The role of poly(A) in the translation and stability of mRNA

A. Sachs

Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA

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Introduction

Twenty years have passed since the discovery of a poly(A) tail at the 3′ end of eukaryotic mRNA (Kates, *Cold Spring Harbor Symp Quant Biol* 1970, 38:743–752, Lim and Canellakis, *Nature* 1970, 227:710–712) yet the experimental confirmation of its involvement in the regulation of translation and mRNA degradation has only been provided in the last few years. Several excellent reviews of different aspects of these and earlier experiments have been published, and the reader is referred to them for further information [1–5]. This review focuses mainly on experiments reported in the past 2–3 years, and tests how closely they adhere to the paradigm that poly(A) metabolism, mRNA degradation, and mRNA translation form a set of interconnecting metabolic events.

Cytoplasmic poly(A): ribonucleoprotein structure and metabolism

In order to understand experiments that examine poly(A) function, a working knowledge of the structure and basic metabolism of cytoplasmic poly(A) is essential. For the purpose of this review, it is sufficient to know that poly(A) is synthesized in the nucleus to a reasonably homogeneous length, which is species-specific, and ranges from 70–90 nucleotides in yeast to 220–250 nucleotides in mammalian cells (Brawerman, *CRC Crit Rev Biochem* 1981, 10:1–38). Although the biochemistry of polyadenylation is becoming clear [4], the reasons for, and the mechanisms of, this control over nuclear length remain unknown.

After synthesis, poly(A) and mRNA in the cell become complexed into a ribonucleoprotein; poly(A) function is therefore intimately linked to the function of this complex. The RNA-binding component of the complex is the poly(A)-binding protein (PAB; Kwan and Brawerman, *Proc Natl Acad Sci USA* 1972, 69:3247–3250; Blobel, *Proc Natl Acad Sci USA* 1973, 70:924–928). PAB exists in both nuclear and cytoplasmic forms, which both havea molecular weight of approximately 65 kD (Baer and Kornberg, *J Cell Biol* 1983, 96:717–721; Sachs and Kornberg, *Mol Cell Biol* 1985, 5:1993–1996). Nuclear PAB appears to arise from cytoplasmic PAB by the cleavage, but not removal, of its 13 kD carboxy-terminus (Sachs *et al.*, *Cell* 1986, 45:827–835). The gene encoding PAB has been isolated from yeast, *Xenopus*, and humans (Adam *et al.*, *Mol Cell Biol* 1986, 6:2932–2943; Sachs *et al.*, 1986; Grange *et al.*, *Nucleic Acids Res* 1987, 15:4771–4787; Zelus *et al.*, *Mol Cell Biol* 1989, 9:2756–2760). PABs from each of these organisms share extensive homology over the first 55 kD region, but diverge at the carboxy-terminus. Within the 55 kD region, PAB contains a tandem repeat of four 90-amino-acid domains, and only one of these is needed for poly(A) binding (Sachs *et al.*, *Mol Cell Biol* 1987, 7:3268–3276; Nietfeld *et al.*, *EMBO J* 1990, 9:3699–3705). The binding site of PAB spans 12 nucleotides, and is arranged on poly(A) like 'beads on a string', with a center-to-center distance of 25 nucleotides (Baer and Kornberg, 1983; Sachs *et al.*, 1987). The multiple-domain structure of PAB allows it to transfer rapidly between strands of poly(A), suggesting that the poly(A)–PAB complex is a dynamic structure in the cell (Sachs *et al.*, 1987).

About 10 years after the discovery of poly(A), it was found that the poly(A) tail is gradually shortened in the cytoplasm (reviewed in Brawerman, 1981). A recent paper by Piper and Aamand [6] has highlighted the compartmentalization of this shortening reaction by analyzing the lengths of poly(A) tails in yeast cells in which mRNA transport is blocked due to the *prp1-1* mutation. As expected, pulse-labeled mRNA has a poly(A)-tail length approximately equal to that of nuclear mRNA, and this length remains unperturbed as long as transport is blocked. After release from the transport block, this pulse-labeled mRNA is presumably transported to the cytoplasm where the poly(A) tail becomes a suitable substrate for the cytoplasmic shortening reaction.

