REVIEW ARTICLE

Molecular mechanisms for the control of translation by insulin

Christopher G. PROUD*[1] and Richard M. DENTON†

*Department of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ, U.K., and †Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.

Insulin acutely stimulates protein synthesis in mammalian cells, and this involves activation of the process of mRNA translation. mRNA translation is a complex multi-step process mediated by proteins termed translation factors. Several translation factors are regulated in response to insulin, often as a consequence of changes in their states of phosphorylation. The initiation factor eIF4E binds to the cap structure at the 5′-end of the mRNA and mediates assembly of an initiation-factor complex termed eIF4F. Assembly of this complex can be regulated by eIF4E-binding proteins (4E-BPs), which inhibit eIF4F complex assembly. Insulin induces phosphorylation of the 4E-BPs, resulting in alleviation of the inhibition. This regulatory mechanism is likely to be especially important for the control of the translation of specific mRNAs whose 5′-untranslated regions (5′-UTRs) are rich in secondary structure. Translation of another class of mRNAs, those with 5′-UTRs containing polypyrimidine tracts is also activated by insulin and this, like phosphorylation of the 4E-BPs, appears to involve the rapamycin-sensitive signalling pathway which leads to activation of the 70 kDa ribosomal protein S6 kinase (p70 S6 kinase) and the phosphorylation of the ribosomal protein S6. Overall stimulation of translation may involve activation of initiation factor eIF2B, which is required for all initiation events. This effect is dependent upon phosphatidylinositol 3-kinase and may involve the inactivation of glycogen synthase kinase-3 and consequent dephosphorylation of eIF2B, leading to its activation. Peptide-chain elongation can also be activated by insulin, and this is associated with the dephosphorylation and activation of elongation factor eEF2, probably as a consequence of the insulin-induced reduction in eEF2 kinase activity. Thus multiple signalling pathways acting on different steps in translation are involved in the activation of this process by insulin and lead both to general activation of translation and to the selective regulation of specific mRNAs.

INTRODUCTION

Insulin plays an important role in the overall regulation of protein synthesis [1]. Some of the effects of the hormone may involve changes in the concentrations of amino acyl-tRNAs [1] and mRNAs [2], but insulin also has important effects on the translation process itself, and it is these effects which are the topic of this Review Article. Not only does insulin cause a global increase in the rate of translation, but, superimposed upon this overall increase, the hormone also brings about marked increases in the translation of specific mRNAs. Examples of this selective regulation of translation include the mRNAs of a number of ribosomal proteins and elongation factor 2 [3], cyclins [4], fatty acid synthase [5] and ornithine decarboxylase [6].

Major advances have been made towards understanding the mechanisms whereby insulin influences the activity of key components in the process of translation. Taken together, these may explain both the effects of insulin on global process synthesis and its selective effects on the translation of specific mRNAs. As will become evident in this Review Article, a common feature is the importance of reversible phosphorylation in the regulation of components which are involved in both the initiation and elongation stages of translation. The signalling pathways which link the insulin receptor to many intracellular events regulated by insulin are also presently the subject of intense research, and we start with a brief overview of current knowledge in this rapidly developing area. As will become apparent, at least three distinct intracellular signalling pathways may be involved in the regulation of mRNA translation by insulin.

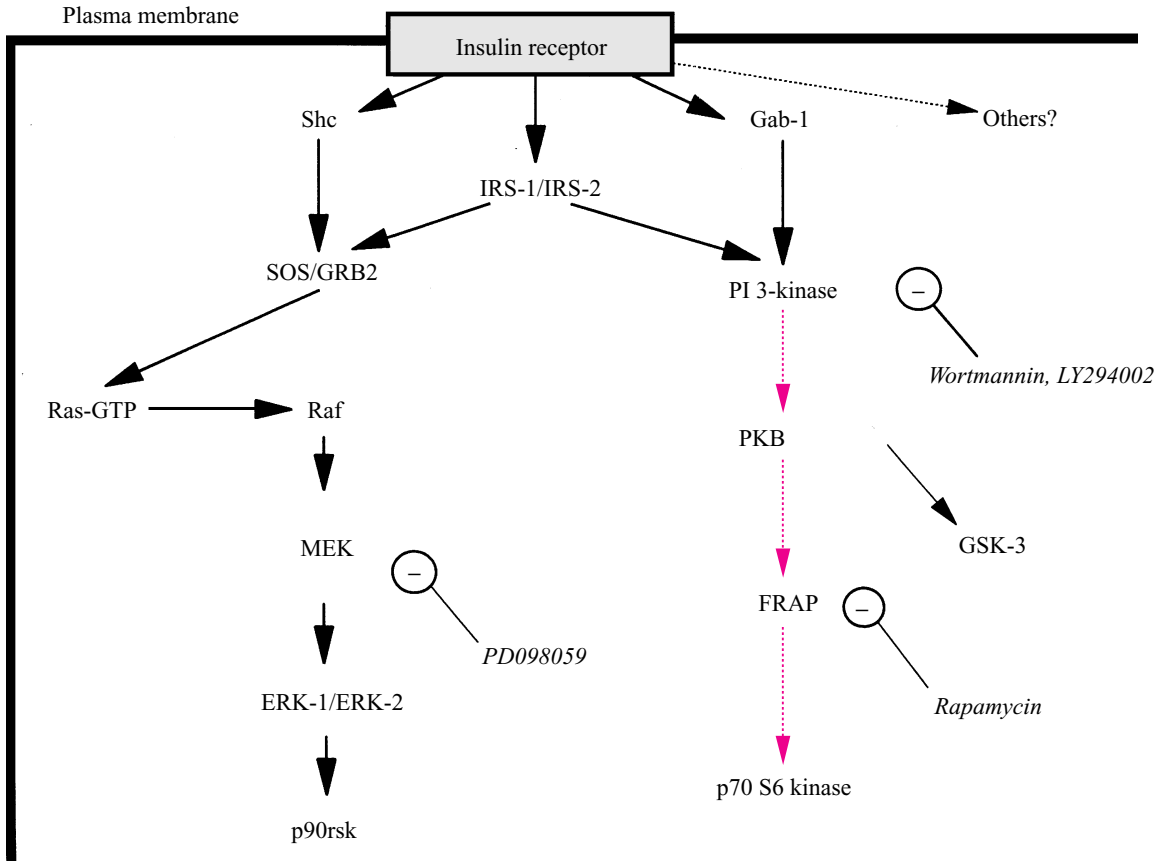
We sympathize in advance with the non-specialist reader for the rather confusing array of different and sometimes seemingly bizarre names given to components of the insulin signalling pathways and, to a lesser extent, to some of those involved in the actual process of translation. In this Review Article, we have only used a single term for each component, but at first mention we have listed the main alternative names!

OVERVIEW OF THE INTRACELLULAR SIGNALLING PATHWAYS INVOLVED IN THE ACTIONS OF INSULIN

Scheme 1 depicts some of the known early events. The binding of insulin to the extracellular α-subunits of the insulin receptor results in the activation of the tyrosine kinase activity intrinsic to the intracellular domain of the β-subunits of the receptor [7,8]. Following the autophosphorylation of specific tyrosine residues within the β-subunits, the receptor phosphorylates a number of

Abbreviations used: 4E-BP, eIF4E-binding protein; 5′-UTR, 5′-untranslated region; Ca/CaM, calcium/calmodulin; CHO, Chinese-hamster ovary; eEF, eukaryotic elongation factor; eIF, eukaryotic initiation factor; eRF, eukaryotic release factor; ERK-1, -2, extracellular-ligand-regulated kinase-1, -2; FKBP, FK506-binding protein; FRAP, FKBP–rapamycin-associating protein; GRB1, growth-factor-receptor-bound protein; GSK-3, glycogen synthase kinase-3; IRS-1, -2, insulin-receptor substrate-1, -2; MAP kinase, mitogen-activated protein kinase; MEK, MAP/ERK kinase; mTOR, mammalian TOR (Target Of Rapamycin); p70 S6 kinase, the 70 kDa ribosomal protein S6 kinase; p90rsk, 'the 90 kDa ribosomal protein S6 kinase' (no longer thought to be important in the phosphorylation of S6 in cells); PDK1, phospholipid-dependent kinase-1; PDK2, phospholipid-dependent kinase-2; PH, pleckstrin homology; PHAS, phosphorylated heat- and acid-stable (protein); PI 3-kinase, phosphatidylinositol 3-kinase; PKB, protein kinase B; PP, protein phosphatase; RAC, related to A- and C-kinases (i.e., to cAMP-dependent protein kinase and protein kinase C); RAFT, rapamycin and FKBP target; SH2, Src homology 2; SH3, Src homology 3; Shc, adaptor protein with homology with Src and collagen; SOS, son-of-sevenless; TOP, tracts of oligopyrimidines; uORF, upstream open reading frame.

[1] Present address and address for print requests: Department of Anatomy and Physiology, University of Dundee, Dundee DD1 4HM, Scotland, U.K.



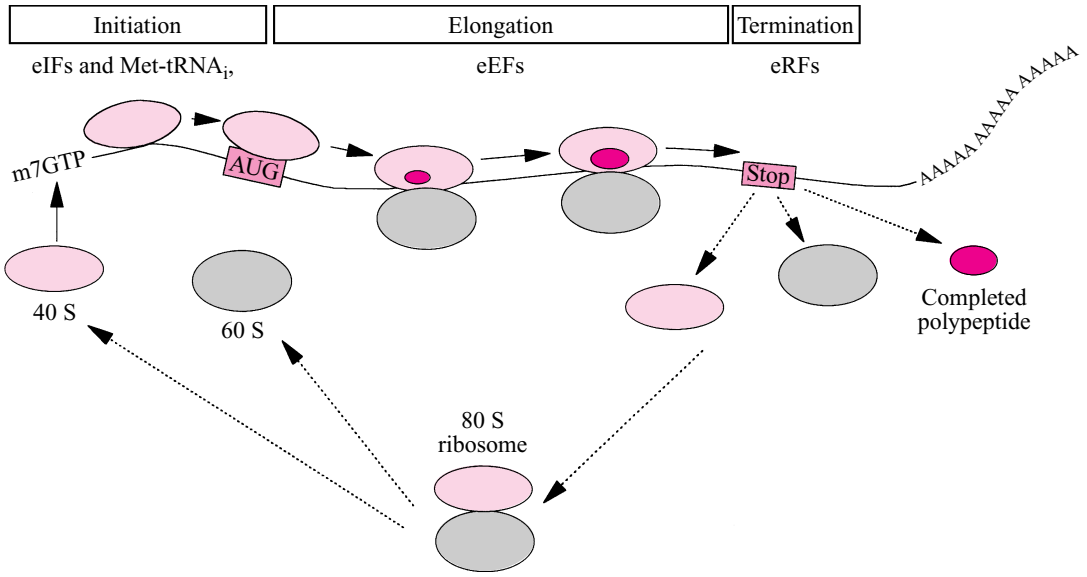
Scheme 1 Early events in insulin signalling

Solid black arrows indicate direct effects, while broken red arrows indicate that other components are, or may be, involved. The sites of action of the inhibitors wortmannin LY294002, rapamycin and PD098059 (in italics) are shown.

intracellular substrates on specific tyrosine residues, and this initiates the intracellular signalling pathways. These substrates include IRS-1 (insulin receptor substrate-1) [7,9], the related IRS-2 [10], Shc [11,12] and GAB1 [13]. The regions in these proteins containing phosphotyrosine residues then act as docking sites for other proteins which contain Src-homology 2 (SH2) domains. The two most important are probably GRB-2 and the 85 kDa subunit of phosphatidylinositol 3-kinase (PI 3-kinase), since these bring about the activation of Ras and PI 3-kinase respectively.

