In the beginning is the end: regulation of poly(A) addition and removal during early development

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The addition of poly(A) tails to nearly all mRNAs within the nucleus was reviewed in the July issue of TIBS. Here we shift focus to the fate of poly(A) tails during early development. At specific times during oogenesis and embryogenesis, the poly(A) tails of certain maternal mRNAs are lengthened, while the tails of a number of other mRNAs are removed. The selective poly(A) addition reactions are regulated by a short, U-rich sequence in the 3' untranslated region, while the removal of poly(A) from specific mRNAs is a 'default state', requiring no specific sequence. These regulated changes in poly(A) length are likely to play a major role in translational regulation in the egg and early embryo.

Although this review will concentrate on studies of oocyte maturation, selective poly(A) addition and removal are by no means confined to this period. Certain mRNAs gain or lose poly(A) after fertilization, during the first several cleavage divisions. In somatic cells, increases in poly(A) tail length are correlated with enhanced translation of insulin, vasopressin and growth hormone mRNA. It is reasonable to speculate that these later changes may occur by a similar mechanism.

A specific example of a general phenomenon: poly(A) addition and removal during oocyte maturation

In the early development of many animal species, translational regulation of several maternal mRNAs is correlated with changes in poly(A) length: mRNAs that receive poly(A) are subsequently translated, while others that lose their poly(A) tails dissociate from ribosomes. Although these correlations are the general rule, they are not without exception. Likewise, the number of mRNAs affected varies enormously between animal species. For example, in the frog, Xenopus, fewer mRNAs undergo selective poly(A) addition and removal, and most of these events are correlated with changes in translation. In the frog, Xenopus, fewer mRNAs appear to be so affected. Yet the generality of the correlation and the wide spectrum of species in which it may be drawn suggests a conserved regulatory process.

Frog oocytes normally advance from first to second meiosis immediately after ovulation and fertilization. This period (oocyte maturation) is ideal for mechanistic studies of poly(A) addition and removal because oocytes can be easily manipulated in vitro. Frog oocytes removed from the mother's abdomen can be induced to mature in vitro. Similarly, excised mouse oocytes undergo spontaneous maturation in vitro unless treated with dibutyryl-cAMP.

The addition of poly(A) tails during oocyte maturation suggests a conserved regulatory process.

![Figure 1](#) Oocyte maturation and changes in poly(A) tail lengths. Frog oocytes advance from first meiotic prophase to second meiotic metaphase in response to progesterone added in vitro. During this process, the short poly(A) tails present on certain maternal mRNAs are lengthened. These RNAs generally become translated. Conversely, poly(A) tails are removed from other mRNAs that leave polysomes. From the standpoint of this review, maturation of mouse oocytes differs only in detail.

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Poly(A) addition during early development

How is a specific maternal mRNA selected to receive poly(A) during oocyte maturation? To address this question, synthetic mRNAs have been injected into frog oocytes. With each mRNA tested thus far, the critical signals lie in the 3′ untranslated region (3′UTR). Transfer of the 3′UTR from an RNA that receives poly(A) to one that does not, results in a chimeric RNA that receives poly(A). Likewise, polyadenylation can be prevented by amputation of the 3′UTR, injected on its own into oocytes, is a competent substrate (Fig. 2). Thus the 3′UTR is both necessary and sufficient to regulate poly(A) addition during maturation.

In Xenopus, the 3′UTRs of several mRNAs that receive poly(A) during maturation contain UUUUUUAU, or a related sequence (Table I), near to the AAUAAA motif that is required for polyadenylation in the nucleus, and which is present in virtually all mRNAs. To determine whether UUUUUUAU or AAUAAA are responsible for polyadenylation during maturation, a variety of synthetic RNAs that end at the natural poly(A) addition site have been injected into the cytoplasm of Xenopus oocytes. In such experiments, both UUUUUUAU and AAUAAA are critical. Deletions of UUUUUUAU abolish polyadenylation, and insertions of UUUUUUAU into otherwise inert RNAs activate it. Similarly, polyadenylation is prevented by point mutations and deletions of AAUAAA.

