Life and death in the cytoplasm: messages from the 3′ end

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The cytoplasmic life of an mRNA revolves around the regulation of its localization, translation and stability. Interactions between the two ends of the mRNA may integrate translation and mRNA turnover. Regulatory elements in the region between the termination codon and poly(A) tail – the 3′ untranslated region – have been identified in a wide variety of systems, as have been some of the key players with which these elements interact.

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Abbreviations
CPE   cytoplasmic polyadenylation element
CPSF   cleavage and polyadenylation specificity factor
LOX   15-lipoxygenase
NMD   nonsense-mediated decay
Pab1p   poly(A)-binding protein
PAP   poly(A) polymerase
SLBP   stem-loop binding protein
UTR   untranslated region
V(D)J   variable (division) joining

Introduction

mRNAs are born and molded in the nucleus. At maturity, they emerge into the cytoplasm and become productive by joining ribosomes. Even as they fulfill their function, mRNAs enter a decay pathway. Ultimately, they vanish. In this and our companion review (Jackson and Wickens, this issue, pp 233–241), we concentrate on the cytoplasmic portion of this life history. Here, we focus primarily on mRNA stability and the regulation of translation during development; the contributions of the 3′ end of the mRNA — the poly(A) tail and 3′ untranslated region (3′-UTR) — are a pervasive theme. In the accompanying review, we discuss recent insights into the molecular mechanisms of translation initiation, focusing on the opposite end of the mRNA. Our goal is not to provide a comprehensive review of these areas but to highlight emerging problems and developments. Citations therefore are not comprehensive but are sufficient to provide an entry point for the reader. We begin by discussing how mRNAs are destroyed. In the process, vital features of an mRNA's adult life emerge.

A functional end-to-end link

Decay of many mRNAs in the yeast *Saccharomyces cerevisiae* involves a pathway comprising three sequential steps (Fig. 1; reviewed in [1]). First, the 3′ poly(A) tail is shortened progressively to a length of about ten adenosine residues, corresponding well with the minimum length required to bind poly(A)-binding protein (Pab1p). Deadenylation beyond this point presumably triggers loss of the last bound Pab1p and thereby elicits the second step in mRNA decay: 'decapping'. In this process, the m7GpppG cap structure at the 5′ end of the mRNA is removed, apparently by hydrolysis of the triphosphate linkage. The *DCP1* gene product is an essential component of this decapping activity and may be the catalytic subunit [2•]. Finally, the decapped mRNA is degraded by the 5′-to-3′ exonuclease activity of the *XRN1* gene product [3,4]. Other decay pathways utilizing 3′-to-5′-exonucleases also exist but appear to be quantitatively minor in yeast [5].

In yeast strains that lack Pab1p, decapping occurs without the need for prior deadenylation [6], implying that, in wild-type cells, the Pab1p/poly(A) tail complex inhibits decapping, raising the possibility of a physical interaction between the Pab1p/poly(A) complex and the 5′ cap of the same mRNA (review in [1,7]). On the basis of studies of the effects of poly(A) on translation *in vitro*, earlier work proposed interactions between the poly(A) tail and eIF4F [8]. More recently, Tarun and Sachs [9•] have proposed a tripartite interaction between the two ends of the mRNA in which Pab1p when bound to the 3′ poly(A) tail interacts with eIF4G — a translation initiation factor — which interacts with another initiation factor, eIF4E, alias cytoplasmic cap-binding protein (Fig. 2). The existence of a tripartite Pab1p/poly(A), eI-4G, eIF4E complex could provide a reasonable explanation for why deadenylation is normally a prerequisite for decapping and decay: in its simplest form, the binding of eIF4E to the cap would be enhanced by the presence of Pab1p on the poly(A) tail, protecting the 5′ end from decapping.

This view suggests that turnover and translational efficiency could be reflections of the same underlying molecular events: the formation of the end-to-end complex and the balance between eIF4E and the decapping enzyme's interaction with the cap (Fig. 2). The poly(A) tail and cap have reciprocal effects on turnover and translation: mRNA decay is initiated by decapping and deadenylation whereas translation requires the cap and eIF4E and is stimulated by the presence of a poly(A) tail (see below). In one simple view, events that depress eIF4E activity might enhance turnover by providing access of

Figure 1
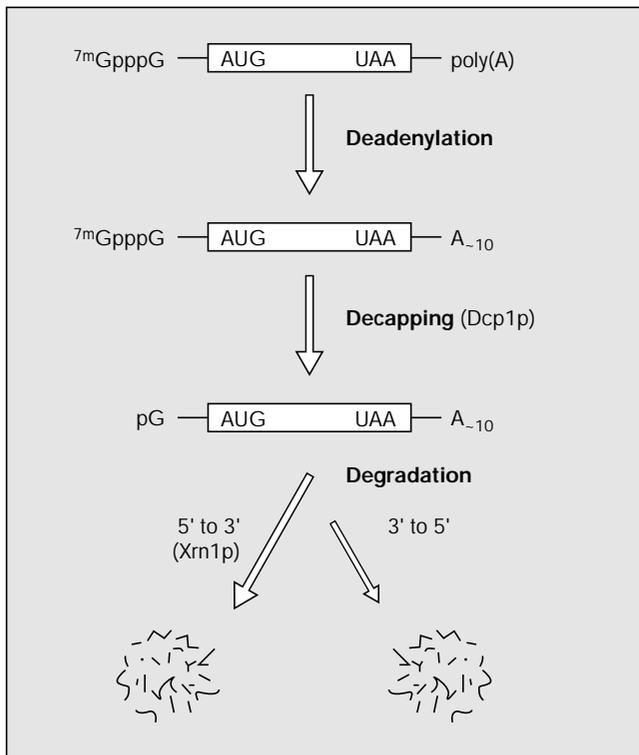
A common pathway of mRNA turnover in yeast: deadenylation-dependent decapping and decay. Cytoplasmic deadenylation, the rate of which is controlled by sequences in the 3′-UTR, progressively shortens the poly(A) tail. Deadenylation beyond a certain length triggers decapping and leads to the 5′ and 3′ exonucleocytic digestion of the mRNA. 3′ and 5′ digestion also occurs but appears less efficient in yeast. (See [1], from which this figure is adapted.) 7mGpppG is the 5′ terminal cap; the open box represents the open reading frame of the mRNA, beginning with AUG and ending with UAA triplets.

the decapping enzyme to the cap, whereas events that depress decapping might have the opposite effect and thereby promote translation. The enhanced decay of *PGK1* mRNA bearing a 5′ terminal site-loop, which should block its translation, is consistent with this notion [5]. More importantly, this hypothesis can also explain the otherwise enigmatic observation that AU-rich elements which destabilize certain mRNAs in mammalian cells instead repress translation in frog oocytes [10–12]. The same underlying molecular events — a shift in the balance away from the eIF4E/cap interaction — could be involved in both cell types; a decrease in the cap/eIF4E interaction might trigger decay in mammalian cells but in oocytes, in which deadenylation does not trigger decay [13,14], the same event might lead only to translational repression.