The activation of Ras, which is located on the inner face of the plasma membrane, appears to involve SOS (son-of-sevenless), which is a guanine nucleotide exchange factor able to promote the exchange of GDP on Ras with GTP, yielding the active form of Ras. SOS is tightly bound to GRB-2 via Src-homology 3 (SH3) domains, and thus it is the GRB-2–SOS complex which binds to specific phosphotyrosine residues within IRS-1, IRS-2 and Shc when these proteins are phosphorylated by the insulin receptor. This binding is thought to bring the GRB-2–SOS complex to the plasma membrane and thus close to Ras. Activated Ras is able to initiate a cascade of activated protein kinases often called the mitogen-activated protein kinase (MAP kinase) cascade [14]. Ras-GTP, but not Ras-GDP, binds to the first kinase of the cascade, Raf-1. This association alone does not appear to cause activation of Raf-1, but rather it appears to bring Raf-1 into juxtaposition with other components of the 'signalling complex'. One such component may be activated PI 3-kinase [15]. Activated Raf-1 phosphorylates another kinase MEK (also called MAP kinase kinase), which is a dual specificity kinase which in turn activates extracellular-ligand-regulated kinase-1 and -2 (ERK-1 and ERK-2) by phosphorylating these kinases on both tyrosine and threonine residues in the sequence TEY. ERK-1 and ERK-2 are very similar and are both expressed in most cells. They are referred to collectively as MAP kinase in this Review Article and have a wide range of potential substrates in cells including transcription factors such as elk-1, c-Jun and other protein kinases, including p90rsk [14]. This kinase becomes activated following phosphorylation by MAP kinase.

PI 3-kinase is activated when its 85 kDa subunit becomes bound to specific phosphotyrosine residues in IRS-1 or IRS-2, and this results in an increase in the product PtdIns(3,4,5)P_3 [16,17]. GAB1, when phosphorylated by the insulin receptor on appropriate tyrosine residues, may also be able to activate PI 3-kinase [13]. The activation of PI 3-kinase appears to be necessary for many of the effects of insulin, including the protein kinase cascades involving protein kinase B (also called RAC or akt) and p70 S6 kinase (Scheme 1) [18–20]. It has been suggested that activation may be the direct result of the binding of PtdIns(3,4,5)P_3 (formed by PI 3-kinase) to the pleckstrin homology (PH) domain in protein kinase B (PKB), but there has been considerable debate over the importance of this [21–27] (see also the legend to Scheme 4 below). Activation of PKB is associated



Scheme 2 The three phases of mRNA translation

The Scheme depicts an mRNA showing its 5′ cap (J-methyl-GTP) and poly(A) tail. The poly(A) tail (AAAA...) may participate in the initiation process (boxed) and may interact with initiation factors [52]. Ribosomes must dissociate into their constituent subunits [40 S (pink) and 60 S (grey)] in order to take part in initiation, which also requires initiation factor proteins. Following assembly of the initiation complex at the start codon (shown AUG on dark pink), the ribosome then proceeds into elongation, (boxed) and the new polypeptide is synthesized (red ellipse). After encountering an in-frame Stop codon (dark pink), termination occurs with release of the completed polypeptide and the ribosomal subunits. Abbreviation m^7GTP, 7-methyl-GTP.

with increased phosphorylation of Thr^{308} and Ser^{473}, and phosphorylation of both sites seems to be required for full activation [28]. Thr^{308} can be phosphorylated, at least *in vitro*, by a novel phospholipid-dependent protein kinase termed PDK1, which is activated *in vitro* by phosphoinositides phosphorylated in the 3 position [29]. Thus PtdIns(3,4,5)P_3 appears to exert a dual effect on PKB: activation involves both direct interaction with PKB and stimulation of the upstream kinase PDK1. PKB is able to phosphorylate glycogen synthase kinase-3 and this *decreases* the activity of this kinase [20]. We will be returning to this below (see also Scheme 4 below). The activation of p70 S6 kinase in cells exposed to insulin is apparently due to its phosphorylation on multiple serine and threonine sites, probably by more than one kinase [30,31]. p70 S6 kinase may be downstream of PKB as indicated in Scheme 1, but it is not a direct substrate [32].

A number of inhibitors have proved useful in dissecting the signalling pathways involved in kinase action (Scheme 1). When used at nanomolar concentrations, the fungal metabolite wortmannin appears to be a reasonably specific inhibitor of PI 3-kinase [33,34], but at higher concentrations it has been shown to also inhibit other enzymes, including phospholipases D and A2, phosphatidylinositol 4-kinase and the protein kinase activity of FK506-binding protein–rapamycin-associating protein/mammalian **T**arget **o**f **R**apamycin (FRAP/mTOR) [35–37]. Most of the metabolic effects of insulin) are blocked in parallel with the inhibition of PI 3-kinase by both wortmannin and the structurally unrelated inhibitor LY294002 [38], indicating the pivotal role of PI 3-kinase in insulin signal transduction. The inhibition of the metabolic effects are associated with the loss of activation of p70 S6 kinase and PKB, as expected if both these kinases lie downstream from PI 3-kinase. However, wortmannin also inhibits the activation of MAP kinase by insulin in many cell types [20,39–41]; the mechanism is not clear, but may stem from an interaction between Ras and PI 3-kinase.

The compound PD098059 acts by binding the inactive form of MEK and blocking its activation. Hence this inhibitor can attenuate the activation of MAP kinase by insulin and growth factors in many cells [20,42,43]. It should be noted that, owing to its mode of action, inhibition of MEK activity within cells may not be complete, and indeed at high concentrations of insulin the effects of this inhibitor may be too small to be useful [42–44].

Rapamycin was first identified as a potent immunosuppressant, but it has effects on many cell types, resulting in the inhibition of growth [45]. It acts by binding to a 12 kDa cytosolic protein (FKBP, FK506-binding protein) and this complex in turn then interacts with a large (about 250 kDa) protein usually called FRAP in mammalian systems, but also referred to as mTOR or rapamycin and FKBP target (RAFT) [46–49]. This protein is related to the yeast protein TOR (target of rapamycin) and contains a domain similar to the catalytic domain of PI 3-kinase, suggesting that it may have protein kinase or lipid kinase activity [46,48,50,51]. The complex of FK506 with FKBP does not bind to FRAP, and thus FK506, by competing with rapamycin, can block the specific effects of rapamycin. In the context of signalling by insulin and growth factors, rapamycin is important because it inhibits a number of their effects on translation, as described below. The signalling components which interact directly with FRAP remain to be established, but rapamycin inhibits the activation of p70 S6 kinase by insulin and other stimuli and therefore it seems reasonable to assume that FRAP is upstream of this kinase (Scheme 1).

THE THREE PHASES OF PROTEIN SYNTHESIS

Translation of mRNA into protein can be conveniently divided into three phases: initiation, elongation and termination (Scheme 2). During initiation, methionyl-tRNA (initiator tRNA) and several initiation factors associate with the 40 S ribosomal subunit to form the 43 S preinitiation complex, this complex binds to mRNA and migrates to the correct AUG initiation codon

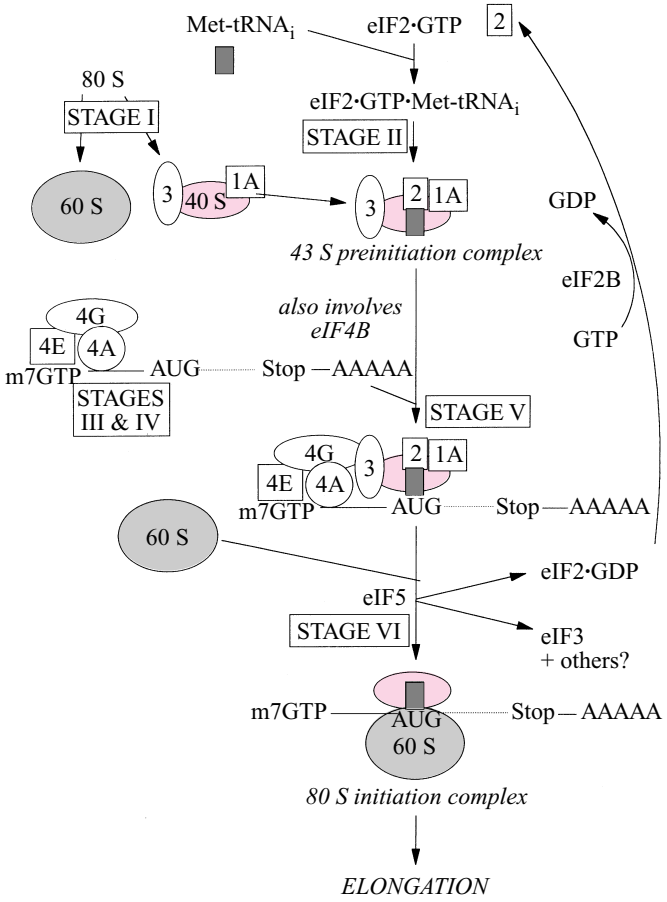
followed by the addition of the 60 S ribosomal subunit. This is then followed by elongation, during which amino acids from amino acyl-tRNAs are added to the growing peptide in the order dictated by the mRNA bound to the ribosome. Eventually the termination phase allows the completed protein to be released from the ribosome. An impressive number of different polypeptides are involved, including about 85 in the 40 S and 60 S subunits and a further 35 or so as the subunits of eukaryotic initiation factors (eIFs), elongation factors (eEFs) and the release factors (eRFs). For further details of these components and their roles the reader is referred to recent reviews on the mechanisms involved in each of the three phases [52–54]. Insulin acts on the initiation and elongation phases, and we will consider these in turn.

REGULATION OF INITIATION BY INSULIN

Overview of peptide-chain initiation

Peptide-chain initiation represents a major control point in gene expression and is subject to control under a variety of conditions. It involves the following principal events, which are summarized in Scheme 3:

- The dissociation of the 80 S ribosome into its component subunits, 40 S and 60 S, which requires the multimeric initiation factor eIF3 and probably also eIF1A (STAGE I)
- The binding of the initiator Met-tRNA$_i$ to the 40 S ribosomal subunit, mediated by eIF2 [55], which binds Met-tRNA only when it is complexed with GTP (STAGE II). This step also involves eIF1A [56] and results in the formation of the 43 S preinitiation complex, in which the Met-tRNA occupies the P-site of the ribosome
- The recognition of the 5′-cap of the mRNA by eIF4E (the 'cap-binding protein') (STAGE III) and subsequently. This, and the next step, probably occur in parallel with the steps described above, rather than subsequently to them
- The interaction of eIF4E with the scaffolding protein eIF4G and thereby with the helicase eIF4A, whose role is thought to be the 'unwinding' of secondary structures (self-complementary stem loops) [57]. The complex of eIF4E with eIF4G and eIF4A is known as eIF4F (STAGE IV). It is not yet clear whether eIF4E binds to the cap alone or as a preformed complex with eIF4G and perhaps other components (see [52] for a detailed discussion of this)
- Interaction of eIF4F with the 43 S preinitiation complex and the subsequent 'scanning' by the 40 S subunit, and associated factors such as eIF4F, from the 5′-end of the mRNA to the AUG codon [58], which is almost certainly identified through its interaction with the anticodon of the Met-tRNA. This process also involves the RNA-binding initiation factor eIF4B, which may help create the ribosome-binding site on the mRNA [56] (STAGE V)
- Addition of the 60 S subunit to form the 80 S initiation complex, which is competent to enter elongation. This step also involves eIF5, the hydrolysis of the GTP bound to eIF2 and the release of eIF2·GDP along with the other eIFs [52] (STAGE VI). Regeneration of active [eIF2·GTP] is mediated by the guanine nucleotide-exchange factor eIF2B, which facilitates the otherwise very slow release of the GDP.