Since all mRNAs contain AAUAAA, it is the UUUUUUAU sequence that distinguishes those frog mRNAs that receive poly(A) from those that do not. In that sense, UUUUUUAU regulates the activity and imparts specificity for particular maternal mRNAs. Whether UUUUUUAU is also required in mouse oocytes remains to be determined.

In Xenopus, although mutations in UUUUUUAU have not yet been analysed, inspection of natural mRNA sequences suggests considerable sequence flexibility (see for example Table I). Several mRNAs contain multiple repeats of UUUUUUAU or related sequences. Furthermore, UUUUUUAU is most commonly located upstream (5′) of AAUAAA, but may also be found downstream. Polyadenylation efficiency can be affected by variation in the sequence that connects AAUAAA and UUUUUUAU, suggesting that these two critical elements may not be the whole story. Thus, although UUUUUUAU is sufficient to promote polyadenylation, it need not be the most potent or shortest sequence to do so; for this reason we shall subsequently refer to this functional sequence as the U-rich element.

Before maturation, a cytoplasmic activity can be detected that adds a small number of nucleotides, presumably oligo(A), to the end of an injected RNA that ends at the poly(A) addition site. This activity requires AAUAAA, and so should act on all mRNAs. Natural mRNAs that receive poly(A) during maturation usually have short poly(A) tails before maturation begins. Presumably, these RNAs received their short tails via AAUAAA-specific polyadenylation, either in the nucleus or cytoplasm. During maturation, it is possible that the short poly(A) segment is recognized, either in addition to, or in place of, AAUAAA. This is reminiscent of nuclear poly(A) addition which proceeds in two phases: the first requires AAUAAA; the second is independent of AAUAAA, but requires the oligo(A) segment already polymerized. By analogy, maturation might activate a second phase of polymerization dependent on a short poly(A) segment, the synthesis of which required AAUAAA earlier in oogenesis. However, the length of oligo(A) present in the natural mRNAs that are substrates for the maturation-induced activity varies between mRNAs, and can be as long as 90 nucleotides. In the nucleus, the second phase begins precisely with the addition of the tenth adenosine.

The activities that add poly(A) to maternal mRNAs during frog oocyte maturation apparently are cytoplasmic, since the reaction is not perturbed by removal of the nucleus. In the nucleus, specificity is conferred by a factor that recognizes AAUAAA. Whether the same factor is responsible for recognition of AAUAAA in the cytoplasm is not known.

Although no data are available on the components that catalyse poly(A) addition during maturation, one may speculate that distinct specificity factors confer specificity for the U-rich element on a cytoplasmic poly(A) polymerase. Poly(A) polymerases in 'cytoplasmic' fractions of cell homogenates have long been reported, but their location and function in vivo are unknown. In general, they appear to be very similar, if not identical to, the nuclear poly(A) polymerase.

Poly(A) shortening during maturation: a default state

Like the Cheshire cat from Alice in Wonderland, many mRNAs vanish tail
The nature of the 3'UTR determines whether an mRNA will undergo poly(A) removal during maturation. Replacement of the 3'UTR of an mRNA that does not lose its poly(A) tail with the 3'UTR of an mRNA that does, results in a chimeric mRNA from which poly(A) is removed\(^2\). Yet, surprisingly, no specific sequence in the 3'UTR is necessary for poly(A) removal: poly(A) is removed from RNAs containing a 'body' of prokaryotic sequence (Fig. 3) (Fox and Wickens, unpublished). Thus deadenylation is a 'default' state during oocyte maturation: in the absence of any information to the contrary, poly(A) tails are removed.

In somatic cells, a UA-rich sequence, first detected in GM-CSF mRNA\(^2\), appears to be essential for deadenylation and the consequent instability of the mRNA\(^2\). In oocytes, however, in which deadenylation is a default state, a 'protecting sequence' must exist that prevents loss of poly(A). The protecting sequences turn out to be the same as those that cause poly(A) addition - namely the U-rich element and AAUAAA. Mutations in either sequence not only prevent poly(A) addition but also cause poly(A) to be removed (Fox and Wickens, unpublished).

