REVIEW

Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview

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Abstract

Expression of a gene can be controlled at many levels, including transcription, mRNA splicing, mRNA stability, translation and post-translational events such as protein stability and modification. The majority of studies to date have focused on transcriptional control mechanisms, but the importance of post-transcriptional mechanisms in regulating gene expression in eukaryotes is becoming increasingly clear. In this short review, selected examples of post-transcriptional gene regulatory mechanisms operating in both lower and higher eukaryotes will be used to highlight the plethora of such mechanisms already identified.

The underlying theme is that post-transcriptional gene regulation relies on specific RNA–protein interactions that either result in the targeted degradation of the mRNA or prevent access of the ribosome to the translation start codon. Such interactions can occur in the 5′ or 3′ untranslated regions of an mRNA or within the decoded portion of the molecule. The importance of these regulatory mechanisms in a range of biological systems is also illustrated.

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Introduction

Translation in eukaryotes is a complex series of steps involving a wide array of protein translation factors that function in conjunction with the ribosome and tRNAs to decode an mRNA, thereby generating the encoded polypeptide chain (reviewed by Merrick & Hershey 1996). The translation process can be divided into three distinct stages (Fig. 1): (i) *initiation*, which involves the assembly of the ribosomal subunits at the initiation (AUG) codon of an mRNA (reviewed by Pain 1996); (ii) *elongation*, the process of tRNA-mediated decoding of the mRNA to form a polypeptide chain (reviewed by Merrick & Hershey 1996); (iii) *termination*, during which a stop codon (UAA, UAG or UGA) signals the release of the polypeptide chain from the ribosome and the subsequent dissociation of the ribosomal subunits from the mRNA (reviewed by Stansfield *et al.* 1995). Each of these stages requires a specific class of translation factor: initiation (eIF), elongation (eEF) and termination (eRF) factors.

The primary target for translational control is at the initiation step, and global regulation of translational initiation can be achieved by post-translational modification of one or more of the initiation factors. Control of translation initiation on individual mRNAs is determined

primarily by the structural properties of the mRNA, particularly the 5′ untranslated region (5′ UTR). However, features of the 3′ untranslated region (3′ UTR), the coding region and interactions between the 5′ and 3′ UTRs of the mRNA also can have profound effects on control. This review will examine the control and regulation of gene expression of post-transcriptional processes in eukaryotic cells, using examples both from simple eukaryotes (especially the yeast *Saccharomyces cerevisiae*) and from more complex mammalian cells, in order to illustrate the range of regulatory mechanisms already described.

mRNA stability

The rate of mRNA synthesis is not the sole determinant of the steady-state levels of an mRNA; the mRNA decay rate (i.e. its chemical half-life) is also a major determinant. The half-life of individual mRNAs within a given eukaryotic cell may vary by several orders of magnitude, from a few minutes to many hours and, in some cases, to days. The vast majority of eukaryotic mRNAs carry a 5′ 7-methylguanosine cap structure and a 3′ poly(A) tail of up to 200 adenosine residues in length, which protect the RNA chain from degradation by 5′→3′ or 3′→5′

Figure 1 An overview of the three stages of translation in eukaryotic cells. The steps in which the initiation (eIF), elongation (eEF) and termination (eRF) factors have a role are indicated. The steps that involve the movement of the ribosome or ribosomal subunits along the mRNA are indicated by the dashed lines.

exonucleases, or both. In addition to the 5' cap structure and 3' poly(A) tail with its associated poly(A)-binding protein (PABP), the intrinsic stability of a given mRNA is also influenced by specific internal sequence elements. A number of such determinants have been identified that exert a destabilising effect on an mRNA and may be located in any region of the transcript. In the mRNAs of the yeast *S. cerevisiae*, for example, such determinants have been found in the 5' UTR (Pierrat *et al.* 1993), in the coding region (Herrick & Jacobson 1992) and in the 3' UTR (Muhlrad & Parker 1992) of different mRNAs. In contrast, the existence of *stabilising* elements in yeast mRNAs is poorly documented.