Identifying all of the components of the end-to-end complex and determining how they interact now seems within our grasp. To date, the physical interactions of yeast proteins related to eIF4G with Pab1p have been demonstrated *in vitro* using yeast extracts and

recombinant proteins [9•]. Although the *in vivo* role and significance of the interactions have not been established yet, genetic tests of their importance will no doubt be performed in the near future. Most obviously, perhaps, the phenotype of strains in which the interaction has been debilitated by mutations in the interacting components will be of considerable interest. Two closely related yeast genes, *Tif4631* and *Tif4632*, have been identified that encode eIF4G-related proteins [15]; both encoded products interact with the Pab1p/poly(A) tail complex *in vitro* [9•]. Double mutants in these two genes are synthetically lethal, suggesting overlapping functions; yet a single mutation in one of these homologs perturbs translation, whereas a mutation in the other does not [15]. This could simply reflect the relative abundance or activities of the two proteins. At this early juncture, however, we do not know whether multiple end-to-end complexes exist with different functions. For that matter, we do not know whether the same complex regulates translation and turnover.

Figure 2

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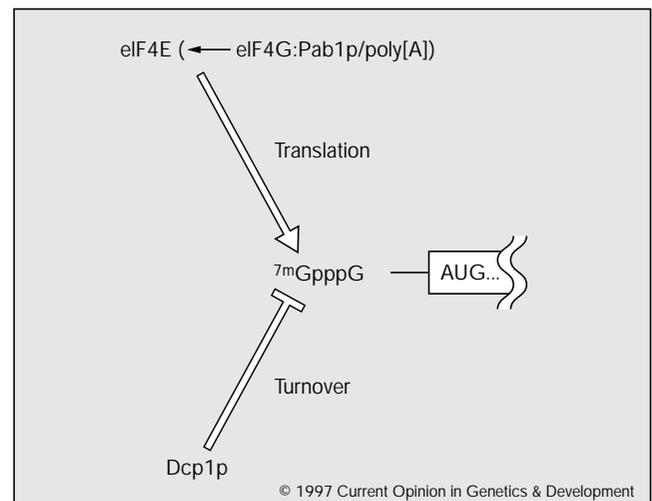
Events at the 5′ end that impact both translation and turnover. eIF4E (cytoplasmic cap-binding protein, a translation initiation factor) binds to the 5′ cap and stimulates translation. eIF4G interacts with eIF4E and probably stimulates its activity in translation. This may be enhanced by the interaction of eIF4G with the Pab1p/poly(A) complex at the 3′ end (see main text for details). Decapping, represented in the figure simply by Dcp1p, negatively regulates expression by causing cap removal and triggering turnover. The balance between the two opposing actions at the cap may be critical in determining the fate of the mRNA (see "Speculations and prospects" section). For example, lack of eIF4E at the 5′ end of a specific mRNA, as might be enhanced by lack of a Pab1p/poly(A) complex, could shift the balance towards decay by enhancing accessibility to the decapping machinery. 7mGpppG is the 5′ terminal cap; the open box represents the open reading frame beginning with the AUG initiation codon.

Genetic analysis of mRNA decay will most likely enable the identification of a diverse group of factors that

influence translation initiation and regulation, as well as deadenylation, decapping, and decay. Mutations in *MRT1* and *MRT3* lead to the accumulation of deadenylated capped RNAs, as does a mutation in *DCP1* [16]. These and other mutants that affect turnover should tap into a constellation of interacting components. For example, if binding of eIF4E and the decapping enzyme are competitive, then mRNA decay could be perturbed by mutations that alter not only the activities of these factors but also any of the other proteins with which they interact, including, potentially, eIF4G and its own array of partners.

Although it is not yet clear in molecular terms how events in translation are connected to turnover, hints of their intimacy are pervasive [17]. Connections may exist at multiple levels. For example, the findings that Upf1p — a factor involved in mRNA decay — is polysome-associated [18] and has genetically separable functions in translation and mRNA decay [19•] suggest that it may operate in and link the two processes. Certain 3′-UTR-borne instability determinants may need to be traversed by a ribosome to cause mRNA decay [20•]. Without a description of the molecules involved, it is difficult to extrapolate whether the diverse array of connections reflect a common mechanism.

It remains to be seen to what extent the yeast decay mechanisms are applicable to higher eukaryotes. As in yeast, mRNA decay in mammalian cells commonly requires prior deadenylation, the rate of which can be accelerated by sequences in the 3′-UTR (reviewed in [17,21,22]). The *trans*-acting factors that interact with these 'instability determinants' have not yet been identified conclusively in any eukaryote, though several candidate proteins have been analyzed in detail. Decapping has been demonstrated only in yeast, though the instability of non-capped mRNAs in vertebrate systems (e.g. *Xenopus* oocytes) suggests that a 5′-to-3′ exonuclease that is sensitive to the structure of the 5′ end exists in such systems and could participate in decapping-dependent decay. Finally, with respect to the nucleases involved, the balance between 5′-to-3′ and 3′-to-5′ activities may differ among organisms or mRNAs. Mammalian histone mRNAs are degraded in the 3′-to-5′ direction [22], in contrast to what appears to be the prominent mode of decay in yeast [3]. A 3′-to-5′ pathway of decay for yeast mRNAs, however, is revealed in cells lacking Xrn1p [5]. The ability to degrade mRNAs through either of two modes provides obvious opportunities for regulation [1].

