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*[1]. 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.

THE FROG EGG is a magnificent biochemical device. After months of oogenesis, at fertilization it abruptly begins a precise choreography of molecular events that divide it into thousands of cells in just a few hours. During this period, key developmental decisions are made, including the positions of future body axes. These decisions require only the larder of mRNAs and proteins formed during oogenesis. Transcription in the embryo does not begin until later.

During early development, changes in translation, together with the selective destruction of certain mRNAs, determine which proteins are synthesized in which cells and when. Certain mRNAs that were translationally inert before fertilization are translationally activated at specific times; other mRNAs show the opposite behavior, and after fertilization their translation ceases. As a result, eggs and early embryos have long been favorite materials for studies of translational regulation.

This review will concentrate on the addition and removal of poly(A) from specific mRNAs early in development, which is one device by which translational control can be asserted and the role that these polyadenylation changes play in regulating translation in the embryo. Poly(A) addition during early development occurs in the cytoplasm and is selective for only certain mRNAs, and so is distinct from the reaction that adds poly(A) in the nucleus to virtually all mRNAs. Furthermore, whereas nuclear poly(A) addition is coupled to a cleavage reaction[1], poly(A) addition during early development is not. I will not provide a general discussion of translational regulation during embryogenesis, nor a summary of all reported functions of poly(A), as several excellent reviews have considered both topics in detail[2–5]. Clearly, translational activation is not likely to be the sole function of poly(A), nor is polyadenylation the only way to activate translation of previously quiescent mRNAs.

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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 (Fig. 1)[2,3,6]. Although these correlations are the general rule, they are not without exception[2,3]. Likewise, the number of mRNAs affected varies enormously between animal species. For example, at fertilization in the surf clam *Spisula*, many different mRNAs undergo selective poly(A) addition and removal, and most of these events are correlated with changes in translation[3,7,8]. 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 before 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 a culture dish in response to progesterone. Similarly, excised mouse oocytes undergo spontaneous maturation *in vitro* unless treated with dibutyryl-cAMP.

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[9,10]. In somatic cells, increases in poly(A) tail length are correlated with enhanced translation of insulin[11], vasopressin[12] and growth hormone[13] mRNAs. It is reasonable to speculate that these later changes may occur by a similar mechanism.

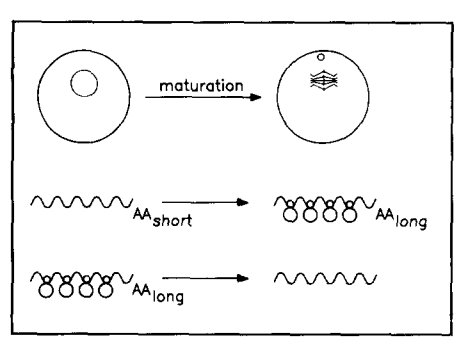

Figure 1
Oocyte maturation and changes in poly(A) tail lengths. Frog oocytes advance from first meiotic prophase to second meiotic metaphase in response to progesterone added *in vitro*. During this process, the short poly(A) tails present on certain maternal mRNAs are lengthened. These RNAs generally 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[14,15] or mouse[6,16,17] 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)[14,16]. Likewise, polyadenylation can be prevented by amputation of the 3'UTR[14,17]. In fact, an appropriate 3'UTR, injected on its own into oocytes, is a competent substrate (Fig. 2)[6,15]. 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 UUUUUAU, or a related sequence (Table I)[14,15], near to the AAUAAA motif that is required for polyadenylation in the nucleus, and which is present in virtually all mRNAs[1]. To determine whether UUUUUAU 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 UUUUUAU and AAUAAA are critical. Deletions of UUUUUAU abolish polyadenylation[14], and insertions of UUUUUAU into otherwise inert RNAs activate it[15]. Similarly, polyadenylation is prevented by point mutations[15] and deletions[14] of AAUAAA.

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

In *Xenopus*, although mutations in UUUUUAU 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 UUUUUAU or related sequences. Furthermore, UUUUUAU is most commonly located upstream (5') of AAUAAA, but may also be able to function downstream[15]. Polyadenylation efficiency can be affected by variation in the sequence that connects AAUAAA and UUUUUAU[15], suggesting that these two critical elements may not be the whole story. Thus, although UUUUUAU 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[15]. 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[2,3]. 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[1,18]: 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[14]. 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[15]. 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.