Scheme 3 Initiation of protein synthesis in mammalian cells

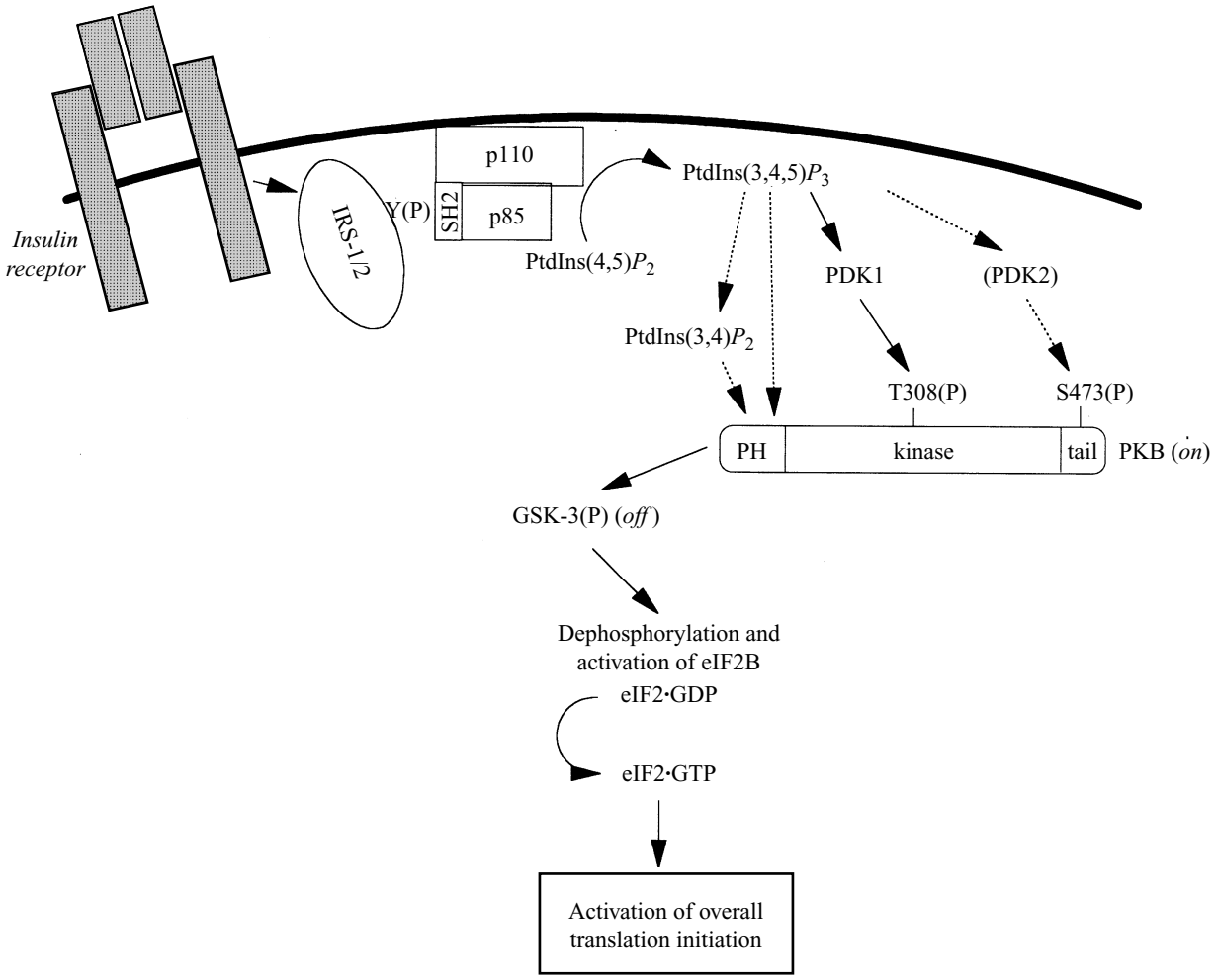
The process is described in detail in the text and is the subject of a recent review by Pain [52]. Abbreviation: m^{7}GTP, 7-methyl-GTP.

Eukaryotic translation initiation has recently been the subject of an excellent review by Pain [52].

Among these steps, two are believed to be particularly important for the regulation of the initiation process. These are STAGES II and III/IV (and perhaps also the associated STAGE V). In each case there is evidence that the activities of the factors involved are altered under conditions where translation is controlled, e.g., in response to insulin or growth factors, and, in each case, the factors involved are subject to, and apparently regulated by, phosphorylation. There are also other mechanisms by which the interaction of specific mRNAs with the translational machinery can be regulated, as is exemplified by the case of those mRNAs, which contain a polypyrimidine tract at their 5′-end ['5′-TOP (Tracts of OligoPyrimidines)' mRNAs].

Regulation of eIF2B activity

Initiation factor eIF2B mediates the recycling of eIF2, the factor which recruits the initiator Met-tRNA to the ribosome [59] (Schemes 3 and 4). As mentioned above, eIF2 is active when bound to GTP and forms a eIF2·GTP·Met-tRNA complex, which binds to the 40 S ribosomal subunit. The GTP is hydrolysed late in the initiation process, and eIF2 is released from the ribosome as an inactive eIF2·GDP complex. eIF2B acts by promoting the release of this GDP, thus allowing it to be replaced by GTP, to regenerate the active eIF2·GTP complex. Hence the level of activity of eIF2B governs the level of active eIF2 (eIF2·GTP) in the cell. Since eIF2 is required for every initiation event, modulating the activity of eIF2B provides a mechanism for controlling overall rates of peptide-chain in-



Scheme 4 Signalling pathway likely to be involved in the regulation of eIF2B by insulin

This Figure is based on recent work concerning the regulation of PKB, GSK-3 and eIF2B. Binding of insulin to its receptor activates the intrinsic tyrosine kinase activity of the receptor, leading to binding and subsequent phosphorylation of IRS1/2. The resulting phosphotyrosine residues include docking sites for the SH2 domains of PI 3-kinase, leading to activation of this enzyme and generation of PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2 [generated by dephosphorylation of PtdIns(3,4,5)P_3] may activate PKB through the protein's pleckstrin homology (PH) domain [24,27,147]. Activation of PKB also involves its phosphorylation at Thr^{308} (T308), in the kinase domain, and Ser^{473}, in the C-terminal tail [27,28]. Phosphorylation of Thr^{308} can be catalysed by an upstream phospholipid-dependent kinase (PDK1), which is activated by PtdIns(3,4,5)P_3 [27,29]. It therefore appears that PtdIns(3,4,5)P_3 plays a dual role in the activation of PKB, via binding at its pH domain and by activating an upstream kinase. The identity of the kinase phosphorylating Ser^{473} (S473) is unknown. Since inhibition of PI 3-kinase also blocks phosphorylation of Ser^{473} [28], it may also be a phospholipid-dependent kinase (hence PDK2). PKB can phosphorylate and inactivate GSK-3 [20]. GSK-3 phosphorylates eIF2B at Ser^{540} in its ϵ-subunit, resulting in inhibition of its exchange activity [76]. Thus inactivation of GSK-3 leads to dephosphorylation of this residue and activation of eIF2B, leading to enhanced nucleotide exchange on eIF2 and increased availability of its active GTP-bound form (eIF2·GTP). This in turn contributes to the overall activation of peptide-chain initiation by insulin.

itiation and is believed to play a key role in the regulation of translation in response to viral infection, amino acid deprivation and other 'stress' conditions [59]. Both eIF2 and eIF2B are multimeric proteins, having respectively three and five non-identical subunits [59]. The functions of their individual subunits have yet to be defined, but it seems likely that the γ-subunit of eIF2 mediates the binding of GTP [60,61], whereas the ϵ-subunit of eIF2B plays a regulatory role through its phosphorylation [62] (see below).

The activity of eIF2B is decreased in muscle of animals rendered experimentally diabetic [63–65], and the factor is acutely activated by insulin in a variety of cells and tissues, including skeletal muscle, which is quantitatively the most important target tissue for insulin's action on protein synthesis [64–67]. Insulin also rapidly activates eIF2B in Swiss 3T3 cells [67].

It has been known for many years that one way of modulating the activity of eIF2B is through the phosphorylation of the α-subunit of eIF2, which can be catalysed by at least two distinct protein kinases in mammalian cells (the haem-controlled repressor and the double-stranded RNA-activated inhibitor [62,68,69]) and which leads to inhibition of the exchange activity of eIF2B (eIF2αP is a strong competitive inhibitor of eIF2B [70]). However, no change in the level of phosphorylation of eIF2α is seen either in diabetes or in cells treated with insulin, suggesting that alternative mechanisms exist for the regulation of eIF2B activity in response to insulin, which seemed likely to involve direct regulation of its activity.

These observations led Welsh and Proud [71] to seek insulin-sensitive protein kinase(s) which might phosphorylate eIF2B, and they identified a kinase which phosphorylates the largest (ϵ)

subunit of eIF2B and was inactivated in response to insulin (and also serum or phorbol esters). They showed that this kinase was glycogen synthase kinase-3 (GSK-3, here specifically the β-isoform [71]), previously identified as an insulin-sensitive ATP-citrate-lyase kinase [72], and that its inactivation appeared to involve phosphorylation of GSK-3, since it was reversed by treatment with protein phosphatase-2A. They and other workers showed that GSK-3 was also inactivated by insulin in cultured human muscle myoblasts [73] and rat fat-cells [40,74] and in response to other agents (see [75]), and that this effect was blocked by pretreatment of cells with wortmannin, an inhibitor of PI 3-kinase [20,39–41,74].

The site in eIF2Bϵ which is phosphorylated by GSK-3 was identified as Ser^{540}, which is conserved in all mammalian species for which sequence data are available [76]. The adjacent sequence contains a second conserved Ser at position +4 relative to the site of phosphorylation by GSK-3 [75], which may act as a priming phosphorylation site for the action of GSK-3 at Ser^{540} (phosphoserine at +4 acts in this capacity in several other substrates of GSK-3 [75,77]). Phosphorylation of eIF2B by GSK-3 results in the inhibition of its GDP/GTP exchange activity [76,78]. Interestingly, the phosphorylation of glycogen synthase by GSK-3 results in inhibition of that enzyme, which is the major regulatory enzyme of glycogen synthesis in mammals. This suggests that the regulation of GSK-3 may provide a way of co-ordinately regulating the activities of regulatory proteins in two major insulin-activated biosynthetic pathways, glycogen synthesis and protein synthesis. GSK-3 can also phosphorylate certain transcription factors at regulatory sites (c-Jun [79], cAMP-response element-binding protein, CREB [80]), suggesting that GSK-3 may also modulate specific gene expression. In order to examine the cellular level of phosphorylation of Ser^{540} in eIF2Bϵ under differing conditions, Welsh et al. raised an antiserum to a peptide corresponding to the region around Ser^{540} and containing a phosphoserine residue at this position. They were then able to demonstrate that insulin rapidly brought about decreased phosphorylation of Ser^{540}, consistent with the model shown in Scheme 4, in which insulin causes inactivation of GSK-3 and hence dephosphorylation of this site in eIF2B, leading to activation of eIF2B and of translation initiation. The insulin-induced phosphorylation of eIF2B was blocked by two inhibitors of PI 3-kinase, wortmannin and LY294002 (G. I. Welsh, C. M. Miller, A. J. Loughlin, N. T. Price, E. J. Foulstone and C. G. Proud, unpublished work).

