Translational Control of Gene Expression

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ABSTRACT

Translational regulation of mRNA is an important step in the control of gene expression. In a general way, the efficiency of the translational apparatus can be influenced either positively or negatively by changing the level or the activity of rate-limiting protein factors taking part in the process of translation. But translational control can also be very specific, affecting only a single mRNA or class of mRNA molecules. In most of these cases regulation takes place at the level of initiation of translation, which is often attributable to structural peculiarities of the mRNA in question, especially of the 5'-untranslated region or leader. This review summarizes the mechanisms which lie at the root of translational control. A better understanding of these mechanisms will eventually provide us with new drugs and antisense oligonucleotide technology, aimed at influencing the level of expression of single proteins. These developments are of interest to basic researchers and clinicians alike, because they may profoundly change the ways in which we treat, *e.g.* viral infections and malignancies in the future. (*Pediatr Res* **37: 681–686, 1995**)

Abbreviations
eIF, eukaryotic initiation factor
IRE, iron-responsive element
IRES, internal ribosome entry site
BP, binding protein
mRNP, messenger ribonucleoprotein
ORF, open reading frame
TNF, tumor necrosis factor

Regulation of mRNA translation, although still less well characterized than the regulation of gene transcription, is now recognized as one of the major regulatory steps in the control of gene expression (1). In recent years, a variety of ways in which translation of specific mRNA molecules can be regulated have been elucidated. It is now clear that translational control is of paramount importance to the regulation of germ cell differentiation, morphogenesis, the cell cycle and iron metabolism. Translational control can be very specific, aimed at translation of only part of the mRNA in the cell or even a single one. A better understanding of the mechanisms involved in translational control will therefore not only have an impact on basic research but, before long, onto clinical practice as well, as it may aid in the development of new drugs aimed at influencing the level of expression of specific proteins.

This review summarizes the ways in which translational control can be mediated. We will limit the discussion to mechanisms of translational control in a rather strict sense. For a discussion of other principles of posttranscriptional regulation of gene expression, for instance regulation of mRNA stability, the reader is referred to a number of excellent reviews (2–4).

THE TRANSLATIONAL MACHINERY

After transcription in the nucleus, a eukaryotic mRNA acquires a cap structure at its 5'-end and a poly(A)-tail at the 3'-end. The RNA strand between these two structures can be thought of as divided into three domains: the 5'-untranslated region or leader, the region encoding the protein, and the 3'-untranslated region or trailer. Of course, in the intact molecule this partition is only artificial: secondary and tertiary interactions take place along the entire strand, within and between these regions. RNA easily forms higher order structures, which as a matter of fact is a prerequisite for the proper fulfilment of its biologic functions (5). A variety of molecules binding to the RNA-strand can change its conformation and this in turn can be expected to have an impact on translation.

Cytoplasmic mRNA molecules are either actively translated by ribosomes with efficiencies that may differ for specific classes of mRNA, or they are translationally repressed, *i.e.* associated with proteins in the form of messenger ribonucleoprotein particles, mRNP (6, 7). To become translated, mRNA molecules must be recruited, or mobilized, from the pool of mRNP. Once mobilized, the mRNA can be translated, a pro-

cess that can be divided basically into three phases: initiation, elongation, and termination.

The goal of initiation is to position the ribosome at the start of the coding region; this phase ends with the formation of the first peptide bond. For most mRNA species the search for the coding region starts at the cap, which is recognized by the cap binding initiation factor eIF-4E. This protein is found in a complex with other initiation factors, among which an RNA-helicase (8). The latter starts unwinding the RNA leader, which enables the so-called initiation complex, composed of the small ribosomal subunit, methionyl-tRNA and the initiation factor eIF2, to begin scanning for the coding region (9). The start of the coding region is marked by an initiation codon, generally an AUG (which encodes methionine). Once the scanning complex encounters a proper initiation codon, it is lined up with the methionyl-tRNA on the small subunit, after which the large ribosomal subunit joins the complex. Elongation of the peptide chain then begins, catalyzed by a domain on the large ribosomal subunit and facilitated by yet another protein, elongation factor 1 (10). The actual processes taking place during elongation, the codon-by-codon decoding of the mRNA and peptide bond formation, proceed at an amazing speed of easily more than 5 aminoacyl residues per second. Finally, termination occurs when a termination codon (UAA, UAG, or UGA) arrives in the active ribosomal site. At that point, the peptide chain and the mRNA are released from the ribosome (11), ignoring the remainder of the mRNA strand.