The cytoplasmic shortening of poly(A) tails on specific mRNAs has been studied in several recent papers; in each of these, the ability to identify newly synthesized mRNA has allowed the time course of deadenylation to be followed (Krowczynska *et al.*, *J Mol Biol* 1985, 181:231–239; Mercer and Wake, *Nucleic Acids Res* 1985, 13:7929–7943; Restifo and Guild, *Dev Biol* 1986, 115:507–510; Harland and Misher, *Development* 1988, 102:837–852; Shapiro *et al.*, *Mol Cell Biol* 1988, 8:1957–1969) [7–9]. In particular, Baker *et al.* [7] have presented the first extensive quantitation of the effects of different conditions on the rate of the poly(A)-shortening reaction, and the work of Herrick *et al.* [9] breaks new ground in using a temperature-sen-

Abbreviation

PAB—poly(A)-binding protein

sitive RNA polymerase II mutation in yeast to measure the half-life of the poly(A) tail in several different mRNAs. In the two quantitative studies, the average rate of poly(A)-tail shortening was between 20 and 40 nucleotides per hour (Mercer and Wake, 1985) [7]. Several of these reports also note that the shortening reaction appears to stop between tail lengths of 30 and 60 nucleotides; this implies either that the poly(A)-shortening enzyme ceases to function at and beyond this length, or that it continues but mRNAs with shorter poly(A) tails are highly unstable (see below). If the deadenylation does stop at this length, then there must be another enzyme(s) responsible for removing the residual adenines.

The discovery that PAB is required for the shortening reaction in yeast [10] has given new insight into the mechanism of cytoplasmic poly(A) tail shortening. Yeast strains depleted of PAB were found to contain cytoplasmic mRNA with poly(A) tails similar in length to those found in the nucleus. The introduction of wild-type PAB into these cells resulted in the reappearance of cytoplasmic poly(A) tails of normal length. These data support the surprising conclusion that poly(A)-tail shortening is stimulated by PAB. The purification of this PAB-stimulated poly(A)-shortening activity will permit the detailed analysis of the role of cytoplasmic poly(A)-tail shortening in mRNA stability and translation.

The degree to which this shortening reaction depends on translation remains unclear. Early work demonstrated some inhibition of shortening with an inhibition of translation (Sheiness *et al.*, *Proc Natl Acad Sci USA* 1975, 72:1077–1081). In agreement with this, translation appears to be required for the shortening of the poly(A) tails of some mRNAs during spermiogenesis [11,12]. However, other studies have strongly suggested that the involvement of translation in the shortening reaction may be message-specific. For instance, inhibiting translation does not seem to affect the shortening of the poly(A) tail of the α1-acid glycoprotein mRNA in cultured hepatic cells [8]. Furthermore, tubulin mRNA undergoes an increased rate of shortening when its translation is blocked by cycloheximide [7].

The cytoplasmic degradation of poly(A) tails appears to have a major role in the regulated control of mRNA expression during oocyte maturation (reviewed in [3]). Dormant *Xenopus* oocytes contain a pool of mRNA with short (10–50 nucleotides) poly(A) tails. Upon induction of maturation, a subset of these become further adenylated, while the remainder have their tails removed (Hyman and Wormington, *Genes Dev* 1988, 2:598–605). This deadenylation reaction appears to be a default pathway for the metabolism of the poly(A) tail, as no specific part of the mRNA is required to induce it, and those mRNAs that are normally polyadenylated become deadenylated if their polyadenylation signal is destroyed (Fox and Wickens, *Genes Dev*, in press; Varnum and Wormington, *Genes Dev*, in press). Many of these poly(A)-deficient mRNAs are not degraded (Paris *et al.*, *Gene* 1988, 72:169–176), and some can be re-adenylated later in development to allow their translational recruitment (Ruiz *et al.*, *EMBO J* 1987, 6:3065–3070). It will be interesting to find out whether this deadenylation reaction is analogous to the shortening reaction seen in other cells, and also whether it requires PAB for activity. If we assume that the poly(A)-deficient mRNAs in the dormant oocyte receive a long poly(A) tail at the time of their nuclear synthesis, it will also be interesting to determine the requirement of the original poly(A)-shortening reaction for PAB.

Recently, several groups [13–15] have obtained important new information about the mechanism of cytoplasmic polyadenylation of specific mRNAs during oocyte maturation (reviewed in [3]). Those mRNAs that are destined to be polyadenylated after maturation contain two sequence elements that allow this. One is the highly conserved AAUAAA sequence that is required for nuclear polyadenylation and is found near the 3′ end of most mRNAs. The second is a less conserved U-rich element in the untranslated 3′ region. Although a detailed discussion of the biochemistry of this reaction is beyond the scope of this review, it is important to recognize that cytoplasmic mRNA can be both adenylated and deadenylated in the absence of new mRNA synthesis.