It is now important to address the upstream control of GSK-3. Cohen and co-workers [20,81,82] found that GSK-3 was a substrate *in vitro* for three insulin-stimulated protein kinases, p70 S6 kinase, $p90^{rsk}$ and PKB (see Scheme 1). All three phosphorylate the same residue in GSK-3, a serine residue at position 9 in the β-isoform of GSK-3 (equivalent to Ser^{21} in GSK-3α) [20,80,81]. Current evidence indicates that PKB may be the enzyme responsible for the inactivation of GSK-3 by insulin; for example, neither rapamycin (which blocks activation of p70 S6 kinase) nor PD098059 (which blocks activation of MAP kinase and hence $p90^{rsk}$) [20,38] affects the ability of insulin to inactivate GSK-3, but this is completely blocked, as noted above, by inhibitors of PI 3-kinase, which is thought to lie upstream of PKB [23].

This suggests a key role for PI 3-kinase in the regulation of both GSK-3 and eIF2B. This is consistent with the data of Mendez et al. [83], who showed that activation of protein synthesis in 32D cells expressing both the insulin receptor and IRS1 was completely dependent upon PI 3-kinase, and that PI 3-kinase-linked signalling pathways were sufficient to mediate this activation. Since rapamycin only partially inhibited the insulin-induced increase in protein synthesis, the FRAP pathway (which includes p70 S6 kinase) appears to play only a minor role in this. Thus another PI 3-kinase-linked signalling pathway must be responsible for this, and this would be entirely consistent with the operation of the pathway depicted in Scheme 4. Welsh et al. [84] have recently shown that PI 3-kinase is required for the activation of eIF2B, using both specific inhibitors of this enzyme and dominant negative mutants of the p85 subunit of PI 3-kinase. In contrast, neither MAP kinase nor the FRAP pathway seem to be required for the activation of eIF2B. These data again support the model depicted in Scheme 4.

eIF2B is also activated under a number of other conditions where translation is stimulated, e.g., in response to mitogenic activation of T-lymphocytes [85] or following exposure of pancreatic islets to elevated glucose concentrations [86]. In the former case, inactivation of GSK-3 is also observed, and this correlates with the activation of eIF2B, but in the latter case no effect on GSK-3 activity was observed, suggesting that another mechanism acts to modulate eIF2B activity in response to glucose.

In yeast, control of eIF2B activity is linked to the regulation of the translation of a specific mRNA, namely that encoding the transcription factor GCN2 [87], which possesses multiple upstream open reading frames (uORFs) in its 5′-untranslated region (5′-UTR). These uORFs are required for the control of the translation of this mRNA as shown in the detailed studies of Hinnebusch [87]. eIF2B might therefore play a similar role in the control of the translation of specific mRNAs in mammalian cells, and a number of mammalian mRNAs are known to possess uORFs which regulate their translation [88]. However, no role for eIF2B has yet been established in their regulation.

Regulation of the eIF4F complex

Overview

eIF4F is a protein complex composed of eIF4E, the cap-binding protein, eIF4G, a large polypeptide with binding sites for a number of other proteins, including eIF4E, and the third component of eIF4F, the ATP-dependent RNA-helicase eIF4A. Insulin brings about the increased association of eIF4E with eIF4G [89]. Various effects may underlie this, including the regulation of the availability of eIF4E by the phosphorylatable eIF4E-binding proteins (4E-BPs, also known as PHAS proteins) [90–93] or the phosphorylation of eIF4E [44], which are increased in response to insulin, or the phosphorylation of eIF4G, which is increased by other conditions which activate translation (reviewed in [94]).

eIF4G is a large multidomain protein which serves as a scaffold upon which initiation-factor complexes are assembled [95]. It contains interaction sites for eIF4E, eIF4A and eIF3. It serves to bring together the cap-binding factor, eIF4E and the helicase, eIF4A, which acts to unwind regions of secondary structure in the 5′-UTR of the mRNA; such regions of secondary structure reduce the translational efficiency of the mRNA, probably by interfering with the movement of the 40 S subunit and its associated factors during scanning. Hence formation of the eIF4F complex is likely to facilitate the translation of mRNAs with 5′-UTRs rich in secondary structure [57], it may well be important in the insulin-increased translation of mRNAs which possess this feature. The best-characterized example of this is ornithine decarboxylase [6], which possesses a highly structured 5′-UTR with a number of potential regulatory features. The interaction of eIF4G (and hence eIF4F) with eIF3 is thought to be important for the association of eIF4F with the 40 S ribosomal subunit, since eIF3 itself interacts with this subunit (and prevents

its reassociation with the 60 S subunit until the appropriate point in the initiation process).

Phosphorylation of 4E-BP1

In 1980, Belsham and colleagues [96] reported the marked insulin-stimulated phosphorylation of a protein in rat fat-cells which had an apparent molecular mass of 22 kDa as judged by SDS/PAGE. The protein had the noteworthy properties of remaining in solution after boiling and after treatment with up to 2% trichloroacetic acid. The phosphorylation observed with insulin was rapid, reversible, occurred on both serine and threonine residues and resulted in changes in electrophoretic migration of the protein [92,97,98]. The role of this protein only became evident after partial amino acid sequencing of the rat protein led to its cDNA being cloned and sequenced by Lawrence and co-workers [99]. The protein had, in fact, a molecular mass of only 12500 Da and was named by the Lawrence group PHAS-I (Phosphorylated-Heat-and-Acid-Stable protein) [99,100]. Sonenberg and colleagues then realized that the sequence of the cDNA for PHAS-I was virtually identical with a cDNA they had cloned by screening a human placental expression library with labelled eIF4E as a probe, and called by them 4E-BP1. This approach also identified the cDNA of another related protein (4E-BP2) which also bound eIF4E [90]. PHAS-I is the rat homologue of human 4E-BP1 and in this Review Article we will refer to these proteins as 4E-BP1 and 4E-BP2.

Subsequent studies showed that binding of 4E-BP1 to eIF4E inhibited cap-dependent translation in HeLa-cell lysates and in cultured cells [90]. This inhibition is not caused by any appreciable diminution in the ability of eIF4E to bind to capped mRNA, but rather the binding of 4E-BP1 to eIF4E blocks the association of eIF4E with eIF4G and hence the formation of the eIF4F complex [101,102]. The binding of eIF4G and 4E-BP1 to eIF4E is mutually exclusive [101,103], and both proteins contain a similar 12-amino-acid sequence which is involved in the binding of these proteins to eIF4E [102].

The phosphorylation of 4E-BP1 which occurs on treatment of rat fat- and other cells with insulin results in the dissociation of 4E-BP1 from eIF4E, presumably allowing the formation of competent eIF4F complexes [90,91,93,98,104,105]. For example, in fat-cells, insulin greatly decreases both the amount of eIF4E which can be precipitated with anti-4E-BP1 antibodies as well as the amount of 4E-BP1 that remains bound to eIF4E separated from extracts by affinity chromatography on m7GTP-resins [90,91,93]. As described above, eIF4E can also become more phosphorylated in cells treated with insulin, but presently available evidence suggests that this may not affect its binding to 4E-BP1, since phosphorylated eIF4E from insulin-treated cells is able to bind exogenous non-phosphorylated 4E-BP1 [90]. Certainly, the phosphorylation of 4E-BP1 in insulin-treated cells is sufficient to decrease greatly its ability to bind exogenous eIF4E [90].

The binding of 4E-BP1 to eIF4E has a stoichiometry of 1:1 ([104]; M. Avison and R. M. Denton, unpublished work). In rat fat-cells there is a considerable excess of 4E-BP1 over the amount of eIF4E. In the absence of insulin, most of the 4E-BP1 in these cells is not phosphorylated, as judged by the incorporation of $[^{32}P]P_i$, and virtually all the eIF4E appears to be bound to 4E-BP1 leaving, however, a substantial amount of non-phosphorylated 4E-BP1 [93]. In the presence of high concentrations of insulin, there is a substantial increase in the phosphorylation of 4E-BP1 and near complete dissociation from eIF4E [93].

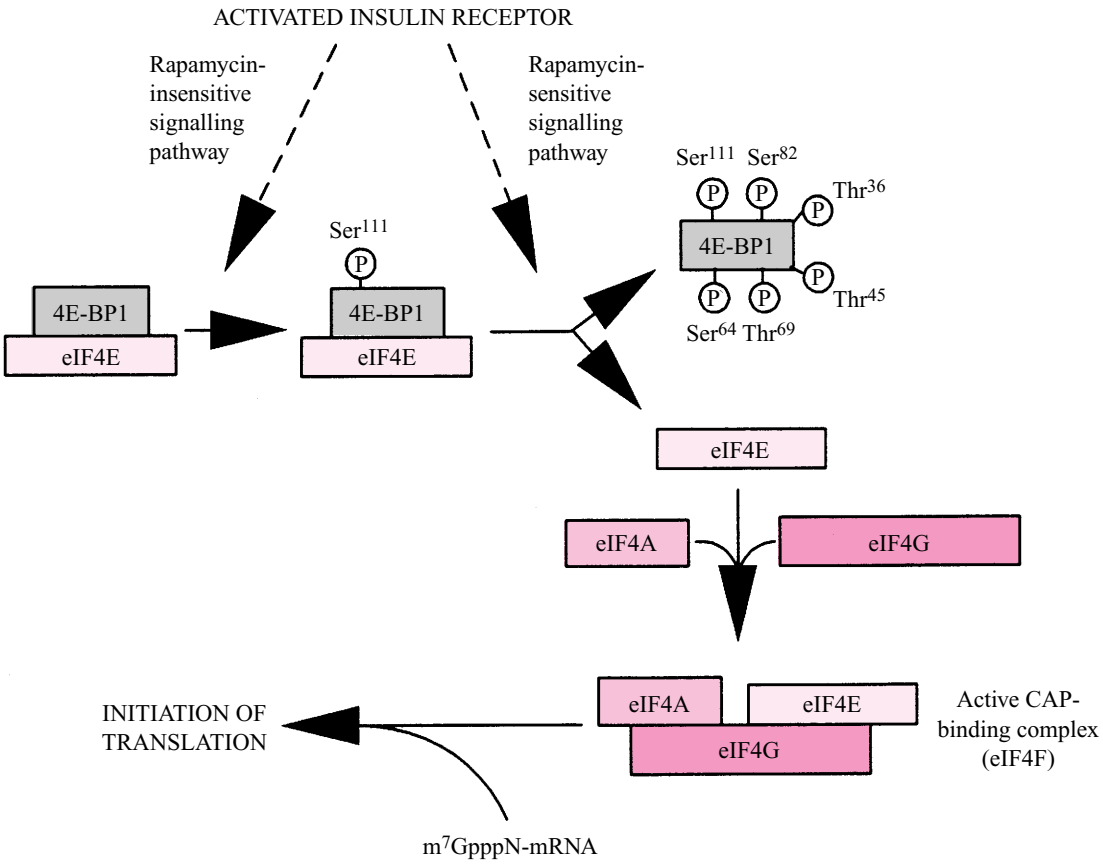
The first hints of the complexity of the phosphorylation of 4E-BP1 in insulin-treated fat-cells came from the multiplicity of forms observed in one- and two-dimensional electrophoresis [98,107] and the fact that at least two major tryptic phosphopeptides containing phosphoserine and phosphothreonine could be separated by two-dimensional TLC analysis [107]. Recent studies by Fadden et al. [108], have indicated that at least five sites may be phosphorylated in insulin-treated fat-cells. These are Thr^{36}, Thr^{45}, Ser^{64}, Thr^{69} and, possibly, Ser^{82}, which all fit a Ser/Thr-Pro motif and are situated on either side of the likely eIF4E-binding site which lies between Arg^{50} and Met^{59}. Fadden et al. [108] suggested that the phosphorylation of Thr^{45} and Thr^{69} may be most important in actually causing release, rather than phosphorylation of Ser^{64}, as had been concluded in an earlier study by this group [109].