GENERAL ASPECTS OF TRANSLATIONAL REGULATION

Translation takes place in the cytoplasm. Some parts of the cytoplasm, however, are so tightly packed with soluble protein and cytoskeleton that ribosomes can be expected to have difficulties diffusing into them. Moreover, some components of the translational machinery are themselves associated with the cytoskeleton (12). It is thus entirely possible that local differences in the activity and composition of the translational machinery exist within the cell. Next to the translational repression by proteins binding to the RNA as mentioned above, this may explain how certain mRNA molecules can (temporarily) be sequestered and kept out of translation, as appears to be the case with a major part of one of the mRNA species of human IGF-II (13).

Regulation of the rate of protein synthesis occurs by influencing rate-limiting steps of the translational process. This can be accomplished by modulation of the available amount of ribosomes or initiation factors (14) or, more often, by a change in the activity of these factors through phosphorylation or dephosphorylation (1, 14). The initiation factor eIF-2, for instance, becomes phosphorylated on its α-subunit in response to a variety of cellular emergencies such as heat shock, hypertonicity and viral infection, and this severely inhibits protein synthesis. Phosphorylation of most other initiation factors, in contrast, correlates with improved protein synthesis (15).

In case of translational regulation of only one or a small class of mRNA molecules other determinants come into play, such as the available amount of the mRNA in question, as

determined by its rate of transcription, processing and turnover, or structural aspects of that particular mRNA or class of mRNA molecules. Almost invariably, regulation at the level of initiation is observed in these cases. In most cells, the availability of the cap-binding protein eIF-4E is the rate-limiting factor in translation initiation (1). Regulation of eIF-4E levels or activity appears therefore a very efficient tool to control the rate of translation, especially of those mRNA transcripts that have difficulties in being translated anyway due to structural peculiarities of their leaders. We come back to this later.

An increasing number of *trans*-acting factors, commonly proteins associated with mRNA in the messenger ribonucleoprotein particles, are now being recognized as modulators of translation. Many of these proteins and their RNA-binding domains have already been characterized (16). The specificity of binding of these proteins varies and in many cases it is not clear whether binding truly modulates translation. It is conceivable that at least some of them play a role in changing the rate of translation of the mRNA molecules to which they bind.

The following main targets of translational control can thus be identified (1) (Fig. 1): *1*) Mobilization *i.e.* the shifting of mRNA between the mRNP fraction and polysomes, as occurs for instance with insulin mRNA in islet cells under high-glucose conditions (17); *trans*-acting factors appear to play a major role in this process. *2*) Alteration of initiation rates, influenced mainly by the availability and activity of translation initiation factors and by *cis*-acting elements, *i.e.* structural peculiarities of the mRNA in question. Typically these are located in the leader or trailer of the mRNA strand. *3*) Modulation of elongation and termination rates appear to be less important in translational control, but frame-shifts and stop codon read-throughs can be used by *cis*-elements in the coding region to regulate gene expression. They will be discussed only briefly. *4*) Elements involved in regulation of mRNA stability are frequently contained within the 3'-untranslated region of mRNA transcripts; as noted in the introduction, this aspect of the control of gene expression is beyond the scope of this review.