Decay of mutant mRNAs: mRNA surveillance and its implications in humans

mRNAs containing nonsense mutations are degraded rapidly through a process termed nonsense-mediated decay (NMD; reviewed in [17,23,24]). In yeast, NMD uncouples decapping and the subsequent 5′-to-3′ exonucleolytic degradation from the requirement for prior deadenylation [25]. NMD requires a *cis*-acting element

with a loose consensus sequence positioned downstream of the termination codon [26] and at least three *trans*-acting proteins: Upf1p, Upf2p and Upf3p [27,28]. These three proteins probably form a complex as Upf1p has been shown to interact with Upf2p [27] and Upf2p with Upf3p [19•]. In *Caenorhabditis elegans*, seven genes — *smg-1* through *smg-7* — have been identified that are required for NMD (reviewed in [29]). Of these, *smg-2* is very similar in sequence to yeast *UPF1*, suggesting that the machinery involved in NMD is most likely conserved among eukaryotes (M Page, B Carr, P Anderson, unpublished data). Indeed, a human cDNA very similar in sequence to *UPF1* has also been identified [30]. The central portion of this sequence, encoding an RNA helicase, complements *UPF1*-deficient yeast when grafted to the amino- and carboxy-terminal portions of Upf1p [30].

NMD is a non-essential system. Null alleles of all three *UPF* genes and at least three *smg* genes have been identified. Mutants that eliminate NMD exhibit only minor phenotypes (see [31]), despite their effects on mRNAs produced from mutant alleles. They do influence the stability of several mRNAs bearing upstream open reading frames and this may be one important function of the system (reviewed in [32]).

A broader role of the NMD system is "mRNA surveillance", as proposed by Pulak and Anderson [33] (Fig. 3). In this view, the system surveys the mRNA population and removes those mRNAs that contain premature nonsense codons, thereby avoiding the deleterious effects of their translation products. The deleterious nature of certain protein fragments is exemplified by several nonsense mutations in the myosin heavy chain gene. Such mutations are recessive in *smg*(+) genetic backgrounds and dominant — paralyzing the worm — in *smg*(−) backgrounds [33]. A simple interpretation of this 'synthetic dominance' is that accumulation of mRNAs bearing premature termination codons in NMD-defective cells can lead to production of toxic fragment polypeptides. Consistent with this view, the several myosin alleles that exhibit synthetic dominance are predicted to express amino-terminal myosin head fragments without attached carboxyl-terminal rod segments. The myosin head fragments predicted from these alleles disrupt sarcomere assembly, possibly because of their ability to interact with actin in an aberrant manner. Importantly, synthetic dominance is not a peculiarity of the myosin gene or protein: in *C. elegans*, dominant mutations in numerous genes are recessive or only weakly dominant when *smg*(+) but strongly dominant when *smg*(−) (B Cali, P Anderson, personal communication).

What are the relevant substrates of NMD in otherwise wild-type animals? Presumably, the role of NMD is not to degrade nonsense mutant mRNAs arising from germline mutations. If such mutations are deleterious, they are effectively eliminated from populations by natural selection. Rather, Pulak and Anderson [33] propose that

Figure 3
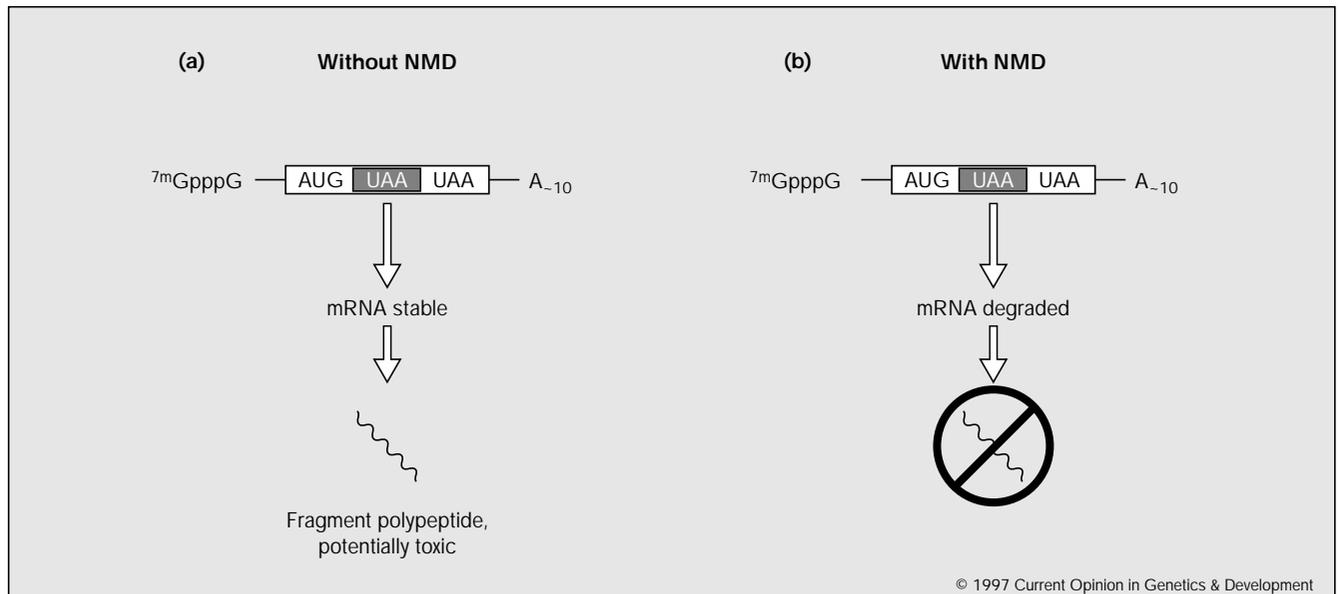
mRNA surveillance prevents the accumulation of toxic polypeptides. The diagram depicts an mRNA bearing a premature nonsense codon (UAA in a gray box). **(a)** In animals lacking the NMD system, the mRNA is stable and leads to the accumulation of a toxic polypeptide. **(b)** In otherwise wild-type animals with a functional NMD system, the mRNA is degraded, avoiding accumulation of the toxic polypeptide and its deleterious effects.

the NMD system removes two broad categories of aberrant mRNAs resulting from non-heritable events: mRNAs generated following errors of gene expression and mRNAs arising from somatic mutation. Gene expression errors that might be removed by NMD include transcriptional mistakes, products of aberrant splicing—including exon skipping and use of cryptic splice sites—and precocious transport of intron-containing mRNAs to the cytoplasm. Indeed, unspliced mRNAs from several intron-containing genes accumulate in yeast lacking the NMD system [34]. Somatic mutant mRNAs that might be removed by NMD include any that contain premature nonsense or frameshift mutations, including 'programmed' aberrant mRNAs such as those generated by out-of-frame V(D)J rearrangements in lymphoid cells [35].