In one-dimensional SDS/PAGE, three bands corresponding to 4E-BP1 can be resolved, and these bands have been designated α, β and γ in order of decreasing electrophoretic mobility. The increase in phosphorylation of 4E-BP1 caused by insulin is associated with a shift from the α to the β and γ bands. The 4E-BP1 bound to eIF4E migrates mainly as the α band with some as the β band, whereas the (phosphorylated) forms of 4E-BP1, which do not bind to eIF4E, migrate as the β and γ bands. Each of the bands probably represent more than one form of 4E-BP1, as eight or more species can be separated by two-dimensional electrophoresis [104,107].

The kinases involved in the phosphorylation of 4E-BP1 in insulin-treated cells have not been definitively identified. Two insulin-activated kinases have been shown to phosphorylate purified BP-1 *in vitro*; these are MAP kinase and casein kinase 2 [104,109,110].

4E-BP1 is an excellent substrate for MAP kinase, and Haystead and colleagues [109] concluded that the kinase may be important in the phosphorylation of the protein in intact cells. In that study, only the phosphorylation of Ser^{64} was identified. Later studies have shown that the kinase is able to phosphorylate all the five proline-directed sites which are phosphorylated in insulin-treated fat-cells [108]. However, it is now clear that MAP kinase may not be important in the effects of insulin on the phosphorylation of 4E-BP1 in intact cells for the following reasons: (i) 4E-BP1 is a very poor substrate for MAP kinase when bound to eIF4E [93,104]; (ii) there is a poor correlation between the extent and time course of 4E-BP1 phosphorylation and the activation of MAP kinase in rat fat- and other cells [93,105,106,111]; (iii) in 3T3-L1 adipocytes, Chinese-hamster ovary (CHO) cells and rat skeletal muscle, the MEK inhibitor PD098059 has little or no effect on 4E-BP1 phosphorylation in the presence of relatively high concentrations of insulin, while blocking the activation of MAP kinase [44,104,106] (although in the absence of insulin [44] or at low concentrations of the hormone [112], some dephosphorylation has been described); (iv) rapamycin inhibits the increase in the β and γ forms of 4E-BP1, and hence its dissociation from eIF4E, in the presence of insulin without blocking the activation of MAP kinase [93,104,105,113].

The effects of rapamycin are of importance, since rapamycin has been shown to inhibit cap-dependent but not cap-independent translation [113]. Rapamycin blocks the phosphorylation of the major proline-directed sites ([108]; M. Avison, K. J. Heesom and R. M. Denton, unpublished work). According to Fadden et al. [108] the effects of rapamycin may be more marked on the phosphorylation of Thr^{45} and Thr^{69} than on Thr^{36} and Ser^{64}, but in other studies using a combination of two-dimensional TLC and HPLC to separate phosphopeptides, this difference in effects of rapamycin on the major proline-directed sites was not evident (M. Avison, K. J. Heesom and R. M. Denton, unpublished work). Certainly, the effects of rapamycin suggest that a protein



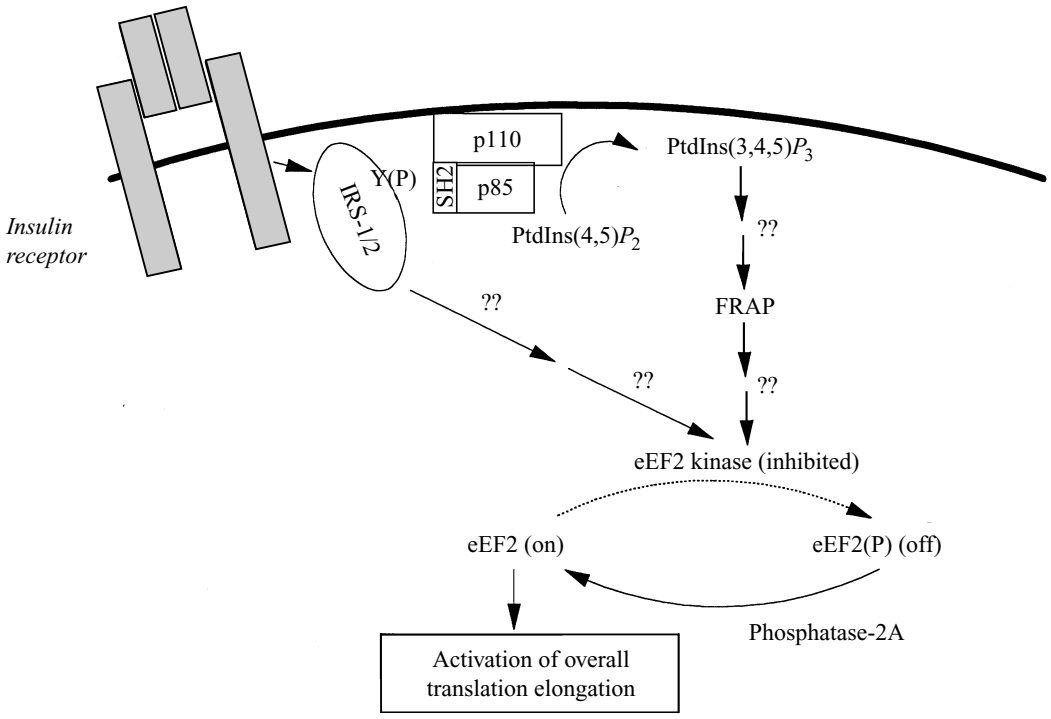
Scheme 5 Regulation of eIF4E, 4E-BP1 and eIF4F assembly

The Figure summarizes our present view of the role of 4E-BP1 (grey) in the regulation of eIF4F complex by insulin ([93,108], K. J. Heesom, M. Avison and R. M. Denton, unpublished work). The first event may be the phosphorylation of 4E-BP1 bound to eIF4E (light-pink) on Ser^{111} by a kinase which is activated by a rapamycin-insensitive pathway. This does not result in the dissociation of 4E-BP1 from eIF4E but may facilitate its phosphorylation on a series of five 'proline-directed' sites (Ser^{82}, Thr^{69}, Ser^{64}, Thr^{45}, Thr^{36}) by a kinase or kinases whose activation is blocked by rapamycin. This latter series of phosphorylation events results in the dissociation of 4E-BP1 from eIF4E which can then bind to eIF4G (dark pink) and hence also eIF4A (mid-pink) to form the eIF4F complex, which is able to facilitate the scanning of the initiation complex along the 5′-UTR. Abbreviation: m^7GpppN-mRNA, capped mRNA.

kinase component of the FRAP pathway (which includes p70 S6 kinase) is likely to be involved in the phosphorylation of the proline-directed sites which causes dissociation of 4E-BP1 and allows the formation of the eIF4 complex (Scheme 5). However, it should be emphasized that p70 S6 kinase itself does not phosphorylate either the free or eIF4E-bound form of 4E-BP1 *in vitro* and thus is probably not involved in intact cells [93]. Recently Lawrence et al. have obtained evidence that FRAP immunoprecipitates contain kinase activity which is able to phosphorylate a number of the proline-directed phosphorylation sites in unbound 4E-BP1 which are phosphorylated in response to insulin [114]. It is not yet clear whether this kinase activity is able to phosphorylate 4E-BP1 bound to eIF4E, as must occur in intact cells. Other recent data indicate that the rapamycin-sensitive signalling events immediately upstream of 4E-BP1 and p70 S6 kinase are distinct, but involve a common proximal activator [115].

By monitoring the incorporation of $[^{32}P]P_i$ into 4E-BP1 in rat fat-cells, it became evident that there is also a rapamycin-insensitive pathway involved in the phosphorylation of 4E-BP1 in response to insulin [93]. 4E-BP1 bound to eIF4E in cells incubated with insulin plus rapamycin was found to be phosphorylated, but this phosphorylation did not result in any change in the mobility of 4E-BP1 on SDS/PAGE; 4E-BP1 still migrated in the α-band despite being phosphorylated. Importantly, this event appears to be restricted to 4E-BP1 bound to eIF4E, and thus the possibility arises that this phosphorylation may be the first event which allows the phosphorylation of the proline-directed sites and hence dissociation to occur. Recent studies have indicated that rapamycin-insensitive phosphorylation occurs on Ser^{111} (M. Avison, K. J. Heesom and R. M. Denton, unpublished work). This is the site phosphorylated by casein kinase 2 *in vitro* ([100]; M. Avison, K. J. Heesom and R. M. Denton, unpublished work) and the eIF4E-bound form of 4E-BP1 is a better substrate than free 4E-BP1 for this kinase [92]. Casein kinase 2 has been shown to be activated to a modest extent in fat-cells by insulin [110]. However, another kinase which is activated more than 10-fold by insulin and which appears to be highly specific for this site has been identified recently in fat-cells. Because the activity of this kinase towards 4E-BP1 complexed to eIF4E is much greater than that of casein kinase 2, it seems likely that this kinase is more important than casein kinase 2 in the phosphorylation of this site (K. J. Heesom, M. Avison and R. M. Denton, unpublished work).

The effects of insulin on 4E-BP2 have not been explored as extensively as those on 4E-BP1. In rat fat-cells, the amount of 4E-BP2 is much less than that of 4E-BP1, but both binding proteins are present in 3T3-L1 adipocytes and in these cells



Scheme 6 Regulation of elongation by insulin

Recent data [145] suggest that the insulin-induced dephosphorylation of eEF2 is a consequence of inactivation of the cognate kinase rather than activation of the phosphatase acting on eEF2 (protein phosphatase-2A [143,144]), and that, at least in the cell type studied, this involves a signalling pathway which is sensitive to the FRAP inhibitor rapamycin [145]. Since phosphorylation of eEF2 inhibits its activity, dephosphorylation will result in activation of eEF2 and hence of overall peptide-chain elongation.

insulin has been shown to cause an increase in the phosphorylation of 4E-BP2 as well as its dissociation from eIF4E [116]. It should be noted that 4E-BP2 is not a substrate for casein kinase 2 and does not contain Ser^{111}, so the priming mechanism suggested for the phosphorylation of 4E-BP1 is not relevant to 4E-BP2.