Does the polyadenylation of cytoplasmic mRNA occur in other cell types? This is still a relatively unexplored area, although it has been recognized for some time that cytoplasmic poly(A) tails in mammals can have up to eight new adenines added to them during their lifetime (Brawerman, 1981). This number represents a lower limit to the extent of cytoplasmic polyadenylation because it reflects a steady-state equilibrium between poly(A) addition and poly(A) removal. One particularly intriguing observation relating to this topic is that the poly(A) tails of vasopressin and oxytoxin mRNAs appear to undergo a substantial lengthening in response to dehydration in rats (Carrazana *et al.*, *Mol Cell Biol* 1988, 8:2267–2274; Zingg *et al.*, *J Biol Chem* 1988, 263:11041–11043) [16]. One possible explanation for this phenomenon lies in the extensive cytoplasmic polyadenylation of existing mRNA. Of course, these and other experiments that document increases in poly(A)-tail lengths due to various physiological stimuli suffer from not knowing whether the response to the stimuli is new nuclear synthesis of mRNA with longer poly(A) tails, the inhibition of the poly(A)-tail-shortening reaction (thereby allowing the basal level of cytoplasmic poly(A) polymerase activity to become detectable), or the stimulation of cytoplasmic polyadenylation.

Poly(A) and mRNA stability

The relationship between poly(A)-tail degradation and mRNA degradation is still far from clear. In general, it is not safe to assume that the deadenylation of mRNA leads to its destabilization, although there are examples of some very unstable mRNAs for which deadenylation does seem to be a prerequisite for rapid mRNA degradation. It is important to recognize that the phenomenon of poly(A)-tail degradation comprises at least two distinct

reactions. The poly(A)-tail-shortening reaction discussed above probably occurs for all mRNAs at some average rate (i.e. 20-40 nucleotides per hour). An acceleration of the deadenylation rate for a particular mRNA may be the result of activation of the PAB-dependent shortening enzyme described above, or the activation of a completely different enzyme. In the second case, rapid poly(A)-tail removal could form part of the mRNA degradation pathway. Because poly(A) removal may be the result of several different reactions, only experiments that resolve these distinct reactions can directly examine the influence of different conditions on any one of them. Such experiments usually study the PAB-dependent poly(A)-tail-shortening reaction on very stable mRNAs, and the poly(A)-removal reaction that is linked to mRNA degradation on very unstable mRNAs. Those mRNAs with intermediate stabilities experience complicated poly(A)-tail metabolism, and as a result, any observed changes in this metabolism are difficult to interpret.

Two well documented members of the class of mRNAs that are stable even though large portions, if not the whole, of their poly(A) tails have been removed are the mRNAs for mouse β-actin and β-tubulin (Krowczynska *et al.*, 1985). Similarly, many other mRNAs lacking poly(A) tails are stable when injected into oocytes (e.g. Galili *et al.*, *J Biol Chem* 1988, 263:5764–5770), in contrast to early experiments suggesting otherwise (Huez *et al.*, *Proc Natl Acad Sci USA* 1974, 71:3143–3146).

The highly unstable mRNAs that contain a UA-rich element in their 3′-untranslated region are members of the class of mRNAs that appear to be destabilized because of the loss of their poly(A) tail. These include the c-*fos* (Wilson and Treisman, *Nature* 1988, 336:396–399), GM-CSF (Shaw and Kamen, *Cell* 1986, 46:659–667), and c-*myc* (Jones and Cole, *Mol Cell Biol* 1998, 7:4513–4521) mRNAs. This UA-rich element is capable of conferring instability to a normally stable message (Shaw and Kamen, 1986), although removing it from an unstable message does not necessarily lead to mRNA stability (Kabnick and Housman, *Mol Cell Biol* 1988, 8:3244–3250) [17]. In particular, the work of Shyu *et al.* [17] supports the existence of two distinct mRNA destabilizing elements in the *c-fos* mRNA.

The analysis of the decay of c-*myc* mRNA *in vivo* has revealed that it is deadenylated before it is degraded [18]. Wilson and Treisman (1988) extended the detail of this analysis by employing a non-denaturing gel system capable of visualizing changes in the poly(A)-tail length. They found that c-*fos* mRNA, like c-*myc* mRNA, undergoes rapid poly(A)-tail removal before the mRNA is destroyed, and showed that translation was required for both rapid removal of the poly(A) tail and mRNA degradation. Similar results on the translational requirements of poly(A)-tail removal and mRNA degradation have recently been obtained for c-*myc* (Laird *et al.*, *Mol Cell Biol* 10:6132–6140). Given that poly(A)-tail degradation occurs before mRNA degradation, these data suggest that poly(A)-tail removal is an early step in the mRNA degradation pathway for this class of messages.

