Translational controls impinging on the 5′-untranslated region and initiation factor proteins

Richard J Jackson* and Marvin Wickens†

Translation of eukaryotic mRNAs is generally initiated by the scanning ribosome mechanism. This can be downregulated by high affinity protein binding to cap-proximal RNA motifs. Translation can also be regulated by short open reading frames within the 5′-untranslated region. A key factor for initiation is eIF4F, in which one of the polypeptide chains, eIF4G, seems to have a bridging function and binds three other factors at separate sites: eIF4E (the cap-binding factor), the helicase eIF4A, and eIF3, which also interacts with 40S ribosomal subunits. Initiation is regulated by the MAP kinase and rapamycin-sensitive signalling pathways, which control phosphorylation of eIF4E and 4E-BP1, a protein which in the dephosphorylated form binds and sequesters eIF4E.

Addresses
*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK
e-mail: rjj@mole.bio.cam.ac.uk
†Department of Biochemistry, 420 Henry Mall, University of Wisconsin, Madison, WI 53706, USA
e-mail: wickens@biochem.wisc.edu

Current Opinion in Genetics & Development 1997, 7:233–241

Electronic identifier: 0959-437X-007-00233

Abbreviations

APOBEC-1	apolipoprotein B mRNA-editing enzyme
HCV	hepatitis C virus
IRE	iron responsive element
IRP	iron regulatory protein
MAP	mitogen-activated protein
NAT1	novel APOBEC-1 target 1
PKR	double-stranded RNA regulated kinase
PTB	polypyrimidine tract binding protein
sORF	short upstream open-reading frame
UTR	untranslated region

Introduction

Whereas our companion review (Wickens, Anderson and Jackson, this issue, pp 220–232) is concerned mainly with how both the 3′-UTR and the poly(A) tail influence mRNA stability and translation, in this review we focus on events at the 5′-end of the mRNA: mechanisms of initiation site selection; regulation of translation by RNA–protein interactions in the 5′-UTR; and regulation of translation by covalent modification of initiation factor proteins or proteins that interact with initiation factors. Although the focus here is mainly on events in somatic cells rather than in—as in the previous chapter—oogenesis or early embryogenesis, there is no absolute demarcation intended: according to current evidence, the events described here are as likely to be relevant to germline cells and early embryogenesis as to somatic cells.

Mechanisms of initiation site selection

With a few exceptions, discussed below, initiation site selection in eukaryotes continues to be interpreted in terms of the scanning ribosome model (reviewed in [1]) and there is no general agreement yet as to how any possible role of poly(A) tails in initiation could be incorporated into this model. The scanning entity, according to generally held opinions [2], is the 40S ribosomal subunit with various initiation factors bound to it: eIF1, which appears to stimulate the overall process of initiation without playing any well defined role; eIF3, which is a high molecular weight complex (>500 kDa) of five (in yeast) or eight (in mammals) polypeptides and has two functions ascribed to it, namely preventing premature 60S subunit joining and potentiating binding to mRNA; and the eIF2/GTP/Met-tRNA$_i$ ternary complex. The bound ternary complex has generally been considered to be necessary for stable binding of the 40S subunit to mRNA, although data from the yeast GCN4 mRNA system discussed below may necessitate revision of this notion. Binding of the primed 40S subunit to mRNA requires ATP hydrolysis and three additional factors [2]: eIF4A, an ATP-dependent RNA helicase activity; eIF4B, an RNA-binding protein which stimulates the helicase activities of eIF4A and eIF4F holoenzyme complex; and eIF4F itself, which consists of three polypeptide chains, eIF4A, eIF4G and the cap-binding protein eIF4E (see below). It is not clear to what extent these factors interact primarily with the mRNA rather than the 40S subunit, nor is it clear to what extent the eIF4F holoenzyme is a fixed entity or dissociates into its constituents on each initiation cycle. Finally, when the 40S subunit is positioned at the selected initiation codon, eIF5 triggers hydrolysis (probably by eIF2) of the bound GTP, 60S subunit joining occurs, and eIF3 and eIF2/GDP dissociate from the ribosome.

The actual mechanism of scanning remains a mystery and the isolation of cDNA clones for the majority of the translation initiation factors [2] has not illuminated this problem. The well known RNA helicase activities of eIF4A and the eIF4F holoenzyme complex (see below) and the recently discovered RNA annealing and strand-exchange activity of eIF4B [3] do not in themselves seem a sufficient explanation. If scanning is not an intrinsic property of the 40S ribosomal subunit itself—but requires the participation of initiation factors—the key to scanning may lie in the complex multi-subunit initiation factor

eIF3, of which only a few of the 8 subunits have been characterized at the cDNA level to date [4–6].

One variant of the linear scanning model is ribosome shunting, in which there is a discontinuity or 'jump' in the scanning process. First proposed in order to explain how the cauliflower mosaic virus 35S mRNA is translated, this model has since been extended to another distantly related plant virus, rice tungro bacilliform virus, where the 'shunt' actually delivers the ribosomes to an AUU initiation codon [7]. Shunting has also been demonstrated recently in the case of the late adenovirus mRNAs which all have in common the tripartite leader 5′-UTR [8]. By insertion of hairpin loops and AUG triplets at various points along this 5′-UTR, it was possible to define fairly precisely the take-off and landing points for this jump or shunt. The 5′-proximal portion of the 5′-UTR is invariably traversed by strictly linear scanning, after which, under normal circumstances, about half the ribosomes continue in linear scanning mode and half appear to shunt. Under conditions of low eIF4F activity, such as late in adenovirus infection or under heat-shock conditions, shunting becomes the exclusive mechanism [9••].

Regulation of translation initiation by RNA–protein interactions in the 5′-UTR

The paradigm for translational regulation by RNA–protein interactions in the 5′-UTR continues to be ferritin mRNA (reviewed in [10,11]), the translation of which is inhibited by the binding of a specific protein, iron regulatory protein (IRP) to a stem-loop structure in the 5′-UTR known as the iron responsive element (IRE). IRP1 is identical to cytoplasmic *cis*-aconitase and has a [4Fe–4S] iron-sulphur cluster in its enzymatically active form [10,11]. This form has only very low RNA-binding activity but treatment of cells with iron-chelating agents results in the complete dissociation of the iron–sulphur cluster, which is accompanied by loss of aconitase activity, acquisition of high affinity binding to the IRE, and consequent inhibition of ferritin mRNA translation. Conversely, incubation of cells with iron or haemin results in loading of the iron–sulphur cluster by an unknown mechanism, gain of aconitase activity, loss of IRE-binding activity and stimulation of ferritin mRNA translation.