Phosphorylation of eIF4E

Insulin and other agents which activate translation bring about increased phosphorylation of eIF4E at a single site now known to be Ser^{209} [94,117–119]. Phosphorylation of eIF4E is reported to increase its affinity for the 7-methyl-GTP cap and for mRNA [120] and may also enhance its ability to form high-molecular-mass complexes, e.g. with eIF4G and eIF4A [121,122]. Flynn and Proud [44] have shown, using the specific MEK inhibitor PD098059, that the MAP kinase pathway is required for the insulin-induced increase in the steady-state level of phosphorylation of eIF4E in CHO cells overexpressing the insulin receptor. In contrast, Mendez et al. [83] reported that insulin's ability to enhance the ^{32}P-radiolabelling of eIF4E in 32D myeloid precursor cells was independent of MAP kinase. Furthermore, Kimball et al. [89] have reported that insulin actually decreases the phosphorylation of eIF4E in gastrocnemius muscle. Despite substantial work to try to resolve this, the role of phosphorylation of eIF4E in the control of translation remains obscure, and was further muddied by the mis-identification of the site of phosphorylation as Ser^{53} rather than Ser^{209}, as subsequently shown by Rhoads and colleagues [118] and by Flynn and Proud [119]. The observation that blocking MAP kinase activation (using PD098059) inhibits the activation of protein synthesis by angiotensin-II in smooth-muscle cells [123] may indicate a role for eIF4E phosphorylation, since eIF4E is so far the only activation factor whose regulation has been linked to the MAP kinase pathway, but no data are yet available on the effect of this compound on the activation of translation by insulin.

In fat-cells, insulin appears to result in a decrease in the amount of eIF4E that is available to interact with the 7-methyl-GTP-affinity resin [93]. This may be due either to increased binding of eIF4E to mRNA (in which state it would not be available to bind to this cap analogue) or to its increased incorporation into initiation complexes which, due to their large size, are inefficiently extracted into supernatant fractions upon lysis of the cells (or a combination of the two).

Regulation of the translation of polypyrimidine-tract-containing mRNAs

Certain mRNAs contain, at their extreme 5′-ends, a sequence of pyrimidine bases which plays a key role in the regulation of their translation [124,125]. These so-called 5′-TOP mRNAs, which encode proteins involved in the process of translation, such as ribosomal proteins and elongation factors, undergo translational up-regulation in response to stimulation of cells. Such mRNAs are largely found in non-polysomal material in serum-starved cells, but shift into large polyribosomes following stimulation of cells (reviewed in [125]). Translation of such mRNAs has been shown to be stimulated in response to a number of stimuli, but little work has so far been carried out on their regulation by insulin. This hormone has, however, been shown to increase the synthesis of elongation factor-2 (eEF2, [126]), whose mRNA belongs to this class, and a number of other unidentified proteins.

The enhanced translation of the S6, eEF1α and eEF2 mRNAs by serum is blocked by rapamycin, pointing to a role for the FRAP pathway [127,128]. The acute translation up-regulation of components of the translational machinery may serve rapidly to increase the cell's overall capacity for protein synthesis. Agents such as insulin thus exert, overall, a dual effect on the translational machinery, first to increase its intrinsic activity (by activating initiation and elongation factors, as described in this Review Article) and, secondly, by ensuring increased synthesis of ribosomes and other components, to enhance the overall maximal activity of translation in the cell.

Addition of a 5′-terminal polypyrimidine tract to a reporter mRNA suffices to confer this pattern of regulation [125]. Such sequences are thus dominant *cis*-acting inhibitors of the translation of mRNAs in serum-depleted cells, and this inhibition is overcome by rapamycin-sensitive events following cell stimulation. Removal of the TOP sequence from an mRNA which normally contains one results in it becoming constitutively translated and present in polysomes even in non-stimulated (quiescent cells) [125].

To date, the mechanisms underlying both the translation repression and its rapamycin-sensitive relief remain obscure. Initial studies suggested a role for eIF4E in this [124,129–131], and the fact that the regulators of eIF4E (4E-BPs) are controlled in a rapamycin-sensitive manner would be consistent with this [44,93,104,105,113]. It has also been suggested that the phosphorylation of ribosomal protein S6 may be involved, since it appears to lie in the ribosome's mRNA-binding site and is phosphorylated in a rapamycin-sensitive manner [132], although direct evidence for a role is lacking. The group of Thomas [133] have used both dominant interfering and rapamycin-insensitive mutants of p70 S6 kinase to explore the role of this enzyme in the control of 5′-TOP mRNA translation. Expression of the interfering mutant, which prevents activation of wild-type p70 S6 kinase, also blocked the activation of 5′-TOP mRNA translation by serum, while the expression of the rapamycin-insensitive T389E mutant of p70 S6 kinase rendered 5′-TOP mRNA translation insensitive to rapamycin. These data are consistent with a role for p70 S6 kinase, and hence potentially of S6 phosphorylation, in regulating 5′-TOP mRNA translation.

It has also recently been reported that another protein of the 40 S subunit, S17, is subject to rapamycin-sensitive phosphorylation, and it is also clearly a candidate for playing a role in the regulation of 5′-TOP mRNAs [134].

Regulation of elongation by insulin

Overview of peptide-chain elongation

In mammalian cells, peptide-chain elongation requires two elongation factors, eEF1 and eEF2. eEF1 mediates the attachment of the amino acyl-tRNAs to the ribosome during peptide-chain elongation, in the form of a complex with GTP (this resembles the role of eIF2, which forms eIF2·GTP·Met-tRNA$_i$ complexes). eEF1 consists of several subunits, α being the subunit which binds GTP and amino acyl-tRNA, while the β, γ and δ subunits function as a guanine nucleotide-exchange factor complex, in an analogous fashion to eIF2B [53]. eEF2, a monomeric protein which also binds GTP, is required for the translocation step of elongation during which the ribosome moves relative to the mRNA and the peptidyl-tRNA migrates from the A- to the P-site of the ribosome [53]. Both eEF1 and eEF2 are phosphoproteins, although the role of phosphorylation of eEF2 is considerably less well understood than that of eEF1. The phosphorylation of eEF2 is regulated by insulin, as described below.

Phosphorylation of eEF2

eEF2 is phosphorylated at threonine residues within its GTP-binding domain by a calcium/calmodulin (Ca/CaM)-dependent protein kinase (formerly called Ca/CaM kinase III, now known as eEF2 kinase) [135–138]. Phosphorylation results in complete inactivation of eEF2 [139], apparently by inhibiting its ability to bind to the ribosome, an interaction that also involves regions within the GTP-binding domain [140]. A cDNA encoding eEF2 kinase has now been cloned and sequenced; the protein appears to be a rather unusual protein kinase, only distantly related to other Ca/CaM kinases [141,142]. The protein phosphatase (PP) responsible for the dephosphorylation of eEF2 appears to be PP-2A [143,144].

In serum-deprived CHO cells, the level of phosphorylation of eEF2 is about 40–50 %, indicating that roughly half the factor is inactive [145]. Treatment of wild-type cells with serum or of cells expressing the insulin-receptor with insulin, results in a very rapid dephosphorylation of eEF2 such that its level of phosphorylation falls to undetectable values within 5 min [145]. Insulin or serum rapidly bring about the inactivation of eEF2 kinase, and this effect, rather than an increase in the activity of PP-2A, appears to be responsible for the dephosphorylation of eEF2. Similar effects of insulin on both eEF2 phosphorylation and eEF2 kinase activity are also observed in fat-cells (N. T. Redpath, T. A. Diggle, K. J. Heasom and R. M. Denton, unpublished work).

Both the dephosphorylation of eEF2 and the inactivation of eEF2 kinase are partially blocked by rapamycin, pointing to a role for the FRAP pathway in the regulation of this process [145], at least in CHO cells (Scheme 6). As expected, wortmannin also blocks these effects. The key question is the relationship of these events to the control of peptide-chain elongation. Rates of elongation are not easy to determine, the standard measure being the 'transit time', an indication of the average time taken for a ribosome to complete a polypeptide chain. In CHO cells, insulin reduces transit times by about one-half, indicative of a doubling of the rate of elongation. This effect is eliminated both by rapamycin and by wortmannin [145]. These data suggest that insulin accelerates the elongation process through a mechanism involving the inactivation of eEF2 kinase (through the FRAP pathway), leading to the dephosphorylation and consequent activation of eEF2.

The mechanism by which insulin (or serum) decreases the activity of eEF2 kinase is unclear. It might involve phosphorylation of the eEF2 kinase protein, although this remains to be demonstrated. Although eEF2 kinase can be phosphorylated by p70 S6 kinase *in vitro*, this does not result in its inactivation [145]. Thus other inputs must exist to control eEF2 kinase activity in response to insulin.

It is not currently clear to what extent the rate of elongation limits the overall rate of protein synthesis, since too few data on the control of elongation, especially in tissues which are physiological targets for insulin, are available. However, it clearly makes sense for agents which accelerate the rate at which ribosomes bind to mRNA (i.e., initiation) also to speed up their progress along mRNAs once bound (i.e., elongation), in order to achieve a co-ordinated activation of protein synthesis.

FUTURE DIRECTIONS AND CONCLUSION

A major question that the reader may well be asking himself or herself having struggled through the above review is: why is it all so complicated? There do now seem to be a lot of regulatory or at least potential regulatory events in eukaryotic translation, and they are modulated by a variety of different signalling

Table 1 Effects of insulin on components involved in translation

Component	Phosphorylation change	Effect	Signalling Pathways
eIF2B	↓ in Ser^{540}	↑ In eIF2-GTP	PK-B/GSK3
4E-BP1	↑ in $Thr^{36/45/69}$ and $Ser^{64/111}$	↑ In eIF4F complex	FRAP plus rapamycin-insensitive
eIF4E	↑ in Ser^{209}	↑ In cap affinity	MAP kinase
S6	↑ in $Ser^{235/236/240/244/247}$	?↑ Translation of 5′-TOP mRNAs	FRAP/p70 S6K
eEF2	↓ in $Thr^{56/58}$	↑ In elongation	FRAP? via eEF2 kinase

pathways (Table 1). One important reason for the multiplicity of control points is that they are not all controlling the same thing(s): eIF2B probably mainly acts to regulate the overall initiation rate in the cell by modulating the supply of active eIF2·GTP, while the regulation of proteins involved in mRNA binding and the unwinding of secondary structure, such as eIF4E, 4E-BP1 and eIF4F, are likely to be important for the regulation of the translation of specific mRNAs. Different regulatory mechanisms, involving distinct signalling pathways and events, serve to modulate the translation of different subsets of mRNAs possessing different regulatory features.

However, as yet only a few mRNAs have conclusively been shown to be regulated at the level of their translation as opposed to stability or nucleocytoplasmic transport, and these are important caveats when it comes, for example, to interpreting the effects of overexpression experiments; Rousseau et al. [146] offer a cautionary tale, since they found that the enhancement of cyclin D1 expression in cells overexpressing eIF4E was related to transport of the mRNA out of the nucleus rather than increased translation of its mRNA *per se*. Studies on the regulation of specific mRNAs must therefore take account not only of changes in mRNA levels (which may be altered due to indirect effects, e.g., on the transcriptional machinery), but also on the behaviour of the mRNA. The most informative approach is to study the distribution of the mRNA of interest between non-polyribosomal, and therefore translationally inactive material, and mRNA associated with ribosomes (active polyribosomal mRNA), including the distribution of the mRNA between polyribosomes of differing sizes. Translational activation of an mRNA should be manifested either as a shift, upon stimulation of the cells, from non-polyribosomal into polyribosomal fractions (which is what is seen for 5′-TOP mRNAs) or from smaller into larger polyribosomes, depending on the type of control being exerted. The identification of mRNAs subject to specific translational control by insulin (and indeed by other agents) is an urgent priority and an important challenge for the future.