Laird *et al.* (1990) have provided further evidence that mRNA degradation is stimulated by poly(A)-tail removal with the observation that the inhibition of both c-*myc* mRNA degradation and poly(A)-tail removal by translation blockers can only beobserved during the early lifetime of the mRNA. If the mRNA is allowed to be in the cytoplasm for a certain period of time before the addition of the translation blocker, the inhibitory effect of the drug is lost and the mRNA is degraded at a normal rate. Presumably, the poly(A) tail on the mRNA has been removed during this period. These data are interpreted to show that poly(A)-tail removal for c-*myc* mRNA is translation-dependent, whereas the degradation pathway for the poly(A)-deficient mRNA is translation-independent. These workers also found that mRNA that is initially stabilized by the lack of translation is eventually degraded at a rapid rate. This might indicate that slow poly(A)-tail shortening of c-*myc* mRNA ultimately creates the proper poly(A)-deficient substrate for the subsequent translation-independent degradation of the mRNA.

The most common class of mRNAs comprises those with intermediate stabilities that have poly(A) tails ranging in size from the long nuclear length to approximately 30 nucleotides. This heterogeneity in length is probably the result of the to the PAB-dependent poly(A)-tail shortening reaction. Several surveys of the relationship between poly(A)-tail lengths and mRNA stability for such mRNAs have appeared (Krowczynska *et al.*, 1985; Santiago *et al.*, *Nucleic Acids Res* 1987, 15:2417–2429; Shapiro *et al.*, 1988) [9]; the general conclusion from these studies is that there is no direct correlation between the poly(A)-tail length of a mRNA and its stability. However, these studies cannot rule out the possibility that the complete removal of poly(A) leads to mRNA destabilization (see below).

A different approach to studying the relationship between the poly(A) tail and mRNA stability is to use an *in vitro* system that accurately reflects the degradation pathway of mRNA *in vivo*. Ross and co-workers (Brewer and Ross, *Mol Cell Biol* 1988, 8:1697–1708) [19] have recently tried to create such a system by studying both the degradation of endogenous mRNA bound to polysomes and the degradation of mRNA synthesized *in vitro* that has been added to polysomes or high-salt washes of polysomes. The validity of these experiments has been based on two observations: first, the stabilities of mRNAs *in vitro* can be ranked identically to the stabilities of the same mRNAs *in vivo*; and, second, the pathways for histone mRNA degradation *in vitro* is identical to that observed *in vivo*.

The *in vitro* system has been used to study the degradation of c-*myc* mRNA bound to polysomes [19]. In this system, the poly(A) tail on the mRNA was also removed before the remainder of the mRNA was degraded. The unstable degradation intermediates liberated messenger 3′-termini located in the UA-rich element of the molecule, suggesting the importance of this region in the degradation process. Exogenous c-*myc* mRNA, when added to the extract, was also deadenylated before it was degraded, although the rate of degradation was much slower than that of the endogenous polysome-associated mRNA. Because the degradation intermediates of the ex-

ogenous mRNA were not characterized in detail, it is not known whether it was degraded along the same pathway as the endogenous mRNA.

The *in vitro* system has also been used to examine the effect of removing PAB on mRNA stability (Brewer and Ross, 1988). In these experiments, the degradation of exogenously added β-globin mRNA that lacked a poly(A) tail was compared with that of β-globin containing a poly(A) tail. This work showed that the poly(A)-tailed β-globin mRNA was stabilized relative to other poly(A) deficient mRNAs in the presence of PAB. The degradation of this mRNA in the absence of PAB results from the activity of a 3′-exonuclease, and it was concluded therefore that PAB normally protects mRNA from degradation by preventing the access of a 3′-exonuclease to the body of the mRNA. The authors suggested that the elements controlling the instability of mRNAs, such as the UA-rich element in c-*fos* and c-*myc* might work by modulating the efficiency of the exonuclease reaction through changes in the affinity of PAB for the poly(A) tail. They proposed that such modulations would lead to the dissociation of PAB and the subsequent degradation of mRNA by the 3′-exonuclease.