A second IRE-binding protein, IRP2, has been described [12] which is similar but not identical to IRP1 and bears a 73 amino acid insert [13]. As IRP1 and IRP2 vary at many positions outside this insert, the two proteins originate from different genes rather than by alternative splicing of the primary transcript of a single gene [13]. IRP2 also differs from IRP1 in having no aconitase activity [10,11]. Both IRPs can bind to the IRE stem-loop structure but *in vitro* selection methods show that the preferred sequences in the 6 residue loop are subtly different for each [14,15]. Moreover, when cells are loaded with iron or haemin, there is accelerated degradation of IRP2 but not IRP1 [16,17], a difference which is attributable to the 73 amino acid insert as IRP2 is stabilized by deletion of this insert and IRP1 is destabilized if the 73 amino acid insert is grafted into it at the appropriate location [18•].

In addition to iron, the system is also sensitive to nitric oxide (NO). NO, like the chelation of available iron, results in the inhibition of ferritin mRNA translation for the same proximal reason, namely disassembly of the IRP iron–sulphur cluster. It is not yet clear if NO affects the cluster directly [10,11]. The kinetics of NO-dependent inhibition of translation are similar to those seen in iron deprivation and both IRP1 and IRP2 are affected [19••]. In contrast, oxidative stress in the form of H_2O_2 or superoxide radicals regulates ferritin translation via a different mode: inhibition is much more rapid, seems to be exerted via IRP1 and not IRP2, and is antagonized by okadaic acid, which has no influence on regulation caused by iron-deprivation or NO [19••,20].

A number of other mRNAs have been identified which have a 5′-proximal IRE and the translation of which appears to be regulated in the same way [21–23]. Not surprisingly, all of them code for proteins intimately involved with iron in one way or another: erythroid 5-aminolevulinate synthase (the first enzyme in the heme biosynthesis pathway), mitochondrial aconitase (an iron-sulphur protein), and the iron–sulphur subunit of *Drosophila* succinate dehydrogenase.

An important feature of the IRE/IRP regulatory system is that it is only really effective if the IRE is located near the capped 5′-end of the mRNA [24]. If the IRE is moved further downstream in the 5′-UTR, the degree of inhibition of translation caused by iron-deprivation is reduced in transfected mammalian cell lines. As the actual binding affinity of IRP for the IRE is independent of position, it is unclear whether the translation machinery overcomes these cap-distal IRE/IRP complexes by a shunting mechanism or by taking advantage of transient dissociation of IRP, or by actively displacing it from the mRNA. Clarification of these issues will necessitate the development of cell-free translation systems which reconstitute the effects seen in transfected cells.

Sucrose gradient analysis of intermediate initiation complexes shows that IRP bound to a cap-proximal IRE inhibits the binding of the 43S intermediate complex (40S subunit with bound eIF3 and eIF2/Met-tRNA$_i$/GTP ternary complex) to the mRNA [24]. This is not a specialized property of IRP but is seen with any cap-proximal high affinity RNA–protein interaction. Thus U1A protein — which normally never impinges on mRNA translation *per se* — can inhibit translation if the mRNA has, in a cap-proximal location, the stem-loop structure of U1 snRNA to which U1A binds with high affinity [24].

Although this type of regulation by RNA–protein interactions in a cap-proximal position was first discovered in

somatic cells, it is also found in the germline. A family of related mRNAs coding for *Drosophila* sperm-tail proteins is held in an untranslated state for several days before finally being activated for translation [25]. This temporal delay is caused by a conserved sequence element found in the same cap-proximal position in all members of the family and, significantly, displacing the element to a more distal position abolishes the temporal delay of translation. In a similar vein, mouse testis expresses a novel form of mRNA coding for copper–zinc superoxide dismutase, transcribed from an upstream promoter with the result that the sequences immediately downstream of the cap are unique to the specific testis form of the mRNA [26]. These sequences are the binding site for a protein that specifically regulates the translation of just the testis-specific mRNA isoform *in vitro* [26] and is the likely explanation of the temporal control of translation of this mRNA species *in vivo* [27].

The regulatory function of short upstream open reading frames

In general, eukaryotic ribosomes can translate just the first cistron of a polycistronic mRNA with two or more cistrons coding for full-sized proteins. Following the translation of a short upstream open-reading frame (sORF), however, there may be a resumption of scanning and the potential to re-initiate at an initiation site further downstream. Detailed examination of the regulation of translation of yeast GCN4 mRNA, which has four sORFs, has shown that such resumption of scanning is dependent on sequences surrounding the termination codon of the sORF [28] and also, according to recent evidence, on sequences upstream of the short reading frame [29]. The location of the sORFs in GCN4 mRNA and their individual characteristics with respect to whether they are either permissive or restrictive to the resumption of scanning [30,31], leads to an exquisitely sensitive specific translational control system whereby the rate of GCN4 synthesis can increase by more than ten-fold in response to a relatively small decrease in the intracellular pool of eIF2/Met-tRNA$_i$/GTP ternary complex (reviewed in [31]). Conditions leading to reduced ternary complex pools include the deletion of some of the four copies of the tRNA$_i$ gene [32••]; partially defective mutants in initiation factor eIF2, or in eIF2B, the guanine nucleotide exchange factor required to recycle eIF2 and prime it for the next initiation event; or phosphorylation of eIF2 in its smallest (α) subunit which, likewise, inhibits the guanine nucleotide exchange reaction [31,32••].

Why and how is scanning resumed after translation of the first sORF in GCN4 mRNA? According to current models [1,2], the scanning 40S ribosomal subunit approaching this sORF is already loaded with initiation factors and Met-tRNA$_i$ but initiation will be coupled with the dissociation of all these initiation factors from the ribosome whereas the initiator tRNA will be released before the second peptide bond is synthesized. Thus the ribosome arrives at the termination codon of this sORF bereft of initiation factors and initiator tRNA. If the ribosome can resume scanning instantaneously, the implication is that the ability to scan is an integral property of the 40S subunit itself, rather than it being dependent on bound initiation factor proteins. An alternative interpretation is that this 40S ribosomal subunit very rapidly reacquires such initiation factors as may be necessary for further scanning and less rapidly acquires the eIF2/Met-tRNA$_i$/GTP ternary complex that would be necessary to allow (re)initiation at a downstream site. Yet another possibility is that we are entirely wrong in our view that all the initiation factors dissociate on initiation at the start of the first sORF. Some factors, particularly those required for scanning — if any such factors exist — may remain ribosome-associated for some time after initiation and dissociate relatively slowly, perhaps being displaced by the growing nascent protein chain. This type of model could explain why resumption of scanning is usually observed only if the ORF is short. The answers to these questions are critical to our understanding of the precise nature of the scanning mechanism and we can only hope that the genetics of regulation of yeast GCN4 mRNA translation may eventually illuminate this issue.