How might these sequences enable certain RNAs to avoid poly(A) removal by the default deadenylase? Two extreme models are depicted in Fig. 4. In the first (compensation) all RNAs undergo poly(A) removal, and only those that can combat loss with poly(A) addition end up with a tail after maturation. In the second (protection) poly(A) added via the U-rich element may be resistant to deadenylation, either because it contains modified adenosines, or because, early in maturation, it is exposed to a different form of poly(A) binding protein than is present during oogenesis. Models that invoke resistance gain credibility both because the polyadenylation activity appears before deadenylation begins, and because the enzyme that removes the poly(A) tail may be a poly(A)-specific exonuclease (Fox and Wickens, unpublished).

The combination of sequence-specific poly(A) addition with default poly(A) removal may simplify the problem of mRNA selectivity during early development. Certain mRNAs lose their poly(A) tails not during maturation, but later, during early cleavages for example. If poly(A) addition prevents loss of the tail, then simply by eliminating addition to a specific mRNA, poly(A) removal would automatically ensue. Thus only poly(A) addition would need to be regulated by specific sequences.

**Poly(A) and translation**

Why do eggs and embryos regulate the length of poly(A) present on certain maternal mRNAs? This problem is tied to the more general question of the functions of poly(A) *in vivo* which even now, 20 years after the discovery of the poly(A) tail, remain controversial. It has been suggested that poly(A) is critical for transport, translation and mRNA stability - in short, if an mRNA does it, evidence has been presented that it does because of poly(A).

Whatever the function of poly(A), it is almost certainly mediated by poly(A)-binding protein (PABP). This protein is associated with poly(A) *in vivo*, binds to poly(A) with high affinity *in vitro* and is essential for cell viability in yeast (see Refs 5, 30, 31 for citations).

Recent experiments strongly suggest that poly(A) and PABP are necessary for efficient translation\(^3\,32\). For example, poly(A) tails enhance translation both in cell-free translation systems\(^3\,32\) and in injected oocytes\(^3\,34\). Furthermore, in yeast, loss of PABP results in dissociation of cellular polyosomes. Remarkably, the lethal effect of lack of PABP in yeast can be overcome by a by-pass suppressor mutation in ribosomal protein, L46\(^3\,36\). In other words, cells without PABP [and so, presumably, without functional poly(A)] survive if they contain an altered translational apparatus\(^3\,35\).

In oocyte maturation, addition of poly(A) to a specific maternal mRNA can cause translational activation of that mRNA. Polyadenylation during maturation can be blocked by removal of UUUAUAU\(^14\), by deletion of a portion of the 3' untranslated region\(^14,16\), or by the presence of a non-extendible 3' deoxyadenosine at the poly(A) site\(^14,16\). Each of these manipulations, designed to prevent poly(A) addition, impairs or prevents translational recruitment. Similarly, removal of the poly(A) tail, conferred by the transfer of a 3'UTR from one RNA to another, causes translational inactivation\(^17\).

**How does poly(A) stimulate translation?**

Poly(A) apparently enhances initiation of translation\(^3\,32\). In that context, the observation of circular polyomes is provocative, as is the presence of poly(A) near the 5' end of certain mRNAs (reviewed in Ref. 31). In
It is not surprising that poly(A) stimulates translation nearly 20-fold in oocytes, but only two- to three-fold in vitro.

Under conditions in which different mRNAs compete for initiation, small differences in the affinity of an mRNA for components of the translation apparatus can result in large differences in translation. Since the frog oocyte’s translational capacity is saturated, the effect of poly(A) may be particularly pronounced.

Possible roles for poly(A) in maturation and early development. Oocyte maturation and fertilization suddenly demand different protein products than those accumulated in the egg during oogenesis. If polyadenylation activates translation, then it is not surprising that it should accompany both events.