There are several different mechanisms of eukaryotic mRNA decay (recently reviewed by Jacobson & Peltz 1996, Ross 1996). A major mRNA decay pathway is initiated by shortening of the poly(A) tail, followed by decapping and subsequent 5'→3' exonucleolytic degradation of the mRNA. A variation of this pathway occurs in which transcripts undergo 3'→5' exonucleolytic decay subsequent to poly(A) shortening. Several sequence elements that promote rapid poly(A) shortening have been identified: for example, sequences within the mammalian *c-fos* coding region and 3' UTR contain an AU-rich element (ARE) (Chen *et al.* 1994) and, in yeast, sequences within the 3' UTR of the *MFA2* mRNA (Muhlrad & Parker 1992) each appear to function to promote poly(A) shortening. A large number of unstable mammalian mRNAs (including proto-oncogene, transcription factor, cytokine and lymphokine mRNAs) have AREs in their 3'

UTRs (Shaw & Kamen 1986, Fig. 2a). When the ARE elements from unstable mRNAs (such as *c-fos* or granulocyte-macrophage colony stimulating factor, GM-CSF) are placed within the 3' UTR of a stable mRNA (such as β -globin), then the normally stable transcript is destabilised (Shaw & Kamen 1986). Furthermore, a family of ARE-binding proteins (such as AUF1) bind to AREs on many mRNAs and regulate their stability (Ross 1996).

mRNA decay can also be initiated by deadenylation-independent decapping and subsequent 5'→3' decay of the transcript. An example of this process can be found in the degradation of the yeast *PGK1* mRNA, which has had a premature nonsense codon introduced by site-directed mutagenesis at the 5' end of its coding region (Muhlrad & Parker 1994). The resulting premature translation termination accelerates decay of the transcript (Peltz *et al.* 1993) and is dependent on *trans*-acting proteins encoded by the *UPF* genes (Leeds *et al.* 1992, Peltz *et al.* 1994). An analogous mechanism also appears to operate in mammalian cells, and is used to degrade incorrectly processed, intron-containing mRNAs that leave the nucleus (reviewed by Jacobson & Peltz 1996). Thus eukaryotic cells have evolved a mechanism for degrading aberrant mRNAs that cannot be translated.

There are also examples of eukaryotic mRNAs that can be degraded via endonucleolytic cleavage before deadenylation. Endonuclease cleavage sites have been identified within the coding sequence and the 3' UTR of several mRNAs. Those within coding regions include mammalian proto-oncogene *c-myc* mRNA (Bernstein

Figure 2 RNA-binding proteins that regulate mRNA stability. (a) A large number of unstable mammalian mRNAs contain AREs in their 3' untranslated regions (3' UTR), and may be bound by regulatory proteins (ARE-binding proteins, ARP), which promote poly(A) tail shortening. (b) The transferrin receptor (*TfR*) mRNA contains five IREs and a functional cleavage site within its 3' UTR (▲). In response to low intracellular concentrations of iron, the IRP has a high affinity for the IREs and prevents access of the nuclease to the cleavage sites. Thus binding by the IRP stabilises the transcript by preventing endonucleolytic cleavage.

et al. 1992) and *Xenopus laevis* albumin mRNA (Dompenciel *et al.* 1995), and those within a 3' UTR include the mammalian transferrin receptor (*TfR*; Binder *et al.* 1994). Endonucleolytic cleavage of some mRNAs is regulated by RNA-binding proteins that bind in the vicinity of the cleavage site(s) and render them inaccessible to nucleolytic attack. In the *TfR* mRNA, five iron-response elements (IREs) are located within the 3' UTR and a functional cleavage site is situated between each of the IREs (Fig. 2b). In response to a decrease in intracellular iron concentrations, the IRE binding protein (IREBP, also called the iron regulatory protein, IRP) has a higher affinity for the IREs and, by binding, prevents access of the nuclease to the cleavage site. When iron concentrations are increased, the IRE–IREBP interaction is diminished and endonucleolytic cleavage occurs (Binder *et al.* 1994).

In summary, the stability of a given mRNA can be controlled by specific intrinsic nucleotide sequences and globally regulated by RNA-binding proteins that bind

many mRNAs or are mRNA-specific. Furthermore, the half-life of many mRNAs can fluctuate in response to developmental or environmental stimuli such as nutrient levels, cytokines, hormones, temperature shifts and viral infection.