The critical features of yeast GCN4 mRNA structure necessary for this regulation are that there should be at least two sORFs with opposing characteristics: a 5′-proximal sORF permissive to the resumption of scanning reinitiation and a distal one that is restrictive [31]. For GCN4 regulation, the amino acid sequences encoded by the sORFs do not seem to be important. There are some cases, however, in which the amino acid sequences are critical. For example, the translation of *Neurospora crassa arg-2* mRNA, or the equivalent *CPA-1* mRNA in *Saccharomyces cerevisiae*, is negatively regulated by arginine via a mechanism that is dependent on the single sORF in the 5′-UTR of these mRNAs [33,34•]. Among mutants showing loss of response to arginine are some in which the amino acid sequence encoded by this sORF has been changed [34•]. In the human cytomegalovirus gp48 mRNA, the second of three sORFs is highly inhibitory to translation of the main reading frame [35]. Mutational analysis suggests that the inhibition is caused by the amino acid sequence of the encoded peptide rather than to the nucleotide sequence *per se* and that this peptide sequence causes the ribosome to stall at the termination codon of the sORF [35,36•]. This most likely explains, in part, why these sORF peptides inhibit only in *cis*, in that the emerging peptide interacts with the ribosome that has just made it. Rather surprizingly, the sORF peptides of gp48, CPA-1 and other candidate mRNAs for this type of regulation are unrelated in sequence. It remains to be seen what fraction of the sORFs found in the 5′-UTRs of eukaryotic mRNAs act through the encoded peptide and what fraction through the yeast GCN4 mRNA precedent.

Initiation by direct internal ribosome entry

Apart from minor deviations to the ribosome scanning model, such as ribosome shunting, there is a major alternative mechanism of initiation site selection, namely internal initiation, in which a specific *cis*-acting RNA element — generally known as the internal ribosome entry segment (IRES) — promotes direct access of the ribosomes to the initiation codon and can even allow translation of the downstream cistron of a dicistronic mRNA if an IRES is inserted as the intercistronic spacer, or allow translation of a covalently closed circular mRNA [37••]. In the latter case, a circular RNA with an IRES directing initiation of translation of an endless reading frame with no in-frame stop codons resulted in the synthesis of proteins several times longer than the size encoded by a single cycle of the circle, which proves definitively that there is no cleavage of the IRES during IRES-dependent initiation.

Internal initiation was first discovered in the animal picornaviruses, which remain the best documented example (reviewed in [38]); it has since been found in another quite distinct family of RNA viruses, hepatitis C virus (HCV) and the pestiviruses [39••,40], in which, unlike the picornaviruses, the IRES extends some 10–15 codons into the viral coding sequences [39••]. The HCV IRES includes a pseudo-knot structure near the authentic initiation site which is essential for internal initiation [41•]. As for cellular mRNAs translated by internal initiation, the short list of previously recognized examples [38] has been enlarged recently by the addition of c-*sis* and initiation factor eIF4G mRNAs [42,43]. Translation of eIF4G by internal initiation may possibly have evolved as an attempt — obviously not an entirely successful one — to combat the virus-induced shut-off of host cell mRNA translation, which is discussed below. As for the other examples of cellular mRNAs translated by internal initiation, there is no obvious reason why these particular RNAs should have evolved in this direction.

Little is known about how IRES elements direct internal initiation. Although they are similar in strictly operational terms to the Shine–Dalgarno sequences of prokaryotic mRNAs, they are very much larger and there is, as yet, no evidence for base-pairing between the IRES element and the 18S rRNA of the eukaryotic small ribosomal subunit. One problem in perceiving any common mechanism is that, although there is conservation of IRES primary sequence and secondary structure within each of the subgroups of picornaviruses [38] and within the HCV/pestivirus group, there is very little homology either between each of these groups or between any of them and the IRESs identified in cellular mRNAs. Current evidence suggests that internal initiation requires all the canonical initiation factors that function in the scanning ribosome mechanism — with some slight qualifications about eIF4F as discussed below — but may also require additional *trans*-acting cellular proteins. Polypyrimidine tract binding protein (PTB), once thought to be a splicing factor, has thus been shown to be required for the function of some but not all picornavirus IRESs [44•] and high concentrations of the autoantigen La stimulate internal initiation which is dependent on the poliovirus IRES [45]. Given that none of these cellular proteins seems to be required universally for all cases of internal initiation and that even quite closely related IRESs differ in whether their function is dependent on PTB, it seems likely that these *trans*-acting factors function by maintaining the correct higher order folded structure of the IRES rather than acting as essential catalysts of internal initiation [44•].

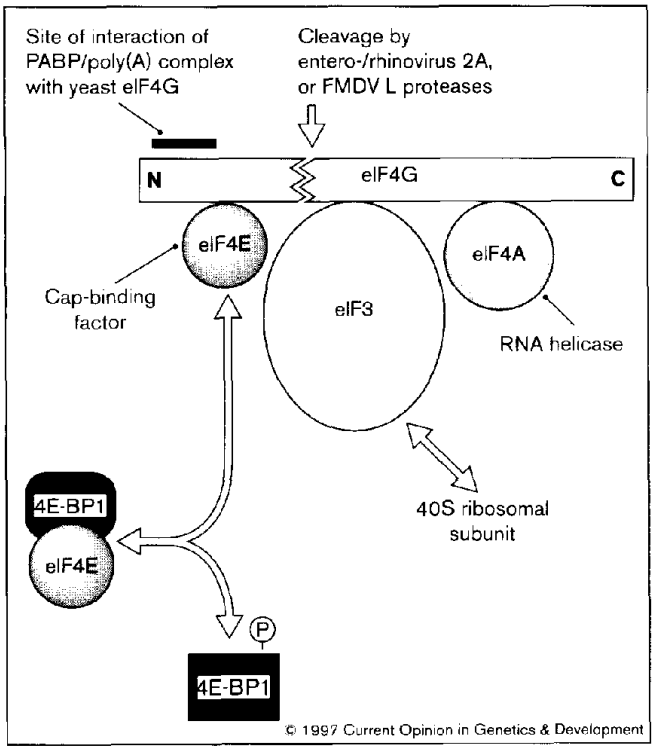
eIF4F structure and function: the emergence of eIF4G as the hub of a constellation of interactions

In the case of the entero-/rhinovirus branch of the picornaviruses and of foot-and-mouth disease virus, the obvious advantage of internal initiation is that these viruses inactivate cap-dependent translation of host cell mRNAs by the scanning mechanism because they encode proteases which cleave the eIF4G subunit of the eIF4F holoenzyme complex. This cleavage reaction has recently helped to illuminate the architecture of eIF4F and its function (Fig. 1). As the entero-/rhinovirus 2A protease and foot-and-mouth disease virus L protease are quite distinct with different specificity they cleave eIF4G at different sites but only seven amino acid residues apart, suggesting that the cleavage sites are in an exposed hinge region [46••]. Curiously, cleavage requires that eIF4E is bound to the amino-terminal portion, perhaps because this interaction with eIF4E renders the hinge more exposed to cleavage by the proteases [47]. This echoes an earlier report that eIF3, which also binds to eIF4G (Fig. 1), is likewise required for cleavage [48].