LEADER-MEDIATED TRANSLATIONAL CONTROL

Because the 5'-untranslated region of an mRNA has to be passed by the scanning ribosome, this is the obvious target for regulating translational efficiency. By definition, the leader ends where the coding region begins and translation starts. As
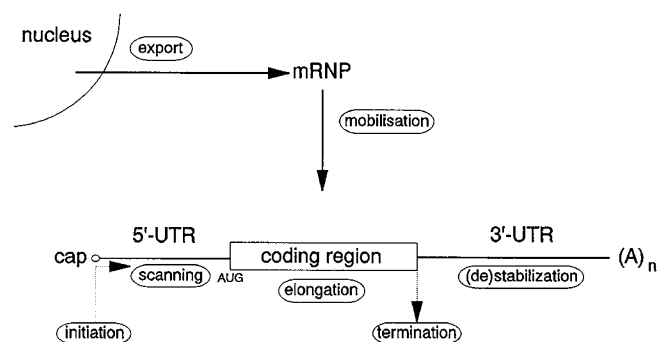

Figure 1. Targets of translational regulation.

discussed above, in the vast majority of cases this is at the first AUG encountered by the initiation complex. To be recognized, this initiator AUG must be in a favorable context, ideally CCPCCAUGG, in which P is a purine (18, 19). Moreover, it must be localized at a sufficient distance from the cap (20). If AUG codons are present in the leader upstream of the genuine initiation codon of the encoded protein, this can severely inhibit translation, especially if these AUGs are in a favorable context. The scanning ribosomes initiating at these "false" initiation codons usually cannot reinitiate at the genuine initiation codon.

An ideally efficient RNA would thus have a leader of moderate length, devoid of secondary structure and upstream initiation codons, followed by an AUG codon in a favorable context. Most proteins that are highly expressed have mRNA molecules that come close to this ideal. Leaders of mRNA molecules encoding proteins with a strictly regulated expression, however, are often much longer than the average of 100–140 nucleotides (18, 19), contain upstream AUG codons and/or are expected to form stable secondary structures by virtue of a high G+C content (21, 22). A large number of mRNA molecules for proteins involved in growth and development fall in this category. Many of these long leaders have been shown to be detrimental to translation (23).

How then can the translational efficiency of these "difficult" leaders containing a lot of secondary structure be improved? One way is by changing the levels or the activity of the limiting factor for translation initiation, eIF-4E, so that even these highly structured leaders get a chance of entering translation (24). The translation of natural mRNA transcripts containing structured leaders, such as ornithine aminotransferase and growth regulating proteins like cyclin D1 and transcription factors, can indeed be induced by eIF-4E overexpression (26) and this is probably how overexpression or enhanced activity of eIF-4E may lead to oncogenic transformation (26, 27).

Another way by which translation of such mRNA transcripts can be improved is by proteins that bind specifically to the leader. Such proteins can influence the structure of the leader, stabilizing, destabilizing, or otherwise altering its conformation. Alternatively, a tightly bound RNA BP can mimick secondary structure by obscuring the cap or stalling the scanning complex in its early stages, thereby hampering translation. The prototype of mRNA-specific translational control by RNA BP is the translational regulation of ferritin expression by iron (28). The leader of ferritin mRNA contains a region of approximately 30 nucleotides that can fold into a stem-loop structure, the iron-response element or IRE. When cellular iron levels are low, a protein binds to this region (the IRE BP). This causes a repression of ferritin-mRNA translation. This makes sense, because there is little need for ferritin under these circumstances. But as soon as cellular iron levels increase, the IRE BP is displaced from its binding site either by iron itself or by hemin (29), so that translation of ferritin mRNA can take place. Most remarkably, the mRNA of another iron-regulating protein, the transferrin receptor, contains five IRE in its 3'-untranslated region, to which IRE BP can bind if cellular iron concentrations are high. This destabilizes the messenger, leading to its increased degradation. The resultant reduction in transferrin receptor expression at the cell surface then limits further iron uptake by the cell (30). Several other mRNA molecules have since been shown to contain an IRE, such as the messengers for 5-aminolevulinate synthetase and aconitase (31, 32). Recently, the second messenger nitric oxide (NO) was shown to be able to regulate the RNA-binding activity of IRE BP (33) but the physiologic significance of this phenomenon is not yet clear.