In humans, analogous 'synthetic dominance' between NMD defects and mutations in other genes might well signify the importance of the NMD system in a variety of clinical conditions. Most simply, recessive alleles in many genes that would have no effect on phenotype in a heterozygote might acquire dominant effects in individuals or cells lacking NMD and thereby cause disease: human equivalents of the paralyzed worm above [33]. Certain dominantly inherited human diseases most likely arise from disruptive nonsense fragment polypeptides (B Cali, P Anderson, personal communication). In individuals lacking NMD, nonsense alleles in many other genes might become dominant and lead to disease. Although analysis

of the consequences of NMD deficiency in *C. elegans* has exploited germline transmission of NMD defects, lack of NMD could also arise by somatic mutation in heterozygous individuals. Thus, mRNA surveillance might parallel 'DNA surveillance' (repair) as a suppressor of a variety of genetic disorders, including cancer. For these reasons, the recent cloning of a human gene similar in sequence to *UPF1* [30] may elicit a burst of exciting clinically-oriented work in mRNA turnover.

In mammalian cells, mRNA associated with the nuclear subcellular fractions is subject to NMD, whereas the fraction which 'escapes' to the cytoplasm is usually turned over at the normal rate [24]. Nevertheless, there is ample evidence that the system which targets the mutant mRNA for accelerated destruction recognizes the nonsense codon after splicing has occurred [36,37], and is dependent on translation of the mutant mRNA (reviewed in [24]). A common presumption is thus that it is translation at or near the nuclear membrane, as the mRNA is transported to the cytoplasm, that triggers the accelerated decay. These observations spark the notion that NMD occurs during the first round of translation, as the mRNA emerges cap-first from the nucleus (reviewed in [24]). Evidence supporting other alternatives, including triplet recognition—and perhaps translation—in the nucleoplasm, has also been presented (e.g. [37]). More prosaically, however, it appears that NMD in yeast can occur on mRNAs associated with cytoplasmic polysomes [38].

The influence of poly(A) tails on translation: yeast and somatic cells

Does the functional link between the two ends of the mRNA, revealed in studies of mRNA turnover, extend to translation? Transfection of of RNA by electroporation into plant protoplasts and other eukaryotic cells has shown that the poly(A) tail acts in synergy with the 5′ cap structure to enhance translation efficiency. Curiously, although the enhancing effect of the poly(A) tail decreased as the length of the 3′-UTR was increased [39]. Translation of mRNAs that lack poly(A) tails (e.g. mammalian histone mRNAs and certain plant viral RNAs) is often enhanced by unusual sequences or structures in their 3′ UTRs [40–42].

Although polyadenylation has relatively little influence on translation efficiency in the rabbit reticulocyte lysate or wheat germ cell-free translation systems, important recent experiments with yeast cell-free systems have suggested that translation is strongly stimulated if the mRNA has a poly(A) tail and that Pab1p is absolutely required for this stimulation [43,44•]. Although the high instability of RNAs in this system complicates the analysis, it appears that translation initiation, particularly the recruitment of the 40S ribosomal subunit, is enhanced by the presence of a poly(A) tail [44•]. This contrasts with earlier reports suggesting that 60S subunit joining was affected [45,46]. The functional connection between the poly(A)/Pab1p complex at the 3′end and the cap at the 5′end, discussed earlier in the context of mRNA turnover, has obvious and attractive parallels here.

The magnitude of poly(A)'s effects on translation *in vivo* in yeast is not clear and it may be an over-interpretation to assume that a poly(A) tail with bound Pab1p is invariably necessary. Although *PAB1* is normally necessary for viability, the lethality of a *pab1* mutation can be suppressed either by mutations in ribosomal proteins or ribosome assembly which result in a slight imbalance in the ratio of the two ribosomal subunits [45], or by null mutations of *XRN1* [6]. Moreover, blocking polyadenylation via a temperature-sensitive mutation in poly(A) polymerase (*PAP1*) does not result in inhibition of protein synthesis despite the fact that most mRNAs have, at most, a very short tail [47]. This experiment may underestimate poly(A)'s role, however, as the total amount of mRNA is decreased by the *PAP1* lesion, leading to an excess of translation machinery and reduced competition among mRNAs. Finally, the endogenous yeast L-A virus and its M(1) satellite, which causes the killer phenotype, encode uncapped mRNAs lacking a poly(A) tail, which are nevertheless translated *in vivo*. Host (yeast) mutations which either increase or decrease expression of the killer phenotype have been described that may help illuminate the question of how the presence or absence of a poly(A) tail can influence translation efficiency [48,49].

Whatever poly(A) does, the S-phase-specific histone mRNAs get by without it. Although these mRNAs never re-ceive a poly(A) tail, they are translated efficiently because a conserved stem-loop structure at their 3′ ends mimics the function of poly(A) in enhancing translation. This RNA structure interacts with a cellular protein, named the stem-loop binding protein (SLBP). Remarkably, the same stem-loop structure is critical in the transport of histone mRNAs from the nucleus and in their regulated degradation. For these reasons, the recent cloning of *SLBP* by two laboratories [50•,51•] is a major development. Although the SLBP/histone stem-loop and Pab1p/poly(A) complexes appear to overlap in function, SLBP bears no sequence similarity to Pab1p and instead contains a short novel RNA-binding motif.

Poly(A) dynamics and translational regulation during early development

In germ cells and early embryos, translational control, rather than regulation of transcription or mRNA degradation, is the key to the changing pattern of protein synthesis (reviewed in [52–54]). During oocyte maturation in *Xenopus*, oocytes advance from first to second meiosis and then await fertilization. During maturation, mRNAs for certain proteins, such as ribosomal proteins or actin, become deadenylated and cease to be translated; another subset of mRNAs, including those coding for c-*mos* and cyclins A, B1 and B2, acquire longer poly(A) tails and become translationally activated ([55]; Fig. 4). Though exceptions exist, the correlation between changes in poly(A) tail length and translational efficiency is extremely widespread. Although much of the work to date has emphasized the regulation of poly(A) length during oocyte maturation, the results are most likely germane at least the entire early period of development, prior to the onset of zygotic transcription.

Both polyadenylation and translational activation are dependent on the same *cis*-acting RNA signal within the 3′-UTR, often known as the cytoplasmic polyadenylation element (CPE). During oocyte maturation, deadenylation and translational inactivation function as a default pathway that affects mRNAs without a CPE (reviewed in [56]). At fertilization, however, deadenylation can also be accelerated by specific sequences in the 3′-UTR [57,58]. Even during maturation, certain mRNAs (e.g. c-*mos*) first receive poly(A), then undergo partial deadenylation [55]. Because poly(A) addition and removal are in competition in the oocyte and early embryo [13,14,57,58], changes in poly(A) length and, hence, translational activity can be achieved by regulating either process.