The cleavage separates eIF4G into an amino-terminal one-third portion — which binds the cap-binding initiation factor eIF4E [46••,49] — and a carboxy-terminal two-thirds fragment binding eIF3 and the helicase eIF4A [46••]. The L-protease digests the carboxy-terminal fragment further, which allows the eIF4A binding site to be assigned to the extreme carboxy-terminal end [46••]. This architecture has lead to the suggestion that eIF4G normally acts as a bridge between eIF4E bound at the 5′-cap, eIF3 which has high affinity for the 40S subunit and eIF4A, thereby positioning the 40S subunit and the helicase together near the 5′-end of the mRNA (Fig. 1). It should be recalled too that, at least in yeast, the counterpart of eIF4G may also interact with Pab1p (Fig. 1) provided the latter is bound to poly(A), an interaction which may play a key role in the end-to-end communication discussed in our companion review (Wickens, Anderson and Jackson, this issue, pp 220–232).

The cleavage of eIF4G by the viral proteases physically separates the cap-binding activity from the helicase function and it is this separation which is presumed

Figure 1



Architecture of the mammalian eIF4F holoenzyme complex and interactions between eIF4E and 4E-BP1. The diagram is approximately to scale in that each component is shown with its area proportional to its original size. The picornavirus proteases cleave eIF4G into two fragments: an amino-terminal one-third fragment (N), which has the eIF4E-binding site, and a carboxy-terminal two-thirds fragment (C), which has the binding sites for eIF3 and eIF4 in roughly the positions shown [46••]. 4E-BP1 binds eIF4E and sequesters it from entry into the eIF4F holoenzyme complex [58] but phosphorylation of 4E-BP1 by the rapamycin-sensitive pathway [63,66•] results in a much-reduced affinity for eIF4E and effectively liberates it for binding to eIF4G [58]. The two species of yeast eIF4G are shorter than the mammalian factor (being 953 and 915 amino acid residues rather than 1402). There is a patchy rather than a continuous homology between the yeast and the mammalian eIF4Gs but the eIF4E-binding site is in approximately the same position relative to the amino terminus. The position of interaction between the PABP/poly(A) complex and yeast eIF4G is shown [72]. Note that this interaction has not yet been proven to occur with the mammalian factor.

to be the explanation for inactivation of translation of capped host cell mRNAs. It seems to have been generally assumed for a long time that the cleaved factor was completely superfluous or redundant for translation of poliovirus RNA. Cleavage, however, actually stimulates translation that is dependent on the IRESs of poliovirus and rhinovirus [50–52,53•], a stimulation which appears to be caused by the carboxy-terminal fragment of eIF4G with associated eIF3 and eIF4A [53•]. In current models, thus, it is suggested that poliovirus RNA translation does require eIF4F but that it does not need the whole of this factor: the carboxy-terminal cleavage product of eIF4G with its bound eIF4A is sufficient and is actually somewhat more efficient than the intact eIF4F. Another surprise is that the translation of uncapped forms of mRNAs which are normally capped *in vivo* can also be stimulated by this cleavage [50] and at present there is no self-consistent explanation for this.

A striking development in this area is the very recent discovery of what appears to be an eIF4G 'decoy'. This came to light in the most unexpected fashion, namely as the result of an investigation into why overexpression of apolipoprotein B mRNA-editing enzyme (APOBEC-1) in the livers of transgenic animals results in rampant hepatocarcinoma [54••]. As this over-expression did not lead to excessive editing of apolipoprotein B mRNA itself, nor any of six other candidate mRNAs which have the consensus 'mooring sequence' recognized by APOBEC-1, differential display was used as a more open-ended search method and a candidate hyper-edited mRNA was found and named novel APOBEC-1 target 1 (NAT1) mRNA. This hyper-editing of NAT1 mRNA in the livers of transgenic animals generates in-frame stop codons at many sites, with the result that there is no detectable full-length NAT1 protein in the livers of transgenic animals [54••]. NAT1 shows blocks of sequence homology with the carboxy-terminal two-thirds of eIF4G and in a yeast two-hybrid system interacts with eIF4A but not eIF4E. Expression of wild-type *NAT1* in COS cells resulted in the inhibition of both scanning-dependent translation initiation and internal initiation dependent on the IRES of encephalomyocarditis virus, whereas, in contrast, expression of the carboxy-terminal portion of eIF4G stimulated internal initiation (but inhibited scanning-dependent initiation).

NAT1 mRNA has a ubiquitous tissue distribution and is curious in that the open reading frame starts with a GUG codon. It is speculated that NAT1 protein acts as a decoy, perhaps sequestering eIF4A or conceivably eIF3, thereby acting as a negative regulator of translation initiation [54••]. It remains to be seen whether this activity of NAT1 protein is controlled, say by phosphorylation, and it is not yet proven that it is the absence of NAT1 protein in the transgenic livers that is the proximal cause of the hepatocarcinoma, although this is a very plausible hypothesis.

Regulation of translation initiation via reversible phosphorylation

It has long been recognized that initiation factor eIF2—which forms a ternary complex with GTP and the initiator tRNA, Met-tRNA$_i$ and effectively delivers the initiator tRNA to the 40S subunit—can be phosphorylated in its smallest (α) subunit by a small number of very specific protein kinases: the haem-controlled kinase hitherto considered a speciality of erythroid cells, the more ubiquitous double-stranded RNA regulated kinase (PKR), and the yeast GCN2 kinase. Phosphorylation of eIF2 does not inhibit its activity *per se* but, as mentioned above, it does inhibit the recycling of the eIF2/GDP complex

catalyzed by the 5-subunit guanine nucleotide exchange factor, eIF2B. Although the haem-controlled kinase and yeast GCN2 kinase seem to play rather specialized roles, PKR may have more general significance in growth regulation as expression of dominant negative PKR mutants, which downregulate the endogenous wild-type enzyme, or expression of eIF2α carrying a Ser-Ala mutation in the phosphorylation site (Ser-51) confer a transformed phenotype on the transfected cells [55]. A homozygous knockout of PKR in mice does not cause such a drastic phenotype, however, but so far seems limited to deficiencies in the response to interferon-γ [56••].