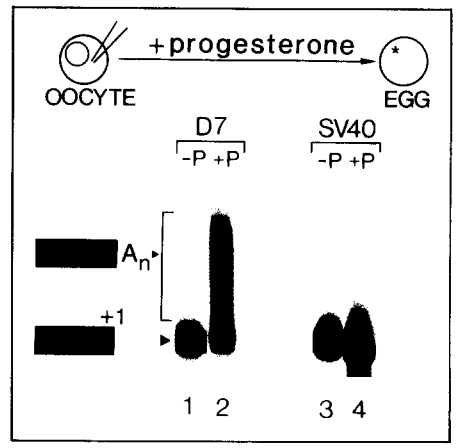

Figure 2
The 3' untranslated region is sufficient for poly(A) addition during maturation. The 3'UTR of a maternal mRNA that receives poly(A) during maturation, called D7, was injected into the cytoplasm of frog oocytes. The 3'UTR of SV40 late mRNA was injected into other oocytes. Oocytes were then either induced to mature with progesterone (+P) or were incubated in the absence of progesterone (–P). The progesterone-treated oocytes, which advance through maturation, polyadenylated the D7 but not the SV40 RNA. (Data taken from Ref. 15.)

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[19,20]. 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

Table I. *Xenopus* mRNAs that receive poly(A) during early development[a]

D7	gccuuc**UUUUUA**cauauuagc**UUUUUAC**cacaAAUAUA –33 nt–poly(A)
G10	**UUUUAAU**auuauuugug**UUUUUAU**aaaggugu AAUAAA –16 nt–poly(A)
B4	guaaaucugg**UUUUUAAU**g**UUUAAU**uucuaua AAUAAA –24 nt–poly(A)
E5	cugcauaccaaugaguc**UUAUAUUUU**gcaaau AAUAAA –3 nt–poly(A)
H4	uuuuu**UUUUUAU**auauaauuuguaaacuuuua AAUAAA –12 nt–poly(A)

[a]A table of this form was first presented in Ref. 14. Each of these RNAs was identified by M. Dworkin and his collaborators, who have generously made them available to others. Sequences have been taken from the following references: D7, Ref. 41; G10, Ref. 14; B4, Ref. 47; E5 and H4, Fox and Wickens, unpublished.

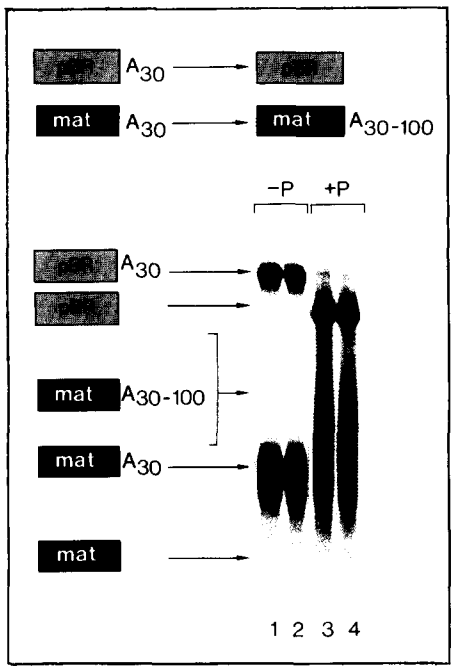

Figure 3

Poly(A) removal is a default state during maturation. A mixture of two RNAs was injected into the cytoplasm of oocytes. The two RNAs have identical 30 nucleotide poly(A) tails but different 'bodies'. The body of one contains prokaryotic sequence (pBR); the other contains the 3'UTR of a maternal mRNA, H4 (mat), that contains UUUUUAU and receives poly(A) during maturation (see Table I). After injection, oocytes were cultured in the presence (+P) or absence (–P) of progesterone. Two oocytes of each type are shown. In the same cell, the poly(A) tail of the 'maternal' RNA is extended while the tail of the 'pBR' RNA is removed. The identities of the products present after maturation were confirmed by injections of each RNA separately. Note that no RNA is detected at the position that would correspond to the deadenylated maternal (mat) mRNA, and that the fully deadenylated 'pBR' RNA is stable. (Data taken from Fox and Wickens, unpublished.)

first, with degradation of the 'body' of an mRNA being preceded by removal of its poly(A) tail[5,21]. Although poly(A) removal may be obligate in the destruction of some mRNAs, others are inactivated by internal cleavages (see for examples Refs 22 and 23).

During oocyte maturation in frogs, the poly(A) tail on certain mRNAs is extended while the poly(A) of other mRNAs is taken off completely. However, poly(A) removal does not necessarily lead to immediate destruction of the mRNA, since tail-less mRNAs persist[24,25] and can even be re-adenylated after fertilization[26].