Other examples of translational regulation by proteins binding to RNA are now emerging, such as the autoregulation of thymidilate synthetase and dihydrofolate reductase activity which may have implications for the design of anti-tumor drugs (34), the regulation of transforming growth factor $\beta 1$ synthesis (35) and the relief of translational inhibition by binding of the Tat-protein of human immunodeficiency virus to its RNA-binding site, TAR (36). We have recently been able to demonstrate stimulation of translation *in vitro* by proteins binding specifically to a circumscript region in one of the four leaders (L3) of IGF-II mRNA; one of these proteins appears to be the 57-kD polypyrimidine tract BP (37).

As discussed above, upstream AUG codons or ORF can be strong repressors of translation. There are two ways in which this repression can be mitigated: *1*) The scanning complex may not always recognize the upstream AUG, especially if it has a suboptimal context, resulting in continued scanning for the next initiation codon. This is called "leaky scanning." *2*) After translation of a small ORF the ribosome is sometimes able to resume scanning for a second AUG. This has only been reported for small ORF and is called resumed scanning or reinitiation.

Normally, neither leaky scanning nor reinitiation is very efficient (38): many mRNA species encoding proteins involved in growth and development contain an upstream ORF which may contribute to their inefficient translation. There are, however, a few cases of cellular mRNA species in which an upstream ORF forms an integral part of a translational regulation mechanism. A beautiful example is the mRNA encoding the β_2-adrenergic receptor, which harbors a short ORF in its leader; the 19-amino acid peptide encoded by this ORF has been shown to inhibit translation of the receptor mRNA (39). A similar mechanism has been proposed for the hexapeptide encoded by a 5'-leader ORF in the mRNA of *S*-adenosylmethionine decarboxylase (40).

The translation of the vast majority of mRNA transcripts depends on the presence of a cap and involves scanning of the whole leader. Yet the mRNA of picorna viruses, although uncapped and endowed with long, highly structured leaders that are riddled with upstream ORF, are translated quite efficiently. These mRNA transcripts initiate via a cap-independent mechanism called internal initiation (41, 42). Here, the initiation complex enters the leader in an IRES instead of at the 5'-end. A genuine IRES will thus direct translation of a downstream ORF, regardless of the secondary structure or upstream ORF that precede it.

The picorna IRES share little sequence homology except for a polypyrimidine tract located approximately 20 nucleotides from the initiation codon, which is essential for internal initiation (43). Two cellular proteins have been shown to be

involved in internal initiation, La protein and polypyrimidine tract BP. Both are primarily nuclear proteins but appear to play a role in translation as well, because their immunodepletion from *in vitro* translation systems inhibits internal initiation (43). Because the viral proteins *per se* play no part in internal initiation, any cellular mRNA with the appropriate structure should be able to translate by internal initiation. The mRNA encoding human immunoglobulin heavy chain binding protein (BiP) was the first cellular mRNA for which translation by internal initiation was reported (44). Recently, internal initiation was also demonstrated to occur *in vitro* in the leader of the adult liver type mRNA of IGF-II mRNA (L1) (45). A computer-fold of this leader indeed shows an IRES-like "platform" of single-stranded nucleotides, as in the BiP and picorna virus leaders (Thomas A, personal communication). No sequence homologies between the putative IGF-II IRES, the BiP IRES, and the picorna virus IRES have been identified (44).