Regulation of translation initiation

Translation initiation is an important step in both global and mRNA-specific gene regulation. Global regulation of protein synthesis is generally achieved by the modification of eukaryotic initiation factors (eIFs), several of which (e.g. eIF4E and eIF2; see below) are phosphoproteins. Translational control of individual mRNAs often depends upon the structural features of the transcript itself, and may include structures in the 5' UTR that inhibit initiation directly, for example by impeding 40S subunit binding or scanning, or indirectly, by acting as receptors for a regulatory RNA-binding protein.

Figure 3 The initiation of translation on eukaryotic mRNAs, identifying the steps at which either global or mRNA-specific regulation is exerted. See text for a full explanation.

Regulation of eIF2 activity

The initiation factor eIF2 has a critical role in translational control (Fig. 3). This factor functions to recruit the initiator Met-tRNA_i as a Met-tRNA_i-eIF2-GTP ternary complex, to the 43S ribosomal complex, which in turn consists of the 40S ribosomal subunit and two other translation initiation factors, eIF3 and eIF4C. The guanine nucleotide exchange factor, eIF2B, catalyses the conversion of eIF2-GDP (which is inactive in translation) to eIF2-GTP in order for eIF2 to be recycled into the initiation process (Goss *et al.* 1984). In mammals, phosphorylation of the α -subunit of eIF2 on Ser51 is induced in response to a number of different stress conditions including iron (haem) deprivation and heat-shock in reticulocytes, viral infection and interferon treatment in humans and mice. The kinase responsible for this phosphorylation is either haem-controlled repressor (HCR; Chen *et al.* 1991) or protein kinase activated by double-stranded RNA (PKR, also known as double-stranded RNA-activated inhibitor, DAI; Meurs *et al.* 1990). Phosphorylation of eIF2 α inhibits guanine nucleotide exchange on eIF2 because the eIF2(α P)-GDP complex has an increased affinity for eIF2B, thus preventing it from catalysing the exchange of GDP for GTP. This results in a decrease in the amount of eIF2B available to recycle the remaining non-phosphorylated eIF2-GDP and leads to a down-regulation of general protein synthesis in cells under various stresses.

Direct regulation of eIF2B activity, independent of changes in eIF2 phosphorylation, can also regulate eIF2 activity and thus translation. A well characterised example involves Chinese Hamster Ovary (CHO) cells over-expressing the insulin receptor, in which insulin increases the eIF2B activity by down-regulating an inhibitory activity (Welsh & Proud 1992, 1993). The inhibitor is believed to be glycogen synthase kinase-3 (GSK-3), which phosphorylates the largest subunit of eIF2B.

Regulation of eIF4E activity

The cap-binding translation factor, eIF4E, is a key component of the eIF4F cap-binding complex that binds directly to the m⁷G cap structure at the 5' end of a eukaryotic mRNA to facilitate 43S complex binding to the mRNA (Fig. 3). Regulation of eIF4E activity in mammalian cells can be regulated in one of three ways:

(i) Cellular concentrations of eIF4E may be the limiting factor compared with other initiation factors and ribosomal subunits (Hiremath *et al.* 1985, Duncan *et al.* 1987). However, recent evidence suggests that the levels of eIF4E may be higher than had previously been reported; for example, the rabbit reticulocyte lysate contains a functional excess of eIF4E (Rau *et al.* 1996).

(ii) eIF4E is a phosphoprotein and the phosphorylated form of mammalian eIF4E has a three- to fourfold greater affinity both for the cap structure (Minich *et al.* 1994) and