There are potentially several problems with these *in vitro* studies. For instance, the rapid deadenylation of mRNAs such as c-*myc* has been shown to require ongoing translation *in vivo* and, as translation is not occurring *in vitro*, it seems unlikely that the reaction is being accurately reconstituted. Furthermore, the specificity of the c-*myc* degradation pathway has not been shown to be responsive to the UA-rich destabilizing element *in vitro* as it is *in vivo* (Shaw and Kamen, 1986) [17].

There are other possible explanations for the observed effects of removing PAB on the *in vitro* decay of exogenous mRNA. For instance, a highly active, non-specific 3′-exonuclease could well be prevented from degrading mRNA only in the presence of PAB. Does this mean that mRNA is normally degraded by this nuclease, or that mRNA can be adequately protected from it by PAB, thereby allowing the specific degradation of mRNA through other enzymes *in vivo*, and possibly *in vitro*? Such non-specific protection is a potential function of PAB, and is an issue that must be addressed before any conclusions about the role of PAB in mRNA stabilization can be drawn.

In summary, the one finding that does seem consistent for most mRNAs *in vivo* and *in vitro* (but see [9]) is that messages with poly(A) tails shorter than 30 nucleotides are very rare. As a result, future studies examining the role of the poly(A) tail in mRNA degradation will need to address the question of why mRNAs with no poly(A) tails are so rare. These studies could ignore the relationship between poly(A)-tail length and stability, and focus instead on the role of a short stretch of poly(A) as a stabilizing element. They will address the issue of whether or not the loss of a poly(A) tail leads directly to a destabilization of the mRNA, and whether or not the loss of a PAB-binding site really makes an mRNA more susceptible to exonucleases. One possible way to rule out the exonuclease protection model would be to stabilize a rapidly deadenylated mRNA (such as c-*fos*) by mutations within the mRNA such that it was still rapidly deadenylated but no longer rapidly degraded.

Other studies that examine the possible indirect mechanisms by which poly(A) tails below 30 nucleotides may destabilize mRNA will also prove valuable. Possible mechanisms include a change in mRNA or ribonucleoprotein structure that exposes an endonucleolytic site, and a reduction in the translational stabilization of an mRNA due to a decrease in the efficiency with which the mRNA is translated in the absence of PAB, (see below). This last model predicts that some mRNAs with poly(A) tails could be stabilized if only their translational efficiency were maintained.

Poly(A) and translation

The recent work of several research groups has shown the importance of poly(A) tails in the initiation of translation. An excellent review of this topic has appeared within the last year [5], and the reader is referred to it for further information and a historical account.

A genetic analysis of mutations of PAB in yeast has provided the first *in vivo* proof that PAB and therefore presumably the poly(A) tail, is required for the initiation of translation as the depletion of PAB *in vivo* results in the accumulation of monosomes and an arrest of cell growth [10]. Extragenic suppressors of the PAB mutations allow translation and growth to resume, and each of these suppressors also modulates the production of the 60S ribosomal subunit. Two of the suppressor mutations have been further characterized by isolating and sequencing their genes. One of them encodes the large ribosomal subunit protein L46, and a deletion of this gene allows translation and growth in the absence of PAB [20]. Another is highly homologous to a family of proteins that are probably ATP-dependent RNA helicases. Mutations in this protein affect the efficiency of large-rRNA processing, ultimately leading to a decrease in the amount and perhaps a change in the structure of the 60S ribosomal subunit [20]. These *in vivo* data suggest that the poly(A) tail and PAB are required for the initiation of translation and that this requirement is mediated by the 60S subunit before it joins the 40S subunit to give the 80S monosome. The data also lead to the hypothesis that one essential function of the poly(A) tail is to mark a mRNA as being intact, thereby preventing the efficient translation of degraded mRNA. This suggests that only a single cleavage within an mRNA is needed to functionally inactivate as such a cleavage would effectively separate the 5′- and 3′-ends.

Concurrent with the discovery that PAB is required for *in vivo* translation, Munroe and Jacobson [21] were analyzing in detail the effects of poly(A) tails on the *in vitro* translation of mRNA. They successfully formed a link between mRNA polyadenylation and *in vitro* translation by showing that poly(A) tails stimulated the translation of

an mRNA twofold, and this was the result of increases *in vitro* in the efficiency of the 60S-subunit-joining step. The lack of any larger effect was attributed to the poor reinitiation efficiency of the *in vitro* system. The efficiency of the 60S-subunit-joining step was found to increase with an increase in poly(A)-tail length, suggesting that longer (i.e. younger) mRNAs have a translational advantage. Similar observations have been made by Gallie *et al.* [22] in plant protoplasts by electroporating synthetic mRNAs containing different poly(A)-tail lengths and measuring the transient expression of the mRNAs. Munroe and Jacobson [21] have also made the intriguing observation that exogenous poly(A) can stimulate the translation of mRNA that lacks a poly(A) tail, and they argue that this indicates that the PAB–poly(A) complex can act as an enhancer of translation.