Apart from these rather specialized systems for the regulation of translation by reversible phosphorylation of initiation factors, there is growing evidence that the more general signal transduction/phosphorylation systems impinge on translation, although this evidence relies extensively on inhibition studies using rapamycin and wortmannin rather than on direct identification of the proximal kinases involved. Prime candidate targets include: eIF4E, the cap-binding initiation factor, in which the phosphorylation site is now recognized to be serine-209 [57]; ribosomal protein S6, which is phosphorylated by both $p70^{S6k}$ and also $p90^{rsk}$, although it is the former that is considered to be the physiologically relevant kinase; 4E-BP1, a protein originally identified in fat cells which binds eIF4E and sequesters it from entering the eIF4F holoenzyme complex [58]; and eIF2B, the activity of which increases rapidly in CHO-T cells exposed to insulin and in T cells treated with mitogens and which is inactivated by phosphorylation of its largest subunit by glycogen synthase kinase-3 [59]. In addition, there are positive correlations but no direct evidence for a cause and effect relationship between phosphorylation of eIF4G and eIF4B and stimulation of translation in response to growth factors and mitogens.

The correlation between phosphorylation of ribosomal protein S6 and the increase in protein synthesis or polyribosome content following stimulation of quiescent cells by serum or specific growth factors appeared to be so tight that for almost twenty years a cause and effect relationship has been generally assumed, even though *in vitro* assays failed to show a consistently higher activity of ribosomes with phosphorylated S6; however, it now appears that this *in vitro* result was largely correct. Inhibition of S6 phosphorylation by rapamycin does not inhibit the serum-induced increase in general protein synthesis rates but did specifically inhibit (incompletely) the translation of a subset of mRNAs that encode ribosomal proteins and translation elongation factors [60], all of which are characterized by having a short 5′-UTR with an oligopyrimidine tract at the extreme 5′-end. Rapamycin treatment was even more effective and selective in inhibiting the mobilization of just one of the two main isoforms of insulin-like growth factor II. This isoform contained the longer 5′-UTR and had not previously been considered to belong to the same class of mRNAs as the ribosomal protein mRNAs [61•]. Thus S6 phosphorylation does not seem to stimulate translation *per se*. Moreover, it is worth noting that even though rapamycin inhibited the translation of a subset of mRNAs, there is no direct proof that this was caused by inhibition of phosphorylation of ribosomal S6 rather than some other rapamycin-sensitive process.

The eIF4E-binding protein 4E-BP1 was discovered initially as a heat- and acid-stable protein of unknown function in fat cells which became rapidly and extensively phosphorylated in response to insulin treatment (hence its original designation as PHAS-I, for 'phosphorylated heat and acid stable [protein]-insulin'). 4E-BP1 was shown subsequently to bind eIF4E with high affinity, mutually exclusive with eIF4E binding to eIF4G; thus 4E-BP1 effectively reduces the amount of eIF4E available to form the eIF4F holoenzyme complex [58••]. Phosphorylation of 4E-BP1 results in dissociation of the eIF4E/4EBP-1 complex and thus brings about an increase in eIF4F holoenzyme concentrations (Fig. 1). This phosphorylation was originally ascribed to the MAP kinase system [62] but subsequent work has shown that even though 4E-BP1 can be phosphorylated by MAP kinase this does not cause release of eIF4E and it now appears to be generally agreed that it is the phosphorylation of 4E-BP1 by the rapamycin-sensitive $p70^{S6k}$ pathway which results in dissociation [63–65]. Even though rapamycin treatment of mammalian cells results in a rapid and complete dephosphorylation of 4E-BP1 [65] (and of ribosomal protein S6 [60]), however, in the short term there is only a slight decrease in general protein synthesis [60,66•]. Rather, it selectively inhibits the translation of a subset of mRNAs [60], though admittedly a subset coding for proteins that are very important for long-term growth. These considerations raise some questions about the significance of 4E-BP1 for the short-term regulation of general translation. In contrast, rapamycin causes a much more severe and rapid decrease in overall translation rates in yeast, likewise probably by interfering with initiation factor interactions at the 5′ cap [67•].

As for the importance of phosphorylation of eIF4E on Ser-209 [57], at present the evidence is mainly correlative rather than direct that the phosphorylation increases eIF4E activity, although a somewhat higher affinity for 5′ cap structures has been reported [68]. Increased phosphorylation correlated with increased translation rates is seen in response to insulin treatment of CHO-T cells [69], or serum stimulation, whereas decreased phosphorylation is observed on heat-shock or late in adenovirus infection [70••]. Inhibitor studies (e.g. [69]) suggest that this phosphorylation of eIF4E is mediated by the MAP kinase pathway rather than the rapamycin-sensitive $p70^{S6k}$ pathway. Phosphorylation of eIF4E would thus seem to be independent of 4E-BP1 phosphorylation and, indeed, although both proteins are dephosphorylated

under heat-shock conditions, adenovirus infection results in dephosphorylation of eIF4E but phosphorylation of 4EBP-1 [70••].

Concluding remarks

Perhaps the most striking innovation in the field over the past two years has been the new insights into how the mitogen-stimulated transmembrane signalling systems impinge on the regulation of translation, mainly via phosphorylation of eIF4E and 4E-BP1. In some ways this was not surprising, as it has been known for over twenty years that stimulation of quiescent cells results in a very rapid increase in the overall rate of translation of pre-existing mRNA and for over five years we have been aware that there was a selective activation of translation of mRNAs coding for ribosomal proteins and other core components of the translation machinery. It was further appreciated by those in the field that this activation of protein synthesis was far too rapid to be explained as a secondary consequence of the transcriptional activation of immediate early genes such as c-*fos*, or delayed early genes in the c-*myc* class. Thus we were well aware that a message was relayed rapidly from the cell surface to the mRNA translation machinery but we were at a loss as to how this 'biochemical e-mail' is routed and what the ultimate recipients of the message that result in activation of translation are. Although these questions still remain unanswered, enough pointers have emerged in the past two years to suggest that we will very soon see rapid progress in this area.

One of the greatest challenges for the future is (still) the exact nature of ribosome scanning; who moves, and what is actually required for the movement? Although there is an agreed model for the sequence of events, there are still many areas of uncertainty as to which components — initiation factors, ribosomal subunits — interact with each other and when these interactions occur. Recent insights into the architecture of the eIF4F holoenzyme complex have been very provocative but there is still controversy over whether this structure is a fixed entity, or whether the different subunits exchange during the initiation step. Answers to these questions are important for gaining a more complete understanding of how upstream sORFs regulate translation. It is true that the translation of ~90% of eukaryotic mRNAs is absolutely straightforward in that the 5′-proximal AUG codon is used as the exclusive or certainly predominant initiation site, entirely in accordance with the scanning ribosome model; however the ~10% of mRNAs that are exceptional include a very interesting subset coding for transcription factors, hormone receptors and components of the transmembrane signalling pathways [71]. Many mRNAs in these classes have short upstream ORFs and the fact that these sORFs are usually conserved in position and length between related species strongly suggests that the default mechanism of translation of these mRNAs is via a (probably regulated) form of ribosome scanning and/or shunting.