for eIF4G, compared with the non-phosphorylated form of eIF4E (Goss 1987). eIF4E is phosphorylated in response to hormones (Morley & Traugh 1990, Flynn & Proud 1996) and growth factors (Bu & Hagedorn 1991, Donaldson *et al.* 1991), but becomes dephosphorylated following heat-shock (Duncan *et al.* 1987, Lamphear & Panniers 1990) and viral infection (Huang & Schneider 1991, Kleijn *et al.* 1996). Changes in the phosphorylation state of eIF4E mediate changes in overall rates of protein synthesis and recruitment of individual transcripts that differ in their dependence on eIF4E. For example, *HSP* mRNAs are efficiently translated in HeLa cells deficient in both eIF4E and eIF4G (Joshi-Barve *et al.* 1992). The phosphorylation site of mammalian eIF4E is Ser209 (Joshi *et al.* 1995). Although eIF4E can be phosphorylated *in vitro* by protein kinase C (PKC), multiple signal transduction pathways appear to be involved, at least in mammalian cells (Flynn & Proud 1996). In contrast, the major phosphorylation sites of yeast eIF4E are Ser2 and Ser15, and these sites can be phosphorylated *in vitro* by caesin kinase II (Zanchin & McCarthy 1995). Furthermore, only a small fraction of yeast eIF4E is phosphorylated *in vivo* and this level appears to be unaffected by heat-shock (Zanchin & McCarthy 1995). The regulation of eIF4E activity in yeast therefore appears to be less dependent upon its phosphorylation state than is mammalian eIF4E.

(iii) Two translational repressors, the eIF4E-binding proteins, 4E-BP1 (also known as phosphorylated heat- and acid-stable protein, insulin stimulated: PHAS-I) and 4E-BP2, regulate eIF4E function (Pause *et al.* 1994). They inhibit eIF4E function by competing with eIF4G for a binding site on eIF4E (Mader *et al.* 1995, Haghghat *et al.* 1995) and their binding to eIF4E is also regulated by phosphorylation; for example, 4E-BP1 becomes hyperphosphorylated in cells after treatment with hormones or growth factors, and subsequently dissociates from eIF4E (Pause *et al.* 1994, Lin *et al.* 1994). However, 4E-BP1 is dephosphorylated upon infection with encephalomyocarditis virus (EMCV) and poliovirus, enabling it to bind to eIF4E (Gingras *et al.* 1996). When 4E-BP1 is bound to eIF4E, phosphorylation by PKC is prevented. Therefore, eIF4E phosphorylation may require the action of two kinases, one to phosphorylate 4E-BPs to free the eIF4E, and then PKC to phosphorylate it (Whalen *et al.* 1996). A model for one pathway leading to eIF4E phosphorylation in mammalian cells is illustrated in Fig. 4.

In yeast, eIF4E is associated with a second protein (p20), which in turn is a repressor of cap-dependent translation initiation (Altmann *et al.* 1997). In common with mammalian 4E-BP1, p20 competes with eIF4G for binding to eIF4E (Altmann *et al.* 1997). p20 can be phosphorylated by caesin kinase II *in vitro*, putative sites of phosphorylation being Ser91, Ser154, or both, and the amount of phosphorylated p20 found associated with eIF4E increases during a heat-shock, but decreases during stationary phase (Zanchin & McCarthy 1995). Thus, the activity of p20

Figure 4 Regulation of eIF4E function in mammalian cells. 4E-BP1 becomes hyperphosphorylated by hormones or growth factors and subsequently dissociates from eIF4E. This, in turn, enables eIF4E to bind to eIF4G and to become phosphorylated by PKC. The phosphorylated eIF4E-eIF4G complex is now able to bind to the 5' cap structure (Whalen *et al.* 1996).

appears to be modulated by phosphorylation in a manner analogous to that of the mammalian eIF4E-BPs. p20 and eIF4E-BPs show little amino acid sequence similarity.

Control of mRNA translation by the 5' UTR

Structural features of the 5' UTR of an mRNA can be important in controlling the level of translation initiation occurring on that transcript. These include:

(i) *The presence of an m⁷G cap structure.* The 5' cap structure is on all known eukaryotic mRNAs (except for organellar mRNAs) and most viral mRNAs (the exceptions being animal picornaviruses and plant retroviruses). This structure consists of a methylguanosine that is attached to the 5' end of the mRNA via a 5'-5' triphosphate linkage and is denoted m⁷G(5')ppp(5')N (where N represents the first nucleotide), or simply as m⁷G. In yeast, only two types of cap structure are known, namely m⁷GpppA and m⁷GpppG (Spirati *et al.* 1976). The m⁷G cap structure protects mRNAs from 5'→3' exonucleolytic degradation (Furuichi *et al.* 1977) and stimulates translational efficiency (Shatkin 1976). A non-methylated cap will not stimulate translation, although it still stabilises the mRNA (Horikami *et al.* 1984). However, mRNAs with long, unstructured leader sequences are less dependent on this cap structure, as demonstrated in vertebrates (Kozak 1991a), plants (Gallie *et al.* 1989) and yeast (Gerstel *et al.* 1992).