Several types of experiment (reviewed in [53,56]) demonstrate that the change in poly(A) length causes the change in translation. For example, if deadenylation is prevented during oocyte maturation, as can be achieved either by ectopic expression of Pab1p, mRNAs that otherwise would have been inactivated continue to be translated [59]. Conversely, the activation of endogenous c-*mos* mRNA translation during maturation is prevented by

Figure 4

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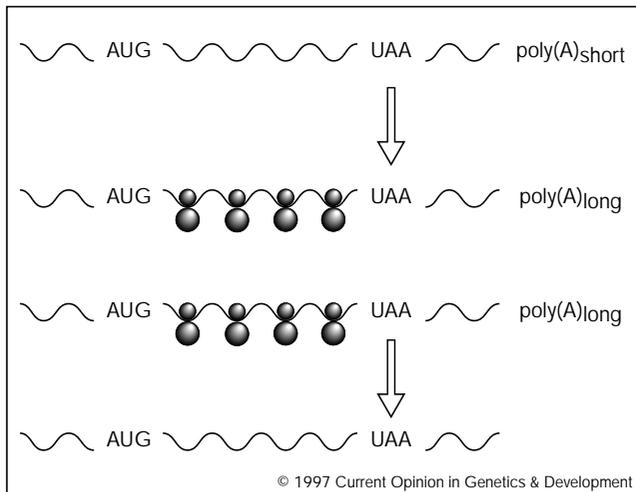
Common correlations between changes in poly(A) length and translational activity during early development. An undulating line in this figure represents an mRNA with its initiation (AUG) and termination (UAA) codons indicated. Shaded circles represent ribosomes. Cytoplasmic lengthening of the poly(A) tail (top) is commonly associated with translational recruitment whereas cytoplasmic removal (bottom) is commonly correlated with translational repression. Although the correlations depicted are widespread, exceptions do exist. (For detailed discussions of these phenomena, see [53,56,71].)

removing the terminal region of its 3′-UTR — including its polyadenylation signals — using RNAseH and antisense oligodeoxynucleotides; translation of c-*mos* mRNA can be rescued by microinjection of a prosthetic RNA designed to anneal to the truncated mRNA and restore its polyadenylation signals [60•].

Although the correlation between polyadenylation and translation has been most closely studied in the *Xenopus* system, it is also well documented in marine invertebrates, nematodes, insects and mammals. In *Drosophila* embryos, for example, several mRNAs that are critical in early developmental decisions are activated early in development but are inactive during oogenesis. For several of these, translational activation is again coupled with an increase in poly(A) tail length [61,62]; *nanos* mRNA is a notable exception, in that its translational activation is achieved without a significant change in the poly(A) tail length [61–63]. Cross-species mRNA microinjection experiments have revealed functional conservation of the *cis*-acting RNA motifs, and presumably the *trans*-acting proteins, regulating polyadenylation and translational activation in *Xenopus*, *Drosophila* and mice [64].

One might have expected that genetic studies of translational regulation in *C. elegans* and *Drosophila* would have revealed CPEs as positive-acting elements in the 3′-UTR, the elimination of which prevents activation of a specific maternal mRNA. CPEs, however, may be required to shorten the poly(A) tail and thereby repress the mRNA

earlier, during oogenesis [65], complicating the issue. If this dual function of the CPE is general, then naturally occurring CPE mutations will appear to define a negative translational control element and not a positive-acting one.

A cautionary note: changes in poly(A) length need not cause changes in translational activity but may occur for other reasons too. As poly(A) addition and removal compete with one another during early development, it may be necessary to add poly(A) merely to avoid its loss and thereby suffer translational inactivation or mRNA decay. Poly(A) tail length can also reflect, rather than cause, a change in translational activity: translational repression by proteins bound to the 5′ UTR causes, but does not require, a shortening of the poly(A) tail (M Muckenthaler *et al.*, personal communication).

Polyadenylation and translational activation: problems and potential solutions

How do changes in poly(A) tail length enhance translation during early development? Do they always do so? The notion that translational efficiency is related directly to achieving a threshold poly(A) tail length is supported by several forms of experiment. For example, injected *bicoid* mRNAs bearing a long poly(A) tail rescue *bicoid* mutant embryos whereas mRNAs with short tails do not [61]. On the other hand, several examples argue that this model is not universal. Ribonucleotide reductase mRNA from the oocytes of a giant surf clam can be de-repressed *in vitro* in the absence of any change in polyadenylation status, even though, *in vivo*, polyadenylation occurs as the mRNA is activated soon after fertilization [66]. *Xenopus* FGFR mRNA, which normally receives a long poly(A) tail during oocyte maturation, is still activated — though much less efficiently — when polyadenylation is prevented (P Culp, TJ Musci, personal communication). *Xenopus* cyclin B1 protein levels increase even when polyadenylation is blocked *in vivo* (S Ballantyne, M Wickens, unpublished data). Yet certain endogenous mRNAs, such as c-*mos*, require polyadenylation for activation [60•,67]. These examples imply that mRNAs differ in their responsiveness to a change in poly(A) length. In light of the end-to-end communication discussed above, it is possible that translation of some mRNAs requires that link whereas translation of others does not.

Aside from specific examples, a simple *a priori* conundrum exists: mRNAs that undergo polyadenylation concomitant with their activation possess respectably long poly(A) tails even when they are silent. For example, prior to oocyte maturation, *Xenopus* c-*mos* mRNA has a poly(A) tail of ~50 residues, yet is inefficiently translated even though a microinjected reporter RNA with a tail of this length is quite actively translated [55]. On maturation, the poly(A) tail of c-*mos* mRNA lengthens to ~120 residues and translation increases dramatically ([55] and references therein). At first glance, it seems unlikely that such an increase in translation efficiency could result from

the modest lengthening of a poly(A) tail from 50 to 120 residues. One solution to this problem is suggested by the observation that the act of polyadenylation, as opposed to the mere length of the tail, is critical in the activation of certain mRNAs [56]. This act appears to cause 2′-O-methylation of the cap structure during oocyte maturation, whereas the mere presence of a long tail does not [68]. Although 2′-O-methylation has been reported as not influencing translational efficiency in other systems, oocytes and embryos might be particularly responsive to this modification. On the other hand, the presence of a poly(A) tail dramatically enhances the translation of mRNAs injected into oocytes prior to maturation (e.g. [55]; reviewed in [69]), when neither cytoplasmic polyadenylation nor cap ribose methylation occur, suggesting strongly that a poly(A) tail can facilitate translation independent of the act of its addition or, by extension, cap modification.