In agreement with these findings, Grossi de Sa *et al.* (*Eur J Biochem* 1988, 176:521–526) have extended the earlier observations made by Jacobson and Favreau (*Nucleic Acids Res* 1983, 11:6353–6368) that free poly(A) preferentially inhibits the *in vitro* translation of poly(A)-tailed mRNA. These workers found that the poly(A) inhibition can be relieved by the addition of purified PAB to the system. These data suggest either a specific role for PAB in translation, or the relief by PAB of a non-specific inhibition by poly(A). However, together with the above *in vivo* and *in vitro* data, it now seems likely that poly(A) is inhibiting the translation system by binding PAB, thereby preventing the association of PAB with poly(A)-tailed mRNA to allow its efficient translation.

Intriguing observations have been obtained by Galili *et al.* (1988) concerning the stimulation of mRNA translation by poly(A) in *Xenopus* oocytes. These workers found that the addition of a poly(A) tail to mRNA does not significantly affect the translational capacity of that mRNA soon after injection into oocytes. However, significant increases in the translation, but not in the amounts, of poly(A)-tailed versus non-poly(A)-tailed mRNAs are found several hours later, and this is associated with an increase in polysome size. These data are interpreted to show that the poly(A) tail allows the efficient reinitiation of a mRNA by the same ribosomes. On the basis of the work described above, this could reflect an increased efficiency in the 60S-subunit-joining step.

Significant progress has also been made in showing that the translational activation of stored oocyte mRNA during maturation is the result of polyadenylation (reviewed in [3]). Although it has been recognized for some time that there is a temporal association of polyadenylation with translation, it has been difficult to prove that the addition of a poly(A) tail to a mRNA was a prerequisite for its translation. It has now been found that mRNAs that cannot be polyadenylated because of a modification of their 3′-end with the chain-terminating compound cordycepin cannot be translated [14,15]. Similarly, mutations within the UA-rich cytoplasmic polyadenylation element that abolish polyadenylation also abolish translation, and the subsequent addition of a poly(A) tail to such mutated mRNAs restores their capacity for being translated [14]. Furthermore, the presence of a poly(A) tail on injected mRNA is sufficient to signal translational recruitment for many mRNAs [15], although some messages may require active polyadenylation if they are to be used efficiently, and others may only be optimally translated if they possess poly(A) tails of a discrete size (Paris and Richter, *Mol Cell Biol* in press) [14]. These new data on the role of poly(A) in the control of translation during oocyte development both complement the information obtained from the genetic and biochemical analyses, and raise new questions about the potential for similar types of cytoplasmic regulation in other cell types.

Conclusions

A working model for poly(A)-tail metabolism and function can be created from the above data. Poly(A) is synthesized in the nucleus as a long precursor and after transport to the cytoplasm, is shortened in a PAB-dependent reaction, and possibly repolymerized by a cytoplasmic poly(A) polymerase. Coupled to this is the poly(A)-tail-removal reaction that is probably part of the degradation pathway of a mRNA. If a mRNA reaches a point in its lifetime where its poly(A) tail becomes shorter than 30 nucleotides, then the enzymes responsible for the degradation of the poly(A)-deficient mRNAs can become important in determining the disappearance of the mRNA. Of course, some mRNAs that lack poly(A) tails are resistant to these enzymes, whereas others are degraded well before their tails achieve this length. As a result, the dependence of the stability of a mRNA on the presence or absence of its poly(A) tail will be message-specific.

The efficient translation of the mRNA requires the presence of the poly(A) tail. This requirement is mediated by the 60S ribosomal subunit, and it allows the efficient joining of the 40S and 60S subunits. Both the poly(A)-tail-shortening reaction and the poly(A)-tail-removal and mRNA-degradation reactions depend on translation in an mRNA-specific manner. Because of this, the recruitment or inhibition of translation will affect the stability of different mRNAs to different degrees.

Future work in the field will probably try to understand these reactions to a greater degree by using *in vitro* systems that accurately reflect the *in vivo* metabolism. With such systems in hand, it should be possible to understand how the different destabilizing and translation–control elements on mRNA contribute to the post-transcriptional control of gene expression.