(ii) *The primary sequence context of the initiation codon.* The efficiency with which the 43S preinitiation complex recognises the AUG codon as the start of initiation is dependent on the nucleotides surrounding that codon (Kozak 1986). A statistical analysis of the sequences surrounding initiator AUGs in eukaryotic mRNAs (Kozak 1986), identified (A/G)CCAUGG as the optimal sequence, although this was derived mainly from vertebrate mRNAs. More recent work has shown considerable variation between eukaryotic groups, but they all share a preference for purines at the -3 position (including yeast; Baim & Sherman 1988) and the identity of this nucleotide

appears to be of functional importance for efficient AUG selection (reviewed by Cavener & Ray 1991). Positions +5 and +6 also appear to be important determinants for initiation, particularly at non-AUG codons such as GUG and CUG (Boeck & Kolakofsky 1994, Grünert & Jackson 1994).

(iii) *The presence of upstream AUGs.* Generally, the eukaryotic 43S preinitiation complex initiates translation at the most 5' proximal AUG codon (Kozak 1987). However, exceptions to this 'first-AUG counts' rule do occur in vertebrates, as a result of reinitiation or leaky 43S complex scanning. Reinitiation can occur at a downstream AUG codon when the 5' AUG codon is followed closely by a termination codon (Kozak 1987, 1995); leaky scanning results when the first AUG codon is located in a suboptimal context such that some 43S complexes bypass this 5' AUG and initiate at a downstream AUG (Kozak 1989a). Recognition of the first AUG can be impaired when it is located close to the cap structure (Kozak 1991b), whereas the presence of upstream out-of-frame AUGs in the 5' UTRs of higher eukaryotic mRNAs tends to be inhibitory to translation (Kozak 1989b). However, these inhibitory effects can be reduced by the presence of a termination codon that is in-frame with the upstream AUG codon (uAUG) such that reinitiation can occur. The frequency of reinitiation is greater with increasing distances between the 5' and 3' cistrons (Kozak 1987).

The first-AUG counts rule also applies to the majority of yeast mRNAs. However, a subset of yeast mRNAs (<5%) have upstream out-of-frame AUGs that do not correspond to sites of translation initiation. They are not continuous with the downstream open reading frame (ORF), prevent initiation at a downstream AUG codon, and are inhibitory to translation. The inhibitory effect of these uAUGs is greater when they are located closer to the authentic AUG codon. Furthermore, the presence of a termination codon in-frame with the uAUG does not facilitate translation in yeast (Yun *et al.* 1996), except in the case of *GCN4* mRNA (Hinnebusch 1984, 1994), which has four short uORFs. Reinitiation of translation of *GCN4* mRNA is dependent on a 10-nucleotide sequence flanking the termination codon of uORF1, indicating that initiation is coupled to the efficiency of termination (Grant & Hinnebusch 1994).

(iv) *The stability and position of secondary structures.* RNA secondary structure positioned between the cap structure and the AUG codon can be inhibitory to translation initiation, with the extent of this inhibition depending on the thermodynamic stability and position of the structure. *In vitro* work, with mammalian cell lysates, has shown that a secondary structure of given stability is more inhibitory when located near the cap structure than when positioned near the AUG codon (Kozak 1989c). This suggests that such secondary structure, near the 5' end, prevents the binding of the 43S preinitiation complex to the cap structure, presumably by steric hindrance. When positioning of

such a structure allows 43S complex binding, it is its thermodynamic stability that determines whether it will inhibit scanning of the 40S ribosomal subunit (Fig. 5a). Secondary structures, positioned downstream from an AUG codon, can actually enhance recognition of the preceding initiation codon. This is optimal when a stem-loop structure is placed 14 nucleotides from the initiator AUG codon, thereby causing the 48S complex to pause with its AUG recognition centre directly over the AUG codon (Kozak 1990). This may be important for the translation of mammalian mRNAs, which initiate translation at an AUG codon in a very weak 5'/3' nucleotide context. Yeast 40S ribosomal subunits appear to be less able to destabilise secondary structures (Baim & Sherman 1988, Cigan *et al.* 1988) than are their mammalian counterpart. Furthermore, secondary structures are more inhibitory to translation in yeast when located near the initiation codon than when they are near the 5' cap structure (Vega Laso *et al.* 1993). The reasons for these differences are unclear, but must reflect differences in the mechanism of translation between higher eukaryotes and yeast.