Poly(A) addition may sustain or amplify de-repression that is achieved by independent means [53]. This hypothesis posits that full de-repression requires two separate steps: a triggering step that is independent of polyadenylation and a second polyadenylation-dependent step. The uncoupling of de-repression and polyadenylation *in vitro*, as with ribonucleotide reductase mRNA and erythroid 15-lipoxygenase (LOX) mRNA (discussed below), reflects the first step. The ability of a poly(A) tail to enhance translation of synthetic mRNAs injected into the cytoplasm reflects only the second step and bypasses the first. Consistent with this two-step view, FGFR mRNA is de-repressed in the absence of polyadenylation but the level of translation achieved is reduced (P Culp, TJ Musci, personal communication).

Injection of synthetic mRNAs into the cytoplasm might miss the first step for a variety of reasons. 'Nuclear experience' may be critical in establishing the repressed state and be bypassed by introducing mRNAs directly into the cytoplasm [70,71]. Although the repressive effect of nuclear experience may be caused in part by FRGY RNA-binding proteins, other sequence-specific repressors may also load on to the RNA in the nucleus and stay on in the cytoplasm. For example, hnRNP E1 and K proteins specifically repress lipoxygenase mRNA by binding to its 3′-UTR, yet are seemingly nuclear [72•]. A simple inference from this line of reasoning is that it may be particularly important to manipulate endogenous rather than injected mRNAs to elucidate the effect of polyadenylation on translation.

Trans-acting factors required for cytoplasmic polyadenylation
Besides *cis*-acting RNA signals, at least three *trans*-acting proteins play a role in the control of cytoplasmic polyadenylation and/or translation. One such protein in *Xenopus* is CPEB, which binds to CPEs and is homologous to the *orb* protein of *Drosophila* [73,74]. Micro-injection of anti-CPEB antibodies inhibits polyadenylation during maturation, suggesting strongly that CPEB is a critical positive-acting polyadenylation/translation factor [74]. During maturation, CPEB is phosphorylated but this event is not required to activate polyadenylation during oocyte maturation (S Ballantyne, D Daniel, M Wickens, unpublished data). A second factor identified biochemically is cleavage and polyadenylation specificity factor (CPSF), a multi-subunit factor that binds preferentially to RNAs containing a CPE and AAUAAA. This factor is required for nuclear polyadenylation but is probably also critical in cytoplasmic polyadenylation: calf CPSF, when mixed with purified poly(A) polymerase, recapitulates *in vitro* the CPE-dependence seen during oocyte maturation [78]. Finally, poly(A) polymerases have been detected immunologically in the cytoplasm of *Xenopus* oocytes, and are likely related to their counterparts in mammalian nuclei [79,80].

The cytoplasmic polyadenylation apparatus is activated early in development. Once activated, polyadenylation continues throughout early development with specific mRNAs receiving poly(A) at distinct times. The mechanism by which polyadenylation is initially activated is not known but in *Xenopus* it does not require either CPEB or PAP phosphorylation, nor activation of the cell cycle regulator, maturation promoting factor (S Ballantyne, D Daniel, M Wickens, unpublished data). The temporal order of polyadenylation events during oocyte maturation may be imposed in part by the fact that the polyadenylation of certain mRNAs requires the prior polyadenylation of others (S Ballantyne, D Daniel, M Wickens, unpublished data).

Negative translational control elements in the 3′-UTR: the search for regulators
The 3′-UTR is a key repository of information for regulating mRNAs in the cytoplasm: signals for controlling translation, stability and localization all can reside in this region of the mRNA (reviewed in [52–54,78,79]). Most of the genes we discuss in the following section were uncovered by geneticists dissecting a key developmental decision, without any particular interest in the control of mRNAs *per se*. The fact that so many studies have converged on the 3′-UTR and translational control argues strongly for their prominence during early development.

In the context of translational regulation, the vast majority of the 3′-UTR signals identified genetically to date are negative: when removed, the mRNA is translated when or where it should not be. As such elements have been discussed elsewhere, we point out only two general principles. First, the regulatory elements are often repeated, sometimes dramatically. For example, the 3′-UTR of LOX mRNA contains ten consecutive repeats of a 19 nucleotide sequence that causes its repression [80] whereas repression of *tra-2* mRNA of *C. elegans* is caused by two precise 28 nucleotide repeats [81]. Second,

these negative elements are often conserved; indeed, the presence of a conserved repeated sequence is now strong *prima facie* evidence for a site through which translation or stability are controlled.

An important first step in determining how such elements repress translation is the identification of the repressors to which they bind. Genetic analysis of pattern formation in *Drosophila* has yielded a particularly large bounty; we begin a discussion of the search for *trans*-acting regulators there. (We highlight recent developments as the role of translational control in development has been reviewed elsewhere [52–54].)

Localized mRNAs, localized regulators

bicoid mRNA encodes a homeodomain transcription factor that activates genes required for 'anterior' structures and is localized to that end of the syncitial embryo by its 3′-UTR. Once *bicoid* mRNA is activated, it generates an anterior–posterior gradient of Bicoid protein. The newly produced Bicoid protein silences the uniformly distributed *caudal* mRNA, which encodes another homeodomain transcription factor [82•,83•], resulting in the accumulation of Caudal protein in the posterior. Remarkably, the homeodomain of Bicoid is required for this translational repression [82•,83•]. This raises the tantalizing possibility that other homeodomain proteins may function through RNA rather than DNA binding.

At the posterior, a superficially analogous cascade of translational regulation occurs. *hunchback* mRNA, which is spread throughout the embryo and encodes a transcriptional regulator of the gap genes, must be repressed in the presumptive posterior. This repression is achieved through the binding of Pumilio to specific sequences in the *hunchback* 3′-UTR [84•]. Although Pumilio protein is present throughout the embryo, repression occurs only in the posterior because it also requires Nanos protein [85], which is present at highest concentrations in the posterior because its mRNA is localized there via its 3′-UTR [86].

The link between translational activity and mRNA localization, however, is more complex than simply positioning the regulator's mRNA; certain mRNAs become active only if they too are in the right place. *Oskar* and *nanos* mRNAs, though present at highest concentrations in the posterior, are also detectable elsewhere; these unlocalized mRNAs do not interfere with pattern formation, however, because only those mRNAs that are in the posterior are translated. Although the molecular mechanisms of this repression are unknown, several of the key players now have been identified.