Another such challenge is the unravelling of the roles played by particular regulatory steps or events in the overall control of translation. The importance of the regulation of individual factors may be expected to differ between cell types and perhaps between stimuli, as well as, certainly, between mRNA species. A surprising feature of the currently available data is that, despite the fact that rapamycin blocks, at least in part, three of the main regulatory mechanisms so far identified as controlling translation in animal cells (the phosphorylation of S6 and of the 4E-BPs, and the activation of eEF2), this drug generally only has modest effects on the rate of protein synthesis, even in stimulated cells [83,113]. In the short term (2 h), rapamycin inhibited serum-stimulated protein synthesis by 25%. The degree of inhibition increased with time after rapamycin addition and approached 50% after 20 h, this may well be due to the cumulative effect of inhibition of ribosomal protein and translation-factor synthesis reducing the cells' overall capacity for translation. The limited effect of rapamycin may well reflect the fact that both S6 (or p70 S6 kinase) and the 4E-BPs probably function primarily to regulate the translation of specific mRNAs rather than the overall rate of translation. It implies that regulating the rate of elongation may not be a major factor in determining the overall rate of translation *in vivo*, although caution must be exerted in extrapolating from data obtained from studies on cell lines to 'real' tissues such as skeletal muscle and fat (and, in any case, rapamycin only partially blocks the effects of insulin or eEF2 kinase).

Investigation of the roles played by individual factors in the overall or transcript-specific control of translation will be facilitated by the fact that we now know the locations of the phosphorylation sites in many of these proteins. However, it is only in a few cases that we know the identity of the protein kinases responsible for their phosphorylation *in vivo*, and the identification of these enzymes (e.g., the kinases acting on the 4E-BPs, eIF4G and eIF4B, and the relevant upstream kinases for p70 S6 kinase and eEF2 kinase) represents another major goal for the future.

REFERENCES

1 DeFronzo, R. A. and Ferrannini, E. (1992) in International Textbook of Diabetes Mellitus, (Alberti, K. G. M. M., Zimmet, P. and DeFronzo, R. A., eds.), pp. 549–593, John Wiley and Sons, Chichester
2 O'Brien, R. M. and Granner, D. K. (1996) Physiol. Rev. **76**, 1109–1161
3 Hammond, M. L. and Bowman, L. H. (1988) J. Biol. Chem. **263**, 17785–17791
4 Rosenwald, I. B., Lazaris-Karatzas, A., Sonenberg, N. and Schmidt, E. V. (1993) Mol. Cell. Biol. **13**, 7358–7363
5 Wilson, S. B., Back, D. W., Morris, S. M. and Swierczynski, J. (1986) J. Biol. Chem. **261**, 15179–15182
6 Manzella, J. M., Rychlik, W., Rhoads, R. E., Hershey, J. W. and Blackshear, P. J. (1991) J. Biol. Chem. **266**, 2383–2389
7 White, M. F. and Kahn, C. R. (1994) J. Biol. Chem. **269**, 1–4
8 Tavare, J. M. and Siddle, K. (1993) Biochim. Biophys. Acta **1178**, 21–39
9 White, M. F. (1996) in Diabetes Annual 9, (Marshall, S. M., Home, P. D. and Rizza, R. A., eds.), pp. 77–89, Elsevier Science, Amsterdam
10 Sun, X. J., Wang, L. M., Zhang, Y. T., Yenush, L., Myers, M. G., Glasheen, E., Lane, W. S., Pierce, J. H. and White, M. F. (1995) Nature (London) **377**, 173–177
11 Pronk, G. J., Devriessmits, A. M. M., Buday, L., Downward, J., Maassen, J. A., Medema, R. H. and Bos, J. L. (1994) Mol. Cell. Biol. **14**, 1575–1581
12 Bonfini, L., Migliaccio, E., Pelicci, G., Lanfrancone, L. and Pelicci, P. (1996) Trends Biochem. Sci. **21**, 257–261
13 Hosomi, Y., Shii, K., Ogawa, W., Matsuba, H., Yoshida, M., Okada, Y., Yokono, K., Kasuga, M., Baba, S. and Roth, R. A. (1994) J. Biol. Chem. **269**, 11498–11502
14 Denton, R. M. and Tavare, J. M. (1995) Eur. J. Biochem. **227**, 597–611
15 Porras, A., Muszynski, K., Rapp, U. R. and Santos, E. (1994) J. Biol. Chem. **269**, 12741–12748
16 White, M. F. (1996) Philos. Trans. R. Soc. London B **351**, 181–189
17 Fry, M. J. (1994) Biochim. Biophys. Acta **1226**, 237–268
18 Kohn, A. D., Kovacina, K. S. and Roth, R. A. (1995) EMBO J. **14**, 4288–4295
19 Ming, X. F., Burgering, B. M. T., Wennstrom, S., Claesson-Welsh, L., Heldin, C. H., Bos, J. L., Kozma, S. C. and Thomas, G. (1994) Nature (London) **371**, 426–429
20 Cross, D. A. E., Alessi, D. R., Cohen, P., Andjelkovich, M. and Hemmings, B. A. (1995) Nature (London) **378**, 785–789
21 Bos, J. L. (1995) Trends Biochem. Sci. **20**, 441–442
22 Kohn, A. D., Takeuchi, F. and Roth, R. A. (1996) J. Biol. Chem. **271**, 21920–21926
23 Hemmings, B. A. (1997) Science **275**, 628–630
24 Franke, T. F., Kaplan, D. R., Cantley, L. C. and Toker, A. (1997) Science **275**, 665–668
25 James, S. R., Downes, C. P., Gigg, R., Grove, S. J. A., Holmes, A. B. and Alessi, D. R. (1996) Biochem. J. **315**, 709–713
26 Frech, M., Andjelkovich, M., Ingley, E., Reddy, K. K., Falck, J. R. and Hemmings, B. A. (1997) J. Biol. Chem. **272**, 8474–8481
27 Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R. J., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F. and Hawkins, P. T. (1997) Science **277**, 567–570
28 Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P. and Hemmings, B. (1996) EMBO J. **15**, 6541–6551
29 Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R. J., Reese, C. B. and Cohen, P. (1997) Curr. Biol. **7**, 261–269