(v) *The length of the 5' leader.* Progressive shortening of the 5' UTR of a eukaryotic mRNA usually promotes leaky scanning, unless there is a well-positioned downstream secondary structure. Such a structure suppresses leaky scanning, possibly by slowing the movement of the scanning 40S ribosomal subunit such that it is able to recognise the first AUG codon (Kozak 1990). Lengthening the 5' UTR can lead to a proportional increase in translational efficiency (Kozak 1991a), which may simply be due to increased loading of the 40S subunits on the longer 5' UTRs. Seventy percent of higher eukaryotic mRNAs are 20–80 nucleotides long, with a mean length of 60 nucleotides. Similarly, 70% of yeast mRNAs have 5' UTRs that are 20–60 nucleotides long, with a mean length of 52 nucleotides (Cigan & Donahue 1987). Yeast 5' UTRs are more A-rich than their vertebrate counterparts and require at least 30 nucleotides for optimal translation (van den Heuvel *et al.* 1989). This may be because formation of the 80S ribosome at the AUG codon on an mRNA with a short 5' UTR sterically hinders access of further 40S subunits to the cap, as the diameter of the eukaryotic ribosome is approximately 30 nucleotides (Kozak & Shatkin 1976). However, the 5' UTR is not absolutely essential; for example, the yeast *TCM1* mRNA, lacking its 5' UTR, but possessing a cap structure at the +1 position, can be translated, albeit inefficiently (Maicas *et al.* 1990).

The importance of non-coding sequences in controlling mRNA translation

5' UTR elements recognised by RNA-binding proteins

In addition to setting the constitutive level of translation of a specific transcript, mRNA secondary structures

Figure 5 Regulation of translation by RNA–RNA and RNA–protein interactions. (a) Secondary structures within the 5' UTR can act as a barrier to the scanning 43S ribosomal complex. (b) Ferritin mRNA contains an IRE in its 5' UTR to which the IRE binding protein (IRP) binds when intracellular concentrations of iron are low, and inhibits its translation. The reduced ferritin concentration leads to a decrease in iron storage. When iron concentrations are high, translation of ferritin is enhanced, resulting in enhanced iron storage. (c) The presence of the selenocysteine insertion sequence, SECIS, within the 3' UTR of a number of eukaryotic mRNAs directs insertion of selenocysteine (SelCys) at in-frame UGA codons.

can also have a regulatory role. For example, RNA structural elements can provide sites for the binding of regulatory proteins, and when such structures are located within the 5' UTR they can impede binding or scanning of the 40S ribosomal subunit by stabilising the structural element, which would not otherwise be inhibitory to initiation. Well-characterised examples are the erythroid 5-aminolevulinate synthase (*eALAs*) and ferritin mRNAs, both of which contain IREs in their 5' UTRs, to which IRPs bind when intracellular iron concentrations are low (Fig. 5b). For the IRE–IRP complex to inhibit protein synthesis efficiently, the IRE must be cap-proximal (Goossen *et al.* 1990, Goossen & Hentze 1992).

Regulation by elements in the 3' UTR

It is becoming increasingly clear that the 3' UTR of an mRNA can have equally as important a role as has the 5' UTR in regulating gene expression post-transcriptionally. In addition to the poly(A) tail, which enhances translation