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[27]. 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[28], appears to be essential for deadenylation and the consequent instability of the mRNA[29]. 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 mRNAs 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[9]. 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 it 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[30,32]. For example, poly(A) tails enhance translation both in cell-free translation systems[31,32] and in injected oocytes[33,34]. Furthermore, in yeast, loss of PABP results in dissociation of cellular polysomes. Remarkably, the lethal effect of lack of PABP in yeast can be overcome by a by-pass suppressor mutation in ribosomal protein, L46[30]. In other words, cells without PABP [and so, presumably, without functional poly(A)] survive if they contain an altered translational apparatus[30,35].

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 UUUUUAU[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[27].

How does poly(A) stimulate translation?

Poly(A) apparently enhances initiation of translation[30–32]. In that context, the observation of circular polysomes is provocative, as is the presence of poly(A) near the 5' end of certain mRNAs (reviewed in Ref. 31). In

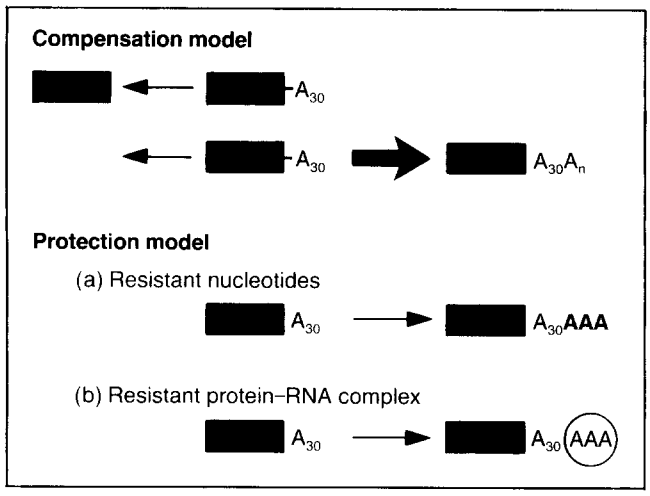

Figure 4
Two extreme models depicting how RNAs containing the U-rich element might escape poly(A) removal by the default deadenylation system. In the compensation model, two RNAs are depicted. Both use poly(A) at the same rate. Only one contains the U-rich element. This RNA therefore receives sufficient poly(A) to compensate for the loss and so contains poly(A) when maturation is complete. Two forms of the protection model are depicted. In (a) the poly(A) added during maturation is covalently different from conventional poly(A), and therefore resistant to nuclease. In (b) a different form of poly(A)-binding protein (present only during maturation and not before) has bound to the new poly(A). This protein–RNA complex is resistant to the nuclease.

oocytes, poly(A) selectively enhances re-initiation, suggesting that it may help shuttle terminating ribosomes back to initiate again[33].

It is very likely that PABP interacts directly with some component of the translational apparatus, but the details of this interaction – including the identity of the interacting molecules – are unknown. It may be possible to identify this molecule (or molecules) genetically, as an allele-specific suppressor or enhancer of a yeast PABP mutant, for example.

The effect of poly(A) on translation is quantitative, not qualitative. Before maturation, certain mRNAs with short poly(A) tails are translated, albeit poorly; then, when they receive more poly(A), their translational efficiency increases. Similarly, histone mRNAs lack poly(A) and yet are translated.

A quantitative effect of poly(A) on initiation clarifies discrepancies in the literature, since experimental systems that initiate poorly will exhibit only a minimal dependence on poly(A). Early reports that poly(A) has no effect on translation *in vitro* were performed with extracts that were relatively inactive (discussed in Ref. 31). Similarly, since initiation in injected oocytes is vastly more efficient than in *in vitro* systems[36], it is not surprising that poly(A) stimulates translation nearly 20-fold in oocytes[33], but only two- to three-fold *in vitro*[33].

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[37]. Since the frog oocyte's translational capacity is saturated[38,39], 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[38,39], 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[3].

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[15], suggesting that their activation by poly(A) addition could be necessary for maturation to proceed[15]. 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[40]. Injection of 3'dATP, which blocks polyadenylation by acting as a chain terminator, apparently prevents maturation[14]. Finally, during maturation, the mRNA that encodes D7 protein, which stimulates maturation, is translationally activated and receives poly(A)[41]. 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)[9,10]. 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[26].

Critical problems and future studies

Eliot had it right 46 years ago[42]:

> 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.

the end of 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 its nuclear counterpart? Is there a distinct specificity factor that recognizes the U-rich element?

Poly(A) 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 faithful poly(A) addition and removal *in vitro* would be invaluable. *Xenopus* egg extracts that support cell cycles *in vitro*, as have recently been reported[43], 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[44]. 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. Ahringer 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[45]. Subsequently, their translation ceases and they disappear[46]. 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[30].

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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