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Annotated references and recommended reading

- • Of interest
- •• Of outstanding interest

1. • BERNSTEIN P, ROSS J: **Poly(A), poly(A)-binding protein and the regulation of mRNA stability.** *Trends Biochem Sci* 1989, 14:373–377.

A short review of the literature concerning poly(A) and PAB and their potential involvement in stabilizing mRNA.

2. • MUNROE D, JACOBSON A: **Tales of poly(A).** *Gene* 1990, 91:151–158.

The authors review those experiments that have addressed the role of poly(A) in translation and mRNA stability.

3. • WICKENS M: **In the beginning is the end: regulation of poly(A) addition and removal during early development.** *Trends Biochem Sci* 1990, 15:320–324.

This review summarizes experiments that have examined poly(A) metabolism and associated changes in mRNA translation during the maturation of oocytes.

4. • WICKENS M: **How the messenger got its tail: addition of poly(A) in the nucleus.** *Trends Biochem Sci* 1990, 15:277–281.

A concise review of the biochemistry of nuclear polyadenylation in eukaryotes.

5. • JACKSON RJ, STANDART N: **Does the poly(A) tail and 3′ untranslated region control mRNA translation?** *Cell* 1990, 62:15–24;.

The authors thoughtfully review key experiments that have examined the role of mRNA 3′-end involvement in translation.

6. • PIPER PW, AAMAND JL: **Yeast mutation thought to arrest mRNA transport markedly increases the length of the 3′ poly(A) on polyadenylated RNA.** *J Mol Biol* 1989, 208:697–700.

This study examines the compartmentalization of the poly(A)-shortening reaction in the *Saccharomyces cerevisae prp1-1* mutant.

7. •• BAKER EJ, DIENER DR, ROSENBAUM JL: **Accelerated poly(A) loss on alpha-tubulin mRNAs during protein synthesis inhibition in *Chlamydomonas*.** *J Mol Biol* 1989, 207:771–781.

A careful and thought-provoking analysis of the metabolism of poly(A) in tubulin mRNA. This study is the first to extensively quantify the effects of different conditions on the poly(A)-shortening reaction.

8. • CARTER KC, BRYAN S, GADSON P, PAPACONSTANTINOU J: **Deadenylation of α-acid glycoprotein mRNA in cultured hepatic cells during stimulation by dexamethasone.** *J Biol Chem* 1989, 264:4112–4119.

The poly(A)-shortening reaction on this mRNA (stable *in vivo*) is examined and shown to be resistant to cycloheximide treatment.

9. •• HERRICK D, PARKER R, JACOBSON A: **Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1990, 10:2269–2284.

This paper is a landmark in studies on mRNA metabolism in yeast. A temperature-sensitive RNA polymerase II mutation is used to measure the half-lifes of many different mRNAs. The results suggest that poly(A)-tail removal may not be an important determinant of mRNA stability in very stable mRNAs.

10. •• SACHS AB, DAVIS RW: **The poly(A)-binding protein is required for poly(A) shortening and 60S ribosomal subunit-dependent translation initiation.** *Cell* 1989, 58:857–867.

The authors present the first *in vivo* proof that PAB is required for the initiation of translation, and that this requirement is probably mediated through the 60S ribosomal subunit. The poly(A)-shortening reaction is also shown to be dependent on PAB.

11. • BRAUN RE, PESCHON JJ, BEHRINGER RR, BRINSTER RL, PALMITER RD: **Protamine 3′-untranslated sequences regulate temporal translational control and subcellular localization of growth hormone in spermatids of transgenic mice.** *Genes Dev* 1989, 3:793–802.

This study identifies an element in the 3′-UTR region of protamine mRNA that is required for translational recruitment during spermiogenesis, and correlates this recruitment with changes in the poly(A)-tail length of the mRNAs.

12. • KLEENE KC: **Poly(A) shortening accompanies the activation of translation of five mRNAs during spermiogenesis in the mouse.** *Development* 1989, 106:367–373.

The storage mRNAs that undergo poly(A)-shortening following their translational recruitment are identified.

13. •• FOX CA, SHEETS MD, WICKENS MP: **Poly(A) addition during maturation of frog oocytes: distinct nuclear and cytoplasmic activities and regulation by the sequence UUUUUAU.** *Genes Dev* 1989, 3:2151–2162.

Elegant experiments are used to identify the sequence requirements for cytoplasmic polyadenylation in the oocyte. These are shown to be the conserved nuclear polyadenylation signal AAUAAA and a cytoplasmic polyadenylation signal in the 3′-UTR. The data obtained raise several important questions about the conservation of polyadenylation reactions in the nuclear and cytoplasmic compartments, as well as the possibility of these reactions sharing several enzymatic components.