30 Weng, Q. P., Andrabi, K., Klippel, A., Kozlowski, M. T., Williams, L. T. and Avruch, J. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 5744–5748
31 Dennis, P. B., Pullen, N., Kozma, S. C. and Thomas, G. (1996) Mol. Cell. Biol. **16**, 6242–6251
32 Burgering, B. M. T. and Coffer, P. J. (1995) Nature (London) **376**, 599–602
33 Yano, H., Nakanishi, S., Kimura, K., Hanai, N., Saitoh, Y., Fukui, Y., Nonomura, Y. and Matsuda, Y. (1993) J. Biol. Chem. **268**, 25846–25856
34 Okada, T., Kawano, Y., Sakakibara, T., Hazeki, O. and Ui, M. (1994) J. Biol. Chem. **269**, 3568–3573
35 Mollinedo, E., Gajate, C. and Flores, I. (1994) J. Immunol. **153**, 2457–2469
36 Cross, M. J., Stewart, A., Hodgkin, M. N., Kerr, D. J. and Wakelam, M. J. O. (1995) J. Biol. Chem. **270**, 25352–25355
37 Brunn, G. J., Williams, J., Sabers, C., Weiderrecht, G., Lawrence, J. C. and Abraham, R. T. (1996) EMBO J. **15**, 5256–5267
38 Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J. and Kahn, C. R. (1994) Mol. Cell. Biol. **14**, 4902–4911
39 Welsh, G. I., Foulstone, E. J., Young, S. W., Tavaré, J. M. and Proud, C. G. (1994) Biochem. J. **303**, 15–20
40 Moule, S. K., Edgell, N. J., Welsh, G. I., Diggle, T. A., Foulstone, E. J., Heesom, K. J., Proud, C. G. and Denton, R. M. (1995) Biochem. J. **311**, 595–601
41 Cross, D. A. E., Alessi, D. R., Vandenheede, J. R., McDowell, H. E., Hundal, H. S. and Cohen, P. (1994) Biochem. J. **303**, 21–26
42 Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T. and Saltiel, A. R. (1995) J. Biol. Chem. **270**, 27489–27494
43 Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J. and Saltiel, A. R. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 7686–7689
44 Flynn, A. and Proud, C. G. (1996) FEBS Lett. **389**, 162–166
45 Abraham, R. T. and Wiederrecht, G. J. (1996) Annu. Rev. Immunol. **14**, 483–510
46 Brown, E. J., Beal, P. A., Keith, C. T., Chen, J., Shin, T. B. and Schreiber, S. L. (1995) Nature (London) **377**, 441–446
47 Chiu, M. I., Katz, H. and Berlin, V. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 12574–12578
48 Sabatini, D. M., Pierchala, B. A., Barrow, R. K., Schell, M. J. and Snyder, S. H. (1995) J. Biol. Chem. **270**, 20875–20878
49 Sabers, C. J., Martin, M. M., Brunn, G. J., Williams, J. M., Dumont, F. T., Wiederrecht, G. and Abraham, R. T. (1995) J. Biol. Chem. **270**, 815–822
50 Abraham, R. T. (1996) Curr. Opin. Immunol. **8**, 412–418
51 Zheng, X. F. and Schreiber, S. L. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 3070–3075
52 Pain, V. M. (1996) Eur. J. Biochem. **236**, 747–771
53 Proud, C. G. (1994) Mol. Biol. Rep. **19**, 161–170
54 Stansfield, I., Jones, K. M. and Tuite, M. F. (1995) Trends Biochem. Sci. **20**, 489–491
55 Merrick, W. C. (1992) Microbiol. Rev. **56**, 291–315
56 Chaudhuri, J., Si, K. and Maitra, U. (1997) J. Biol. Chem. **272**, 7883–7891
57 Sonenberg, N. (1996) in Translational Control (Hershey, J. W. B., Matthews, M. B. and Sonenberg, N., eds.), pp. 245–269, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
58 Rhoads, R. E., Joshi, B. and Minich, W. B. (1994) Biochimie **76**, 831–838
59 Price, N. T. and Proud, C. G. (1994) Biochimie **76**, 748–760
60 Flynn, A., Oldfield, S. and Proud, C. G. (1993) Biochim. Biophys. Acta **1174**, 117–121
61 Gaspar, N. J., Kinzy, T. G., Scherer, B. J., Humbelin, M., Hershey, J. W. and Merrick, W. C. (1994) J. Biol. Chem. **269**, 3415–3422
62 Clemens, M. J. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp. 139–172, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
63 Kimball, S. R., Vary, T. C. and Jefferson, L. S. (1994) Annu. Rev. Physiol. **56**, 321–348
64 Kimball, S. R. and Jefferson, L. S. (1988) Biochem. Biophys. Res. Commun. **156**, 706–711
65 Jeffrey, I. W., Kelly, F. J., Duncan, R., Hershey, J. W. and Pain, V. M. (1990) Biochimie **72**, 751–757
66 Karinch, A. M., Kimball, S. R., Vary, T. C. and Jefferson, L. S. (1993) Am. J. Physiol. **264**, E101–E108
67 Welsh, G. I. and Proud, C. G. (1992) Biochem. J. **284**, 19–23
68 Proud, C. G. (1995) Trends Biochem. Sci. **20**, 241–246
69 Chen, J.-J. and London, I. M. (1995) Trends Biochem. Sci. **20**, 105–108
70 Rowlands, A. G., Panniers, R. and Henshaw, E. C. (1988) J. Biol. Chem. **263**, 5526–5533
71 Welsh, G. I. and Proud, C. G. (1993) Biochem. J. **294**, 625–629
72 Hughes, K., Ramakrishna, S., Benjamin, W. B. and Woodgett, J. R. (1992) Biochem. J. **288**, 309–314
73 Borthwick, A. C., Wells, A. M., Rochford, J. J., Hurel, S. J., Turnbull, D. M. and Yeaman, S. J. (1995) Biochem. Biophys. Res. Commun. **210**, 738–745
74 Moule, S. K., Welsh, G. I., Foulstone, E. J., Heesom, K., Edgell, N., Proud, C. G. and Denton, R. M. (1997) J. Biol. Chem. **272**, 7713–7719
75 Welsh, G. I., Wilson, C. and Proud, C. G. (1996) Trends Cell Biol. **6**, 274–279
76 Reference deleted
77 Welsh, G. I., Patel, J. C. and Proud, C. G. (1997) Anal. Biochem. **244**, 16–21
78 Singh, L. P., Denslow, N. D. and Wahba, A. J. (1996) Biochemistry **35**, 3206–3212
79 Nikolakaki, E., Coffer, P. J., Hemelsoet, R., Woodgett, J. R. and Defize, L. H. (1993) Oncogene **8**, 833–840
80 Fiol, C. J., Williams, J. S., Chou, C., Wang, Q. M., Roach, P. J. and Andrisani, O. M. (1994) J. Biol. Chem. **269**, 32187–32193
81 Sutherland, C. and Cohen, P. (1994) FEBS Lett. **338**, 37–42
82 Sutherland, C., Leighton, I. A. and Cohen, P. (1993) Biochem. J. **296**, 15–19
83 Mendez, R., Myers, M. G., White, M. F. and Rhoads, R. E. (1996) Mol. Cell. Biol. **16**, 2857–2864
84 Welsh, G. I., Stokes, C. M., Wang, X., Sakaue, H., Ogawa, W., Kasuga, M. and Proud, C. G. (1997) FEBS Lett. **410**, 418–422
85 Welsh, G. I., Miyamoto, S., Proud, C. G. and Safer, B. (1996) J. Biol. Chem. **271**, 11410–11413
86 Gilligan, M., Welsh, G. I., Flynn, A., Bujalska, I., Proud, C. G. and Docherty, K. (1996) J. Biol. Chem. **271**, 2121–2125
87 Hinnebusch, A. G. (1994) Trends Biochem. Sci. **19**, 409–414
88 Geballe, A. P. and Morris, D. R. (1994) Trends Biochem. Sci. **19**, 159–164
89 Kimball, S. R., Jurasinski, C. V., Lawrence, J. C. and Jefferson, L. S. (1997) Am. J. Physiol. Cell Physiol. **41**, C754–C759
90 Pause, A., Belsham, G. J., Gingras, A.-C., Donzé, O., Lin, T. A., Lawrence, J. C. and Sonenberg, N. (1994) Nature (London) **371**, 762–767
91 Lin, T.-A., Kong, X., Haystead, T. A. J., Pause, A., Belsham, G. J., Sonenberg, N. and Lawrence, J. C. (1994) Science **266**, 653–656
92 Belsham, G. J., Brownsey, R. W. and Denton, R. M. (1982) Biochem. J. **204**, 345–352
93 Diggle, T. A., Moule, S. K., Avison, M. B., Flynn, A., Foulstone, E. J., Proud, C. G. and Denton, R. M. (1996) Biochem. J. **316**, 447–453
94 Proud, C. G. (1992) Curr. Top. Cell Regul. **32**, 243–369
95 Hentze, M. W. (1997) Science **275**, 500–501
96 Belsham, G. J., Brownsey, R. W. and Denton, R. M. (1980) Diabetologia **18**, 307–312
97 Denton, R. M., Brownsey, R. W. and Belsham, G. J. (1981) Diabetologia **21**, 347–362
98 Blackshear, P. J., Nemenoff, R. A. and Avruch, J. (1983) Biochem. J. **214**, 11–19
99 Hu, C., Pang, S., Kong, X., Velleca, M. and Lawrence, J. C. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 3730–3734
100 Lawrence, J. C., Fadden, P., Haystead, T. A. J. and Lin, T.-A. (1997) Adv. Enzyme Regul. **37**, in the press
101 Haghighat, A., Mader, S., Pause, A. and Sonenberg, N. (1995) EMBO J. **14**, 5701–5709
102 Mader, S., Lee, H., Pause, A. and Sonenberg, N. (1995) Mol. Cell. Biol. **15**, 4990–4997
103 Rau, M., Ohlmann, T., Morley, S. J. and Pain, V. M. (1996) J. Biol. Chem. **271**, 8983–8990
104 Lin, T.-A., Kong, X., Saltiel, A. R., Blackshear, P. J. and Lawrence, J. C. (1995) J. Biol. Chem. **270**, 18531–18538
105 Graves, L. M., Bornfeldt, K. E., Argast, G. M., Krebs, E. G., Kong, X., Lin, T. A. and Lawrence, J. C. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 7222–7226
106 Azpiazu, I., Saltiel, A. R., DePaoli-Roach, A. A. and Lawrence, J. C. (1996) J. Biol. Chem. **271**, 5033–5039
107 Diggle, T. A. and Denton, R. M. (1992) Biochem. J. **282**, 729–736
108 Fadden, P., Haystead, T. A. J. and Lawrence, J. C. (1997) J. Biol. Chem. **272**, 10240–10247
109 Haystead, T. A. J., Haystead, C. M. M., Hu, C., Lin, T. A. and Lawrence, J. C. (1994) J. Biol. Chem. **269**, 23185–23191
110 Diggle, T. A., Schmitz-Peiffer, C., Borthwick, A. C., Welsh, G. I. and Denton, R. M. (1991) Biochem. J. **279**, 545–551
111 Von Manteuffel, S. R., Gingras, A. C., Ming, X. F., Sonenberg, N. and Thomas, G. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 4076–4080
112 Scott, P. H. and Lawrence, J. C. (1997) FEBS Lett. **409**, 171–176
113 Beretta, L., Gingras, A.-C., Svitkin, Y. V., Hall, M. N. and Sonenberg, N. (1996) EMBO J. **15**, 658–664
114 Brunn, G. J., Hudson, C. C., Sekulic, A., Williams, J. M., Hosoi, H., Houghton, P. J., Lawrence, J. C. and Abraham, R. T. (1997) Science **277**, 99–101
115 von Manteuffel, S. R., Dennis, P. B., Pullen, N., Gingras, A.-C., Sonenberg, N. and Thomas, G. (1997) Mol. Cell. Biol. **17**, 5426–5436
116 Lin, T. A. and Lawrence, J. C. (1996) J. Biol. Chem. **271**, 30199–30204
117 Flynn, A. and Proud, C. G. (1996) Cancer Surv. **27**, 293–310
118 Joshi, B., Cai, A. L., Keiper, B. D., Minich, W. B., Mendez, R., Beach, C. M., Stolarski, R., Darzynkiewicz, E. and Rhoads, R. E. (1995) J. Biol. Chem. **270**, 14597–14603

119 Flynn, A. and Proud, C. G. (1995) J. Biol. Chem. **270**, 21684–21688
120 Minich, W. B., Balasta, M. L., Goss, D. J. and Rhoads, R. E. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 7668–7672
121 Bu, X., Haas, D. W. and Hagedorn, C. H. (1993) J. Biol. Chem. **268**, 4975–4978
122 Morley, S. J., Rau, M., Kay, J. E. and Pain, V. M. (1993) Eur. J. Biochem. **218**, 39–48
123 Servant, M. J., Giasson, E. and Meloche, S. (1996) J. Biol. Chem. **271**, 16047–16052
124 Levy, S., Avni, D., Hariharan, N., Perry, R. P. and Meyuhas, O. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 3319–3323
125 Meyuhas, O., Avni, D. and Shama, S. (1996) in Translational Control, (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp. 363–388, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
126 Levenson, R. M., Nairn, A. C. and Blackshear, P. J. (1989) J. Biol. Chem. **264**, 11904–11911
127 Jefferies, H. B. J., Reinhard, G., Kozma, S. C. and Thomas, G. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 4441–4445
128 Terada, N., Patel, H. R., Takase, K., Kohno, K., Nairn, A. C. and Gelfand, E. W. (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 11477–11481
129 Kaspar, R. L., Rychlik, W., White, M. W., Rhoads, R. E. and Morris, D. R. (1990) J. Biol. Chem. **265**, 3619–3622
130 Hammond, M. L., Merrick, W. and Bowman, L. H. (1991) Genes Dev. **5**, 1723–1736
131 Bommer, U. A., Lazaris-Karatzas, A., De Benedetti, A., Nurnberg, P., Benndorf, R., Bielka, H. and Sonenberg, N. (1994) Cell. Mol. Biol. Res. **40**, 633–641
132 Jefferies, H. B. J. and Thomas, G. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp. 389–409, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
133 Jefferies, H. B. J., Fumagalli, S., Dennis, P. B., Reinhard, C., Pearson, R. B. and Thomas, G. (1997) EMBO J. **16**, 3693–3704
134 Patel, H. R., Terada, N. and Gelfand, E. W. (1997) Biochem. Biophys. Res. Commun. **227**, 507–512
135 Nairn, A. C. and Palfrey, H. C. (1987) J. Biol. Chem. **262**, 17299–17303
136 Price, N. T., Redpath, N. T., Severinov, K. V., Campbell, D. G., Russell, J. M. and Proud, C. G. (1991) FEBS Lett. **282**, 253–258
137 Ovchinnikov, L. P., Motuz, L. P., Natapov, P. G., Averbuch, L. J., Wettenhall, R. E., Szyszka, R., Kramer, G. and Hardesty, B. (1990) FEBS Lett. **275**, 209–212
138 Nairn, A. C. and Palfrey, H. C. (1996) in Translational Control (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp. 295–318, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
139 Redpath, N. T., Price, N. T., Severinov, K. V. and Proud, C. G. (1993) Eur. J. Biochem. **213**, 689–699
140 Carlberg, U., Nilsson, A. and Nygard, O. (1990) Eur. J. Biochem. **191**, 639–645
141 Redpath, N. T., Price, N. T. and Proud, C. G. (1996) J. Biol. Chem. **271**, 17547–17554
142 Ryazanov, A. G., Ward, M. D., Mendola, C. E., Pavur, K. S., Dorovkov, M. V., Wiedmann, M., Erdjument-Bromage, H., Tempst, P., Parmer, T. G., Prostko, C. R. et al. (1997) Proc. Natl. Acad. Sci. U.S.A **94**, 4884–4889
143 Redpath, N. T. and Proud, C. G. (1990) Biochem. J. **272**, 175–180
144 Redpath, N. T. and Proud, C. G. (1991) Biochim. Biophys. Acta **1093**, 36–41
145 Redpath, N. T., Foulstone, E. J. and Proud, C. G. (1996) EMBO J. **15**, 2291–2297
146 Rousseau, D., Kaspar, R., Rosenwald, I., Gehrke, L. and Sonenberg, N. (1996) Proc. Natl. Acad. Sci. U.S.A **93**, 1065–1070
147 Klippel, A., Kavanaugh, W. M., Pot, D. and Williams, L. T. (1997) Mol. Cell. Biol. **17**, 338–344