(reviewed by Munroe & Jacobson 1990a), there are an increasing number of examples of other sequences within the 3' UTR that regulate mRNA stability or translational efficiency, or both. Examples of translational regulation by the 3' UTR include a domain within the pseudoknot in the 3' UTR of the tobacco mosaic virus (TMV) RNA, which regulates translation (Gallie & Walbot 1990, Leathers *et al.* 1993), interaction between an RNA-binding protein and a pyrimidine-rich motif in the 3' UTR of the 15-lipoxygenase (*LOX*) mRNA (Ostareck-Lederer *et al.* 1994), an AU-rich sequence within the 3' UTR of the human cytokine mRNA, which inhibits translation (Kruys *et al.* 1989), the presence of the selenocysteine (SelCys) insertion sequence (SECIS) within the 3' UTR of a number of eukaryotic mRNAs, which directs insertion of (SelCys) at in-frame UGA codons (Low & Berry 1996; Fig. 5c), and mammalian histone mRNAs, which terminate in stem-loop structures that are functionally similar to the poly(A) tail (Gallie *et al.* 1996). Examples in which the 3' UTR functions in developmental

Figure 6 A model for the proposed interaction of the 5' and 3' ends of an mRNA by binding of PABP with eIF4G. The initiation factors, eIF4E and eIF4G (denoted 4E and 4G respectively), may form a bridge between the 5' cap structure and one or more PABP molecules bound at the 3' end of the mRNA.

regulation include *Xenopus c-mos* and cyclin mRNAs (Sheets *et al.* 1994). The mechanism(s) by which 3' UTR-mediated regulation is achieved remains to be established, but is likely to involve specific RNA–protein interactions.

Regulation by 5'–3' interactions

Recently, the role of molecular communication between the 5' and 3' ends of an mRNA in the regulation of translation of that mRNA has received considerable attention. It is becoming evident that there is both a physical and a functional interaction between the two ends of an mRNA. Certainly mRNAs with base complementarity between the 5' and 3' UTRs and which form a stable secondary structure, are translated poorly, if at all, in COS cells (Kozak 1989c) and in yeast (Vega Laso *et al.* 1993), although in both cases mRNA degradation is also inhibited. However, in plant mRNAs, the cap and poly(A) tail act synergistically to achieve a high translational level *in vivo* (Gallie 1991). This may be due to the interaction of PABP with eIF4G, eIF4E and, possibly, other initiation factors (Gallie & Tanguay 1994), thereby allowing for more efficient cap-dependent translation.

There is an enhancing activity on translation initiation by the poly(A) tail of mRNAs in yeast (Sachs & Davies 1989, Gerstel *et al.* 1992), in rabbit reticulocyte lysates and in wheat germ (Rubin & Halim 1987), which may exert this action through the effects of its associated PABP (reviewed by Munroe & Jacobson 1990a). The poly(A) tail appears to stimulate translation through the association of PABP with eIF4G, in which the mRNA can be circularised by the bridging interaction between eIF4G with eIF4E and PABP (Sachs & Buratowski 1997), as illustrated in Fig. 6. The 3' poly(A) tail and the 5' cap structure may share the same function in translation initiation, namely to

stimulate 40S (Tarun & Sachs 1995) or 60S, or both (Sachs & Davis 1989) ribosomal subunit joining to the mRNA. Those mRNAs of which the translation is to some extent eIF4E-independent may be more poly(A) tail-dependent and, in turn, may be regulated by poly(A) tail length or accessibility. Evidence supporting circularised mRNAs includes electron micrographs of polysomes with interacting 5' and 3' ends (for example Christensen *et al.* 1987) and experiments showing that exogenous poly(A) is capable of working *in trans* to stimulate translation of capped poly(A)[−] mRNA (Munroe & Jacobson 1990b).

Coupling of mRNA translation and stability

The processes of mRNA stability and translation are closely coupled rather than being separate events in space and time. Not only do the poly(A) tail and PABP protect the transcript from exonucleases attack, but they also enhance translation initiation (reviewed by Munroe & Jacobson 1990a). The decay of many nonsense-codon-containing mRNAs requires components of the translational apparatus (reviewed by Jacobson & Peltz 1996).

In conclusion, studies on gene regulation to date have quite rightly focused on events occurring at the level of transcription initiation. Nevertheless, the often-held assumption that this is the *only* level at which gene expression can be regulated is no longer tenable. As briefly reviewed above, there are a plethora of mechanisms operating post-transcriptionally (even excluding mRNA processing events that take place in the nucleus) to regulate gene expression either specifically or globally. Increasingly important is the realisation that such events are the key to the successful outcome from highly ordered developmental processes in complex organisms.

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