The crucial *cis*-acting elements reside in the 3′-UTRs of *nanos* [63,87–89] and *oskar* [90] mRNAs. Translational repression of *oskar* requires short repeated sequences in its 3′-UTR with which an 80 kDa protein, Bruno, apparently interacts ([90]; PJ Webster *et al.*, personal communication).

Repression of *nanos* mRNA away from the posterior requires stem-loop structures in its 3′-UTR [63,88,89] that most likely interact with Smaug protein [88]. Activation of *nanos* mRNA in the posterior requires Vasa protein, a posteriorly localized RNA helicase [91]. Thus Vasa protein might remove a uniformly distributed repressor by destabilizing an RNA secondary structure to which the repressor binds.

In *C. elegans*, spatial and temporal control of *glp-1* and *pal-1* translation are required for anterior–posterior cell fate determination. *glp-1* mRNA is present throughout a four-cell embryo but is translated only in presumptive anterior blastomeres [92] whereas *pal-1* mRNA is translated only in a specific posterior blastomere [93]. Repression of *pal-1* translation requires the Mex-3 protein and since that protein contains KH-domain homology suggestive of an RNA-binding protein, the two may interact directly [94].

Regulatory RNAs

Though proteins are commonly expected to interact with regulatory sites in 3′-UTRs, RNAs can do the job too. The *C. elegans lin-14* mRNA is repressed by a short RNA encoded by the *lin-4* gene [95]. The repressor RNA appears to bind to complementary sequences in the 3′-UTR of its target mRNA [96,97•]. A bulged cytosine residue in the hybrid structures is essential for repression [97•]. This subtle structural requirement hints that the hybrids may be recognized or assembled by an as yet unidentified regulatory protein but does not alter the fundamental importance of the RNA in repression.

It is too early to tell whether other comparable RNA regulators exist in other systems, particularly as their identification might be difficult using either conventional genetic or biochemical approaches (see [98]). However, the number of non-coding RNAs with important biological functions in other systems continues to increase; these discoveries highlight our ignorance of RNAs as regulators, and suggest that searches for *trans*-acting factors should be designed to accommodate RNA.

Regulation via the 3′-UTR in somatic cells

Translational control involving regulatory elements in the 3′-UTR is not confined to oocytes or embryos. In mammalian cells, erythroid LOX mRNA is repressed until late in erythroid differentiation, after the nucleus has been silenced; the enzyme then helps degrade the mitochondrial membrane. The repression is most likely caused by the regulation by a specific binding factor, termed LOX-BP. This erythroid cell specific factor binds to a repeated sequence in the 3′-UTR of LOX mRNA and inhibits its translation *in vitro* [80]. LOX-BP has now been identified as hnRNP E1 and K; either recombinant protein on its own will specifically repress translation of LOX mRNA *in vitro*, though a mixture of the two is more effective [72•]. Protamine mRNAs in spermatids are translationally repressed until chromosome condensation,

again through sequences in their 3′-UTRs and the proteins that bind to them (e.g. [99,100]).

Although these examples demonstrate regulation via 3′-UTRs in cells other than oocytes, the cells are so peculiar that they beg the question: do somatic cells with a normal, active nucleus also display such control? In a few cases, clearly the answer is yes. In *C. elegans*, for example, *tra-2* and *lin-14* mRNAs are both translationally controlled via their 3′-UTRs in a variety of somatic cells [81,95,96]. As yet, it is unresolved how many mRNAs are translationally regulated via their 3′-UTRs in somatic cells and to what extent?

For many mRNAs, both in somatic cells and the germline, the 3′-UTR is extraordinarily long and has motifs with strong phylogenetic conservation, arguably more so than the 5′-UTR. Although many such conserved motifs may be involved strictly in localization or regulation of mRNA degradation, it would be remarkable if the specific mechanisms that have evolved to regulate translation during oogenesis and early embryogenesis were reserved solely for these special situations.

Speculations and prospects

In this review, we have highlighted repressors, activators, and their interactions, as well as the connection of translational activity to turnover and RNA localization. Some of the crucial *trans*-acting translational regulators have been cloned and more are on the way. In several cases, genetic candidates have been identified but the directness of their involvement in repression is unclear. Although the pursuit of regulatory proteins will yield factors that actually touch the RNA, it will also yield proteins that participate in other events in the pathway. For example, Nanos and Pumilio proteins are both required for repression of *hunchback* mRNA though only Pumilio appears to bind to the mRNA. Connecting the fascinating biological decision-making — such as pattern formation or sex determination — with the molecular details of the control of translation and stability is an exciting area for the future and seems well poised for dissection.

In those cases in which a repressor has been identified unambiguously, other factors may need to join directly with them to achieve regulation *in vivo*. For example, repression of *lin-14* probably requires an unidentified protein, not just *lin-4* RNA. Surely many other RNA-binding coregulators will emerge, much as they have in studies of transcriptional regulation.

From a biochemical standpoint, the identification of *trans*-acting factors is needed to facilitate the reconstitution of 3′-UTR-based regulation *in vitro*. Translational repression of LOX by LOX-BP has clearly pioneered this area and remains one of the very few reported instances of specific repression via a 3′-UTR in a cell-free system. Reconstitution in the test tube of potentially more complex systems — such as those involved in spatially restricted repression and activation in *Drosophila* — may be difficult but is required to usher in a new level of detailed molecular analysis.

Changes in poly(A) tail length are so commonly associated with altered translational activity that they present central issues for understanding translational control during development. We discuss two that are particularly pressing. Firstly, how does a change in poly(A) length enhance translation in the oocyte and early embryo? In particular, is the common connection between a change in poly(A) tail length and translational activity in embryos a reflection of the same molecular events that underlie translational stimulation by the poly(A) tail in yeast extracts? Secondly, do certain negative translational control elements in the 3′-UTR — as have been identified in so many critical regulatory genes — act by directly controlling poly(A) tail length (Fig. 5)? That such elements do control poly(A) tail length is unambiguous in certain cases; remove the element genetically, and the tail is longer. But is the change in length the cause of the altered translational activity or vice versa (Fig. 5)? These possibilities are not mutually exclusive and it seems inevitable that we will soon discover examples of both types.