14. •• MCGREW LL, DWORKIN RE, DWORKIN MB, RICHTER JD: **Poly(A) elongation during *Xenopus* oocyte maturation is required for translational recruitment and is mediated by a short sequence element.** *Genes Dev* 1989, 3:803–815.

Evidence is presented that polyadenylation is the cause of translational recruitment of mRNA during oocyte maturation, and that some mRNAs may only be recruited if they are in the process of being polyadenylated. Translation of synthetic mRNA is blocked when polyadenylation is prevented by using cordycepin, or when mRNA is mutated in the cytoplasmic polyadenylation element. This element and the conserved AAUAAA sequence are identified as being essential for cytoplasmic polyadenylation.

15. •• VASSALLI J-D, HUARTE J, BERLIN D, GUBLER P, VASSALLI A, O'CONNELL ML, PARTON LA, RICKLES RJ, STRICKLAND S: **Regulated polyadenylation controls mRNA translation during meiotic maturation of mouse oocytes.** *Genes Dev* 1989, 3:2163–2171.

This report contains evidence that polyadenylation is the cause of translational recruitment of mRNA during oocyte maturation. Key experiments reported include the blockage of polyadenylation using cordycepin-modified 3′-ends to prevent translation, and the *in vitro* polyadenylation of mRNA to allow translational recruitment. The 3′-UTR is identified as the region that controls the adenylation status of tissue-type plasminogen activator mRNA.

16. • CARTER DA, MURPHY D: **Independent regulation of neuropeptide mRNA level and poly(A) tail length.** *J Biol Chem* 1989, 264:6601–6603.

The authors document the regulation of the poly(A)-tail length of oxytocin mRNA by dehydration in rats.

17. •• SHYU AB, GREENBERG ME, BELASCO JG: **The c-*fos* transcript is targeted for rapid decay by two distinct mRNA degradation pathways.** *Genes Dev* 1989, 3:60–72.

Shyu *et al.* report the discovery of more than one destabilizing element in this highly unstable mRNA. One element identified is the AU-rich sequence, and the other lies within the coding sequence of the mRNA. This finding raises the possibility that mRNA destabilization can be controlled by more than one pathway in eukaryotes.

18. • SWARTWOUT SG, KINNIBURGH AJ: **c-*myc* RNA degradation in growing and differentiating cells: possible alternate pathways.** *Mol Cell Biol* 1989, 9:288–295.

Careful analysis of c-*myc* mRNA degradation *in vivo* shows that poly(A)-tailed mRNA loses its poly(A) tail before the remainder of the mRNA is degraded

19. •• BERNSTEIN P, PELTZ SW, ROSS JR: **The poly(A)–poly(A)-binding protein complex is a major determinant of mRNA stability *in vitro*.** *Mol Cell Biol* 1989, 9:659–670.

This report examines the effects of PAB removal on β-globin mRNA stability in an *in vitro* mRNA-degradation system. The authors suggest that the affinity of mRNA for PAB may be controlled by elements within the mRNA.

20. •• SACHS AB, DAVIS RW: **Translation initiation and ribosomal biogenesis: involvement of a putative rRNA helicase and RPL46.** *Science* 1990, 247:1077–1079.

The deletion of a ribosomal protein or mutations within a pre-rRNA-processing enzyme allows cell growth in the absence of PAB. This suggests that the ability of *spb* mutants to suppress a deletion of PAB is realized through changes in the structure of the 60S subunit.

21. •• MUNROE D, JACOBSON A: **mRNA poly(A) tail, a 3′ enhancer of translation initiation.** *Mol Cell Biol* 1990, 10:3441–3455.

In vitro translation of vesicular stomatatis virus N- and β-globin-mRNA is stimulated by polyadenylation. Polysome analysis of translational intermediates is used to identify the 60S-subunit-joining step as the target for the effects of poly(A). It is also shown that capped non-poly(A)-tailed mRNA translation is stimulated by free poly(A), presumably through a PAB–poly(A) complex.

22. • GALLIE DA, LUCAS WJ, WALBOT V: **Visualising mRNA expression in plant protoplasts: factors influencing efficient mRNA uptake and translation.** *Plant Cell* 1989, 1:301–311.

Poly(A) tails are shown to stabilize electroporated β-glucuronidase mRNA moderately and effect a much larger increase in the expression of the mRNA through translational stimulation.