Aside from these considerations, a more complete understanding of the mechanics of scanning and the changing interactions of the initiation factors with each other and with the ribosome is surely necessary to catalyze new ideas and the design of experiments pertinent to the fascinating questions discussed in our companion review (Wickens, Anderson and Jackson, this issue, pp 220–232) of how poly(A) tails and protein–RNA interactions in the 3′-UTR can influence initiation events at the 5′-end.

Acknowledgements

We thank Matthias Hentze, Thomas Innerarity, and Theo Ohlmann for communicating the results of their work currently in press and also for verifying the accuracy of some of the statements we have made, although any remaining errors of fact and interpretation are entirely our responsibility. We also thank Niki Gray for helpful comments and suggestions on the manuscript. Work from the laboratories described herein was supported by grants from the Wellcome Trust (to RJ Jackson) and from the National Institutes of Health (to M Wickens).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jackson RJ: **A comparative view of initiation site selection mechanisms.** In *Translational Control.* Edited by Hershey JWB, Mathews MB, Sonenberg N. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1996:71–112.

2. Merrick WC, Hershey JWB: **The pathway and mechanism of eukaryotic protein synthesis.** In *Translational Control.* Edited by Hershey JWB, Mathews MB, Sonenberg N. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1996:31–69.

3. Altmann M, Wittmer B, Methot N, Sonenberg N, Trachsel H: **The *Saccharomyces cerevisiae* translation initiation factor Tif3 and its mammalian homolog, eIF-4B, have RNA annealing activity.** *EMBO J* 1995, **14**:3820–3827.

4. Naranda T, Macmillan SE, Hershey JWB: **Purified yeast translational initiation factor eIF-3 is an RNA-binding protein complex that contains the PRT1 protein.** *J Biol Chem* 1994, **269**:32286–32292.

5. Danaie P, Wittmer B, Altmann M, Trachsel H: **Isolation of a protein complex containing translation initiation factor Prt1 from *Saccharomyces cerevisiae.*** *J Biol Chem* 1995, **270**:4288–4292.

6. Garcia-Barrio MT, Naranda T, Vazquez de Aldana CR, Cuesta R, Hinnebusch AG, Hershey JWB, Tamame M: **GCD10, a translational repressor of *GCN4*, is the RNA binding subunit of eukaryotic translation initiation factor-3.** *Genes Dev* 1995, **9**:1781–1796.

7. Futterer J, Potrykus I, Bao Ym, Li L, Burns TM, Hull R, Hohn T: **Position-dependent ATT initiation during plant pararetrovirus rice tungro bacilliform virus translation.** *J Virol* 1996, **70**:2999–3010.

8. Dolph PJ, Huang J, Schneider RJ: **Translation by the adenovirus tripartite leader: elements which determine independence from cap-binding protein complex.** *J Virol* 1990, **64**:2669–2677.

9. •• Yueh A, Schneider RJ: **Selective translation initiation by ribosome jumping in adenovirus-infected and heat-shocked cells.** *Genes Dev* 1996, **10**:1557–1567.
Describes the first example of ribosome shunting in mammalian systems and gives an exemplary account of how insertion of hairpin loops and upstream AUG triplets can be used to define the positions of take-off and landing in the shunt.

10. Rouault TA, Klausner RD, Harford JB: **Translational control of ferritin.** In *Translational Control.* Edited by Hershey JWB, Mathews MB, Sonenberg N. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1996:335–362.

11. Hentze MW, Kuhn LC: **Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress.** *Proc Natl Acad Sci USA* 1996, **93**:8175–8182.

12. Henderson BR, Seiser C, Kuhn LC: **Characterization of a second RNA-binding protein in rodents with specificity for iron-responsive elements.** *J Biol Chem* 1993, **268**:27327–27334.

13. Samaniego F, Chin J, Iwai K, Rouault TA, Klausner RD: **Molecular characterization of a second iron-reponsive element-binding protein, iron regulatory protein-2.** *J Biol Chem* 1994, **269**:30904–30910.

14. Henderson BR, Menotti E, Bonnard C, Kuhn LC: **Optimal sequence and structure of iron-responsive elements: selection of RNA stem-loops with high-affinity for iron regulatory factor.** *J Biol Chem* 1994, **269**:17481–17489.

15. Henderson BR, Menotti E, Kuhn LC: **Iron regulatory proteins 1 and 2 find distinct sets of RNA target sequences.** *J Biol Chem* 1996, **271**:4900–4908.

16. Pantopoulos K, Gray NK, Hentze MW: **Differential regulation of two related RNA-binding proteins, iron regulatory protein (IRP) and IRP_B.** *RNA* 1995, **1**:155–163.

17. Henderson BR, Kuhn LC: **Differential modulation of the RNA-binding proteins IRP-1 and IRP-2 in response to iron.** *J Biol Chem* 1995, **270**:20509–20515.

18. • Iwai K, Klausner RD, Roualt TA: **Requirements for iron-regulated degradation of the RNA binding protein, iron regulatory protein 2.** *EMBO J* 1995, **14**:5350–5357.
Shows that the iron-dependent degradation of IRP2 is caused by its unique 73 amino acid insert and that comparable instability can be conferred on IRP1 by grafting this insert in the appropriate position.

19. •• Pantopoulos K, Weiss G, Hentze MW: **Nitric oxide and oxidative stress (H_2O_2) control mammalian iron-metabolism by different pathways.** *Mol Cell Biol* 1996, **16**:3781–3788.
Demonstrates that the regulation of IRP/IRE interactions by oxidative stress is quite different from regulation by NO or iron-deprivation, in that is much more rapid and is sensitive to okadaic acid.

20. Pantopoulos K, Hentze MW: **Rapid responses to oxidative stress mediated by iron regulatory protein.** *EMBO J* 1995, 14:2917–2924.

21. Melefors O, Goossen B, Johansson HE, Stripecke R, Gray NK, Hentze MW: **Translational control of 5-aminolevulinate synthase mRNA by iron-responsive elements in erythroid cells.** *J Biol Chem* 1993, **268**:5974–5978.

22. Kohler SA, Henderson BR, Kuhn LC: **Succinate dehydrogenase b mRNA of *Drosophila melanogaster* has a functional iron-responsive element in its 5′-untranslated region.** *J Biol Chem* 1995, **270**:30781–30786.

23. Gray NK, Pantopoulos K, Dandekar T, Ackrell BAC, Hentze MW: **Translation regulation of mammalian and *Drosophila* citric acid cycle enzymes via iron-responsive elements.** *Proc Natl Acad Sci USA* 1996, **93**:4925–4930.

24. Gray NK, Hentze MW: **Iron regulatory protein prevents binding of the 43S translation pre-initiation complex to ferritin and eALAS mRNAs.** *EMBO J* 1994, **13**:3882–3891.

25. Schäfer M, Nayernia K, Engel W, Schäfer U: **Translational control in spermatogenesis.** *Dev Biol* 1995, **172**:344–352.

