above, mRNAs with a poly(A) tract less than 8 nucleotides long would appear in the poly(A)$^-$ mRNA class. Therefore these results suggest that some poly(A)$^-$ mRNAs may derive from the gradual degradation of the poly(A) segment of some poly(A)$^+$ mRNAs.

9. Possible relationships of poly(A)$^-$ mRNAs to hnRNA

Finally, very little is known about the possible relationship of poly(A)$^-$ mRNA to hnRNA. Nevertheless, it has been reported that the complexity of poly(A)$^-$ hnRNA is 10-fold greater than the complexity of poly(A)$^-$ mRNA [88]. Furthermore, two independent studies report that poly(A)$^-$ mRNA sequences are absent from poly(A)$^+$ hnRNA, although no results have yet been published [30,110].

10. Concluding remarks

Poly(A)$^-$ mRNA appears to occur naturally on polysomes and is translated into protein in vivo. This class of mRNA does not appear to be derived from poly(A)$^+$ mRNA by deadenylation in vivo or in vitro.

Although some abundant proteins are encoded by both poly(A)$^+$ and poly(A)$^-$ mRNAs, the majority of poly(A)$^+$ and poly(A)$^-$ mRNAs share little sequence homology, suggesting that they are derived from two distinct populations of genes. Until now, most studies have examined the population of genes coding for poly(A)$^+$ mRNA. It is possible that the structure and regulation of the genes coding for poly(A)$^-$ mRNA is different from that of the genes coding for poly(A)$^+$ mRNA. Therefore, it would be of interest to compare the regulation of these two gene populations.

The availability of DNA probes (cDNA [30] and expressed DNA [84,87]) complementary to poly(A)$^-$ mRNA should allow the detailed study of the transcription, processing and translation of poly(A)$^-$ mRNAs. Furthermore, the study of changes in the expression of poly(A)$^-$ mRNA during cellular growth, development and viral transformation could be extended.

The recent development of recombinant DNA technology should allow the examination of the structure of genes coding for specific poly(A)$^-$ mRNAs. In particular, the presence of introns in the genes coding for some particular poly(A)$^+$ mRNAs [111] raises the question as to whether the genes coding for poly(A)$^-$ mRNA also contain introns.

The comparison of the genes coding for poly(A)$^+$ and poly(A)$^-$ mRNAs may therefore lead to a greater understanding of the regulatory mechanisms involved in eukaryotic gene expression.

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