TRANSLATIONAL CONTROL MEDIATED BY THE CODING REGION

Apart from recognition of the initiation codon, regulation of translation by sequences in the coding region is not a widespread phenomenon. Although some coding regions contain "ribosomal pause" sites, probably caused by secondary structure in the coding region or by codons requiring rare or depleted aminoacyl-tRNAs, the elongation rate under nonstarvation conditions is considered to be near-maximal. An exception is the increase in elongation rate of the heat shock protein HSP70 during heat shock, both *in vivo* and *in vitro* (46).

A growing number of eukaryotic genes, among which basic fibroblast growth factor and several mammalian transcription factors such as Pit-1 and c-*myc*, employ multiple initiation codons to produce N-terminally extended or truncated protein products (47–49). This probably occurs by leaky scanning. Regulation of the ratio of the products of alternative initiation has been reported, for instance for c-*myc* mRNA (49). Although this regulation is still poorly understood, it may have profound biologic consequences because of the differences between the biologic activities of these products.

Some sequences in ORF can induce a frameshift at a specific codon with a high frequency. This is frequently found in viral genomes (50), but it has also been discovered in a mammalian mRNA, ornithine decarboxylase antizyme (51). This protein functions as a repressor of ornithine decarboxylase, the rate-limiting enzyme of polyamine synthesis. The normal in-frame translation of ornithine decarboxylase antizyme leads to a premature stop, but in the presence of polyamines this premature stop is bypassed by a +1 frameshift.

TRAILER AND POLY(A) TAIL-MEDIATED TRANSLATIONAL CONTROL

The 3'-untranslated region or trailer is often the largest part of an mRNA. Yet it has long been considered a meaningless link between the stop codon and the poly(A) signal. In the last several years this region has turned out to be a treasure trove of posttranscriptional regulation. RNA processing, export from the nucleus, mRNA stability, and intracellular localization and translation can all be regulated by the trailer (52, 53). Recently, the importance of this region was underscored by the discovery that the trailers of muscle structural mRNA molecules can induce differentiation and suppress oncogenicity in myogenic cells, as well as suppress proliferation of fibroblasts, all without the benefit of a coding region (54).

All eukaryotic cellular mRNA transcripts, with the exception of histone messengers, are provided with a poly(A) tail in the nucleus. After entering the cytoplasm, the poly(A) tail is progressively shortened as the mRNA ages. Some very unstable mRNA species, such as those encoding the *myc* and *fos* proto-oncogenes, are deadenylated far more rapidly than the average mRNA (55). A wealth of evidence indicates that, next to having a stabilizing influence on mRNA, long poly(A) tails are stimulatory for translation, both *in vivo* and *in vitro* (56). A *trans*-acting factor, the poly(A) tail BP, is involved in this stimulation (57, 58). Surprisingly, the poly(A)-poly(A) tail BP complex influences an event taking place far upstream in the mRNA-molecule: the binding of the large ribosomal subunit to the small subunit at the initiation codon (58). The mechanism by which this is achieved is still incompletely understood.

The length of the poly(A) tail can also be increased in the cytoplasm. This occurs for example during early oocyte development in a large number of animals, including *Xenopus laevis* and the mouse. Some of the maternal mRNA transcripts in the oocytes of these animals are initially not translated, but become activated, or "unmasked," at later stages of oocyte maturation or early embryogenesis. This is accompanied by an increase in the length of their poly(A) tails (59, 60). The signal for this cytoplasmic polyadenylation is located in the trailer and consists of the normal polyadenylation signal, AAUAAA and a U-rich sequence located upstream of it (52, 60). So far, translational activation by cytoplasmic polyadenylation has been demonstrated only in germ cells, but somatic cells as well do have some adenylation activity in their cytoplasm (61). Interestingly, in two somatic mRNA species, encoding the neuropeptides vasopressin and oxytocin, a strong correlation has been found between induction of peptide production and extension of the poly(A) tail. Whether this coincides with translational activation is still unresolved (62).