26. Gu W, Hecht NB: **Translation of a testis-specific Cu/Zn superoxide dismutase (SOD-1) mRNA is regulated by a 65-kilodalton protein which binds to its 5′-untranslated region.** *Mol Cell Biol* 1996, **16**:4535–4543.

27. Gu W, Morales C, Hecht NB: **In male mouse germ cells, copper-zinc superoxide dismutase utilizes alternative promoters that produce multiple transcripts with different translation potential.** *J Biol Chem* 1995, **270**:236–243.

28. Grant CM, Hinnebusch AG: **Effect of sequence context at stop codons on efficiency of reinitiation in *GCN4* translational control.** *Mol Cell Biol* 1994, **14**:606–618.

29. Grant CM, Miller PF, Hinnebusch AG: **Sequences 5′of the first upstream open reading frame in *GCN4* messenger RNA are required for efficient translational reinitiation.** *Nucleic Acids Res* 1995, **23**:3980–3988.

30. Grant CM, Miller PF, Hinnebusch AG: **Requirements for intercistronic distance and level of eIF-2 activity in reinitiation on *GCN4* mRNA varies with the downstream cistron.** *Mol Cell Biol* 1994, 14:2616–2628.

31. Hinnebusch AG: **Translational control of *GCN4*: gene-specific regulation by phosphorylation of eIF2.** In *Translational Control.* Edited by Hershey JWB, Mathews MB, Sonenberg N. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1996:199–244.

32. •• Dever TE, Yang WM, Astrom S, Bystrom AS, Hinnebusch AG: **Modulation of $tRNA_{(i)}$, eIF-2, and eIF-2B expression shows that *GCN4* translation is inversely coupled to the level of eIF-2·GTP·Met-$tRNA_{(i)}$ ternary complexes.** *Mol Cell Biol* 1995, **15**:6351–6363.
A definitive work which brings together in a single paper all the different ways in which GCN4 expression can be stimulated, all of which are common in that they have a reduced pool of ternary complexes.

33. Luo ZL, Sachs MS: **Role of an upstream open reading frame in mediating arginine-specific translational control in *Neurospora crassa*.** *J Bacteriol* 1996, **178**:2172–2177.

34. • Freitag M, Dighde N, Sachs MS: **A uv-induced mutation in *Neurospora* that affects translational regulation in response to arginine.** *Genetics* 1996, **142**:117–127.
Genetic evidence that the amino acid sequence of the sORF in *arg-2* mRNA is a determinant of the regulation of translation of this mRNA by arginine.

35. Cao JH, Geballe AP: **Translational inhibition by a human cytomegalovirus upstream open reading frame despite inefficient utilization of its AUG codon.** *J Virol* 1995, **69**:1030–1036.

36. • Cao JH, Geballe AP: **Coding sequence-dependent ribosomal arrest at termination of translation.** *Mol Cell Biol* 1996, **16**:603–608.
Direct evidence that ribosomes stall at or near the termination codon of an inhibitory short upstream ORF.

37. •• Chen CY, Sarnow P: **Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs.** *Science* 1995, **268**:415–417.
An extremely elegant experiment showing that mammalian ribosomes can initiate translation on a covalently circularized RNA provided that the RNA includes an IRES. If all in-frame stop codons are eliminated, the ribosomes will continue elongation for many cycles.

38. Jackson RJ, Kaminski A: **Internal initiation of translation in eukaryotes: the picornavirus paradigm and beyond.** *RNA* 1995, 1:985–1000.

39. •• Reynolds JE, Kaminski A, Kettinen HJ, Grace K, Clarke BE, Carroll AR, Rowlands DJ, Jackson RJ: **Unique features of internal initiation of hepatitis C virus RNA translation.** *EMBO J* 1995, **14**:6010–6020.
The first ten codons of viral coding sequence are an important part of the IRES, in contrast to the precedents of picornavirus IRESs.

40. Poole TL, Wang CY, Popp RA, Potgieter LND, Siddiqui A, Collett MS: **Pestivirus translation initiation occurs by internal ribosome entry.** *Virology* 1995, **206**:750–754.

41. • Wang CY, Le SY, Ali N, Siddiqui A: **An RNA pseudoknot is an essential structural element of the internal ribosome entry site located within the hepatitis C virus 5′-noncoding region.** *RNA* 1995, 1:526–537.
Compensatory mutations show that there is a pseudoknot in the 5′-UTR of HCV RNA that is essential for the function of the IRES.

42. Bernstein J, Shefler I, Elroy-Stein O: **The translational repression mediated by the platelet-derived growth factor 2/c-*sis* mRNA leader is relieved during megakaryocytic differentiation.** *J Biol Chem* 1995, **270**:10559–10565.

43. Gan WN, Rhoads RE: **Internal initiation of translation directed by the 5′-untranslated region of the mRNA for eIF4G, a factor involved in the picornavirus-induced switch from cap-dependent to internal initiation.** *J Biol Chem* 1996, **271**:623–626.

44. • Kaminski A, Hunt SL, Patton JG, Jackson RJ: **Direct evidence that polypyrimidine tract binding protein (PTB) is essential for internal initiation of translation of encephalomyocarditis virus RNA.** *RNA* 1995, 1:924–938.
Direct evidence from affinity column depletion experiments that PTB is essential for the activity of some, but not all, picornavirus IRESs and that it is not required for internal initiation promoted by the hepatitis C virus IRES.

45. Svitkin YV, Meerovitch K, Lee HS, Dholakia JN, Kenan DJ, Agol VI, Sonenberg N: **Internal translation initiation on poliovirus RNA:**

further characterization of La function in poliovirus translation *in vitro*. *J Virol* 1994, **68**:1544–1550.

46. •• Lamphear BJ, Kirchweger R, Skern T, Rhoads RE: **Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases: implications for cap-dependent and cap-independent translational initiation.** *J Biol Chem* 1995, **270**:21975–21983.
Key experiments exploiting the cleavage of eIF4G by picornavirus proteases in order to determine the architecture of the eIF4F holoenzyme complex.

47. Ohlmann T, Pain VM, Wood W, Rau M, Morley SJ: **The proteolytic cleavage of eukaryotic initiation factor (eIF) 4G is prevented by eIF4E binding protein (PHAS-I; 4E-BP1) in the reticulocyte lysate.** *EMBO J* 1997, **16**:844–855.

48. Wyckoff EE, Hershey JWB, Ehrenfeld E: **Eukaryotic initiation factor 3 is required for poliovirus 2A protease-induced cleavage of the p220 component of eukaryotic initiation factor 4F.** *Proc Natl Acad Sci USA* 1990, **87**:9529–9533.

49. Mader S, Lee H, Pause A, Sonenberg N: **The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4g and the translational repressors 4E-binding proteins.** *Mol Cell Biol* 1995, **15**:4990–4997.