The emerging view that the 5′ and 3′ ends of an mRNA are linked via protein–protein interactions raises a collection of key questions, including the delineation of the players and precise contacts involved. How does the presence of a poly(A) tail associated with Pab1p stabilize the mRNA and facilitate translation? Is the same complex directly involved in both processes? Although the effects of poly(A) on turnover are unambiguous, it is sobering that its effects on translation in yeast *in vivo* are not. Putting that aside, it is not immediately apparent, a priori, how an interaction between ends promotes association with the ribosome. The end-to-end complex may recruit the ribosome or 40S subunit bypassing scanning from the cap, rather as does an internal ribosome entry site ([44•]; see companion review, this issue, pp 233–241). Alternatively, the end-to-end complex could facilitate cycling of ribosomes that have traversed the termination codon and continued into the 3′-UTR. It is apparent from work on *GCN4* regulation that translation past termination codons can occur in yeast [99] and may also occur on certain mRNAs in mammalian cells [20•].

Studies of mRNA decay continue to illuminate key features in an mRNA's cytoplasmic life. How deadenylation triggers decapping and whether that is related to the end-to-end complex are now central problems. Similarly, the mechanism by which the presence of a premature nonsense codon triggers decapping in the absence of deadenylation will inevitably shed light on the metabolism of wild-type mRNAs. The ribosome must distinguish a premature nonsense codon from a

Figure 5
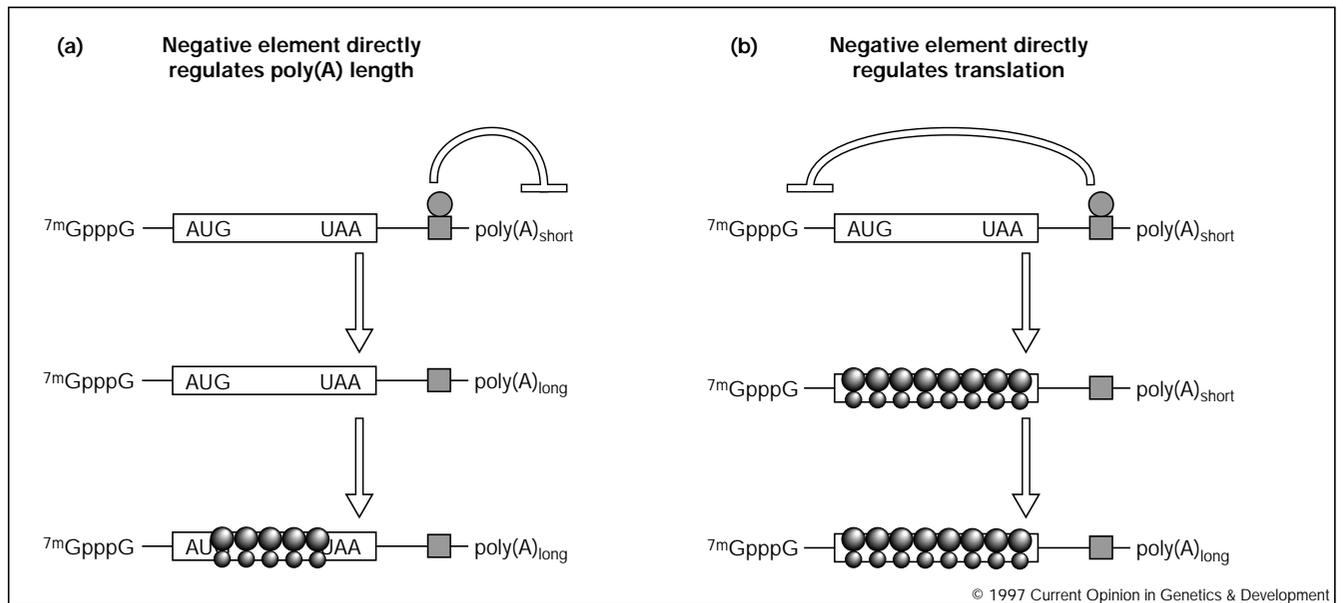
Two mechanisms by which negative translational control elements in the 3′-UTR might regulate poly(A) length. **(a)** A protein bound to a negative translational control element (shaded circle on a square) in the 3′-UTR directly represses poly (A) length. This might be accomplished by inhibiting cytoplasmic polyadenylation or by enhancing cytoplasmic deadenylation. When the repression of poly(A) length is relieved, the tail gets longer. This results in translational activation. **(b)** A protein bound to a negative translational control element represses translation independent of any effect on poly(A) length. When the repression is relieved, translation begins. This leads secondarily to the acquisition of a longer poly(A) tail.

natural one. The recent identification of eukaryotic translation termination factors, eRF1 and eRF3, should provide access to the detailed mechanisms of normal and premature termination. eRF1 actually promotes termination of translation and is identical to the yeast *SUP45* protein [101]. A second polypeptide, eRF3 (alias *SUP35*), interacts with eRF1 and is probably responsible for the GTPase-dependent recycling of eRF1 [102–104]. *Trans*-acting factors needed for NMD may have roles in normal elongation and termination and interact with these 'universal' termination factors as well as with factors required for deadenylation-dependent decay.

A far-reaching aspect of NMD arises from the discovery that, in its absence, toxic polypeptides accumulate from nonsense-containing alleles [33]. As discussed in the text, it seems inevitable that individuals defective in NMD will display a range of clinical conditions, in which mutations that would normally be recessive instead become dominant and thereby cause disease. With the finding that humans, worms and yeast share closely related components in NMD ([30]; see above), the stage is set for an exciting period in understanding the human implications of NMD defects.

Whatever the components of the end-to-end complex, its mere existence suggests an array of mechanisms by which 3′-UTR-borne translational repressors and activators, and regulators of turnover, might function. These hypothetical mechanisms are dependent, conceptually, only on the importance of protein–protein interactions in forming the complex. Activators might enhance formation of the complex; for example, cytoplasmic polyadenylation might present a larger 'landing pad' for Pab1p, or evict proteins that interfere with the formation of the end-to-end complex. Repressors bound to the 3′-UTR might interfere with translation by disrupting formation of the end-to-end complex by touching any of the interacting molecules. Perhaps the assembly of an end-to-end complex is much like the assembly of a transcription complex in eukaryotic cells. The ultimate end is the recruitment of RNA polymerase II or a ribosome. That ultimate end relies on the formation of a network of interactions that can be enhanced and interfered with in a multitude of ways. Vague though such an analogy may be, it suggests that we now see only the smallest fraction of what is to come and that regulators of translation, like those of transcription, will have their unique nuances and points of intervention but regulate a common pathway.

Acknowledgements

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