Other forms of translational stimulation by trailer elements are as yet poorly understood. Interestingly, a form of the lysosomal storage disease aspartylglucosaminuria has been described resulting from a homozygous deletion of most of the trailer part of the aspartylglucosaminidase mRNA. This deletion caused the synthesis of a stable, truncated but polyadenylated mRNA with a completely intact coding region, which was nevertheless untranslated *in vivo*, whereas no defect in translational efficiency could be demonstrated *in vitro* (63). Another example is provided by the mRNA for the amyloid protein precursor, which has two forms produced by alternative polyadenylation. The longer mRNA translates more efficiently than the shorter one, and the available evidence indicates that the region between the two polyadenylation sites is stimulatory for translation (64).

In contrast to translational stimulation by the trailer, the number of mRNA transcripts that is translationally repressed by *cis*-acting elements in their trailers is expanding and by no

means restricted to germ cell systems. Such repression can be conferred by AU-rich elements, for instance in the trailers of certain cytokines, human interferon β, granulocyte-macrophage colony-stimulating factor and c-*fos* (65, 66). Translational control of another cytokine, TNF, takes part in the up-regulation of TNF production in macrophages in response to bacterial endotoxin (67). Here also, the translational regulation is conferred by the trailer and the AU-rich element is essential both for repression in nonactivated cells and derepression in activated cells. Dexamethasone suppresses TNF translation, which is also mediated by the trailer (68). Translational repression can also be conferred by proteins binding to the trailer, *e.g.* in the case of protamine mRNA during spermatid maturation (52) or of 15-lipoxygenase mRNA during reticulocyte maturation (69).

CONCLUDING REMARKS

This overview of the known mechanisms of mRNA-specific translational control illustrates both the great diversity of translational regulation and the essentially limited number of steps that are affected. Most of the examples given were disclosed during the last 6 or 7 y, and the number of mRNA molecules subject to translational control is still rapidly increasing. Although many of the mechanisms involved are still far from being completely understood, the principles of translational regulation will soon find their way into basic research as well as clinical practice.

In research, for instance, the efficiency of recombinant protein biosynthesis can be improved by clever engineering of expression plasmids, *e.g.* by optimizing the length and composition of leaders and trailers, the context of the initiation codon, and the stability of the transcript. Elements such as the IRE in ferritin mRNA will provide constructs with a molecular switch mechanism enabling the voluntary expression of the protein under study, and a further characterization of elements conferring tissue and/or development-specific regulation at the translational level will lead to additional refinements in expression patterns, *e.g.* in transgenic animals.

In clinical practice, the ability to interfere with translational processes may completely change the ways in which we treat viral and malignant diseases (70). Clinical trials with antisense oligonucleotides have already started, aimed at blocking translation of very specific classes of mRNA, be they viral, bacterial or aberrant human ones. By binding to the mRNA in question they may directly obstruct translation, *e.g.* by interfering with cap recognition or RNA unwinding, or they may prevent the binding of *trans*-acting factors that modulate translation. In addition, they can stimulate endonucleolytic cleavage and destruction of the mRNA. This is a highly promising novel way of treating viral infections (*e.g.* human immunodeficiency virus) and malignancies, especially if used as an adjunct to the regular antiviral and cytostatic drugs. Without being unrealistic, a substantial refinement of our therapeutic possibilities in nonmalignant growth disturbances and many viral and genetic diseases can be expected to result from a further increase in our knowledge of the mechanisms of translational control.

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Erratum

In the article "Deconvolution Analysis of Spontaneous Nocturnal Growth Hormone Secretion in Prepubertal Children with Preterminal Chronic Renal Failure and with End-Stage Renal Disease" by Burkhard Tönshoff et al. (Pediatr Res 37:86–93, 1995) each value in "mg/L" should be "μg/L". In addition, in Table 2, "GH production/m^2 body surface area (mg/m^2/10 h)" should be "GH production/m^2 body surface area (μg/m^2/10 h)". The authors regret this error.