50. Ohlmann T, Rau M, Morley SJ, Pain VM: **Proteolytic cleavage of initiation-factor eIF-4g in the reticulocyte lysate inhibits translation of capped mRNAs but enhances that of uncapped mRNAs.** *Nucleic Acids Res* 1995, **23**:334–340.

51. Ziegler E, Borman AM, Deliat FG, Liebig HD, Jugovic D, Kean KM, Skern T, Kuechler E: **Picornavirus 2A proteinase-mediated stimulation of internal initiation of translation is dependent on enzymatic activity and the cleavage products of cellular proteins.** *Virology* 1995, **213**:549–557.

52. Ziegler E, Borman AM, Kirchweger R, Skern T: **Foot-and-mouth disease virus Lb proteinase can stimulate rhinovirus and enterovirus IRES-driven translation and cleave several proteins of cellular and viral origin.** *J Virol* 1995, **69**:3465–3474.

53. • Ohlmann T, Rau M, Pain VM, Morley SJ: **The C-terminal domain of eukaryotic protein synthesis initiation factor (eIF)4G is sufficient to support cap-independent translation in the absence of eIF4E.** *EMBO J* 1996, **15**:1371–1382.
Evidence that internal initiation of translation requires at least the carboxy-terminal two-thirds fragment of eIF4G, which has the eIF4A and eIF3 binding sites.

54. •• Yamanaka S, Poksay KS, Arnold KS, Innerarity TL: **A novel translational repressor mRNA is extensively edited in livers containing tumors caused by the transgene expression of the apoB mRNA-editing enzyme.** *Genes Dev* 1997, 11:321.
Unexpected discovery of an eIF4G 'decoy' resembling the carboxy-terminal two-thirds of eIF4G, which binds eIF4A but not eIF4E and which is inhibitory to both scanning-dependent and IRES-dependent initiation when it is over-expressed in transfected cells.

55. Donze O, Jagus R, Koromilas AE, Hershey JWB, Sonenberg N: **Abrogation of translation initiation factor eIF-2 phosphorylation causes malignant transformation of NIH 3T3 cells.** *EMBO J* 1995, 14:3828–3834.

56. •• Yang YL, Reis LFL, Pavlovic J, Aguzzi A, Schafer R, Kumar A, Williams BRG, Aguet M, Weissmann C: **Deficient signalling in mice devoid of double-stranded RNA dependent protein kinase.** *EMBO J* 1995, 14:6095–6106.
First insights into the real *in vivo* role of PKR.

57. Joshi B, Cai AL, Keiper BD, Minich WB, Mendez R, Beach CM, Stepinski J, Stolarski R, Darzynkiewicz E, Rhoads RE: **Phosphorylation of eukaryotic protein synthesis initiation factor 4E at Ser-209.** *J Biol Chem* 1995, **270**:14597–14603.

58. Pause A, Belsham GJ, Gingras AC, Donze O, Lin TA, Lawrence JC, Sonenberg N: **Insulin-dependent stimulation of protein-synthesis by phosphorylation of a regulator of 5′-cap function.** *Nature* 1994, **371**:762–767.

59. Welsh GI, Miyamoto S, Price NT, Safer B, Proud CG: **T-cell activation leads to rapid stimulation of translation initiation-factor eIF2B and inactivation of glycogen synthase kinase-3.** *J Biol Chem* 1996, **271**:11410–11413.

60. Jefferies HBJ, Reinhard C, Kozma SC, Thomas G: **Rapamycin selectively represses translation of the 'polypyrimidine tract' mRNA family.** *Proc Natl Acad Sci USA* 1994, **91**:4441–4445.

61. • Nielsen FC, Ostergaard L, Nielsen J, Christiansen J: **Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway.** *Nature* 1995, **377**:358–362.
A very striking influence of rapamycin on the initiation of translation of just one of the two main isoforms of IGF-II mRNA, which differ in the length of their 5′-UTRs.

62. Lin TA, Kong XM, Haystead TAJ, Pause A, Belsham G, Sonenberg N, Lawrence JC: **PHAS-1 as a link between mitogen-activated protein kinase and translation initiation.** *Science* 1994, **266**:653–656.

63. Diggle TA, Moule SK, Avison MB, Flynn A, Foulstone EJ, Proud CG, Denton RM: **Both rapamycin-sensitive and -insensitive pathways are involved in the phosphorylation of the initiation factor-4E-binding protein (4E-BP1) in response to insulin in rat epididymal fat-cells.** *Biochem J* 1996, **316**:447–453.

64. Azpiazu I, Saltiel AR, Depaoliroach AA, Lawrence JC: **Regulation of both glycogen synthase and PHAS-I by insulin in rat skeletal muscle involves mitogen-activated protein kinase-independent and rapamycin-sensitive pathways.** *J Biol Chem* 1996, **271**:5033–5039.

65. Vonmanteuffel SR, Gingras AC, Ming XF, Sonenberg N, Thomas G: **4E-BP1 phosphorylation is mediated by the FRAP-p70^{s6k} pathway and is independent of mitogen-activated protein kinase.** *Proc Natl Acad Sci USA* 1996, **93**:4076–4080.

66. • Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N: **Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap- dependent initiation of translation.** *EMBO J* 1996, 15:658–664.
Rapamycin results in a very rapid dephosphorylation of 4E-BP1 but only a slow and incomplete inhibition of translation in mammalian cells.

67. • Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN: **TOR controls translation initiation and early G_1 progression in yeast.** *Mol Biol Cell* 1996, **7**:25–42.
Rapamycin causes an exceedingly rapid and largely complete inhibition of translation in yeast.

68. Minich WB, Balasta ML, Goss DJ, Rhoads RE: **Chromatographic resolution of *in vivo* phosphorylated and nonphosphorylated eukaryotic translation initiation factor eIF-4E: increased cap affinity of the phosphorylated form.** *Proc Natl Acad Sci USA* 1994, **91**:7668–7672.

69. Flynn A, Proud CG: **Insulin-stimulated phosphorylation of initiation factor 4E is mediated by the MAP kinase pathway.** *FEBS Lett* 1996, **389**:162–166.

70. •• Feigenblum D, Schneider RJ: **Cap-binding protein (eukaryotic initiation factor 4E) and 4E-inactivating protein BP-1 independently regulate cap-dependent translation.** *Mol Cell Biol* 1996, **16**:5450–5457.
This report shows that the phosphorylation of eIF4E and of 4E-BP1 are not necessarily co-ordinated under conditions of heat-shock or infection by adenovirus and thus it is likely that quite distinct pathways are involved.

71. Kozak M: **An analysis of vertebrate mRNA sequences: intimations of translatonal control.** *J Cell Biol* 1991, **115**:887–903.

72. Tarun SZ, Sachs AB: **Association of the yeast poly(A) tail binding protein with translation initiation factor eIF4G.** *EMBO J* 1996, **15**:7168–7177.