But why remove poly(A) from other mRNAs? This may be more than translational parsimony. Since mRNAs compete for translation in oocytes, deadenylation of superfluous mRNAs will not only remove them from the translated ‘pool’, but should enhance the translation of mRNAs that still have poly(A). In this respect, the default deadenylation system may be viewed as a translational purge. This view is particularly attractive in Spisula and Urechis, in which many mRNAs undergo translational inactivation.

Translational regulation via poly(A) may be essential for proper oocyte maturation and early development. Several maternal mRNAs that encode proteins required for maturation contain UUUUUAU or a closely related sequence near AAUAAA, suggesting that their activation by poly(A) addition could be necessary for maturation to proceed.

The hypothesis that polyadenylation is essential for maturation is supported by circumstantial evidence. For example, maturation is induced by injection of polyadenylated RNA extracted from oocytes after maturation, but not before. Injection of 3’dATP, which blocks polyadenylation by acting as a chain terminator, apparently prevents maturation. Finally, during maturation, the mRNA that encodes D7 protein, which stimulates maturation, is translationally activated and receives poly(A). Although none of these experiments is definitive, they suggest that further tests are warranted.

After fertilization, poly(A) addition and removal probably continues to regulate translation of certain maternal mRNAs. For example, several mRNAs that become translationally active during early Xenopus development acquire poly(A)10. The detailed mechanism of poly(A) addition during early development may differ from that during oocyte maturation, since the same mRNA (Xfin) apparently can both lose poly(A) during maturation, and gain it again after fertilization.

Critical problems and future studies. Eliot had it right 46 years ago:

Eliot had it right 46 years ago:

What we call the beginning is often the end.
And to make an end is to make a beginning.
The end is where we start from.

What does this involve? The mRNA does influence the start of translation, and this, in turn, may be particularly important in the embryo, at life’s beginning. Yet many important questions remain.

The mechanism by which poly(A) is added to certain mRNAs during maturation is a key unsolved problem. To what extent are these cytoplasmic events analogous to polyadenylation in the nucleus? Is the poly(A)-polymerizing activity in the cytoplasm similar to the poly(A) polymerase in the nucleus? What cytoplasmic factor recognizes AAUAAA, and how does it compare with that in the nucleus? These are important questions that require further investigation.

Polypeptide addition and removal are both induced by maturation. How? Does this involve covalent modification of pre-existing polymerases, specificity factors, or poly(A) removal enzymes?

To answer these questions, a cell-free system that supports the translation of poly(A) is needed. This system would be invaluable.

Xenopus egg extracts that support cell cycles in vitro, as have recently been reported, seem a likely source for the activities.

The discovery that sex determination...
in *Drosophila* hinges on regulated splice site choice has provided an extremely powerful approach to the mechanism of alternative splicing. Studies of regulated poly(A) addition and removal would profit comparably from analogous genetic approaches. Where should one look?

Any maternal mRNA whose poly(A) tail length or translation changes during early development could provide a useful starting point. For example, in the nematode *Caenorhabditis elegans*, the * fem-3* gene, a key gene in sex determination, encodes a maternal mRNA that receives poly(A) early in development. Furthermore, single nucleotide changes in the 3'UTR of * fem-3* mRNA result in premature lengthening of the poly(A) tail and in gain-of-function phenotypes consistent with improper translation (J. Ahrringer and J. Kimble, pers. commun.). In *Drosophila*, several maternal mRNAs critical in early development (e.g. *bicoid* mRNA) are translationally inactive in the oocyte but become translated soon after fertilization. Subsequently, their translation ceases and they disappear. It seems likely that, for at least some of these mRNAs, translational activation will be caused by poly(A) addition, and inactivation and mRNA disappearance by poly(A) removal.

However poly(A) is added or removed, the problem of how poly(A) stimulates translation remains. Here, identification of the factor(s) with which PABP interacts to stimulate translation is of paramount importance. Yeast genetics may prove very useful, since conditionally lethal mutations of PABP that prevent translation have already been described.

The conclusions discussed here stem from the serendipitous convergence of genetics, biochemistry and developmental biology on problems of common interest. Many new questions have arisen. The same diversity of approaches should rapidly provide new answers.

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**References**