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Activation of translation initiation factor eIF2B by insulin requires phosphatidyl inositol 3-kinase

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Abstract Eukaryotic initiation factor eIF2B mediates a key regulatory step in peptide-chain initiation and is acutely activated by insulin, although it is not clear how. Inhibitors of phosphatidylinositide 3-kinase blocked activation of eIF2B, although rapamycin, which inhibits the p70 S6 kinase pathway, did not. Furthermore, a dominant negative mutant of PI 3-kinase also prevented activation of eIF2B, while a Sos-mutant, which blocks MAP kinase activation, did not. The data demonstrate that a pathway distinct from MAP and p70 S6 kinases regulates eIF2B. Glycogen synthase kinase-3 (GSK-3) phosphorylates and inactivates eIF2B. In all cases, eIF2B and GSK-3 were regulated reciprocally. Dominant negative PI 3-kinase abolished the insulin-induced inhibition of GSK-3. These data strongly support the hypothesis that insulin activates eIF2B through a signalling pathway involving PI 3-kinase and inhibition of GSK-3.

Key words: Initiation factor; Protein synthesis; Insulin; Phosphatidylinositide 3-kinase; Wortmannin; Glycogen synthase kinase-3

1. Introduction

Protein synthesis is activated at the level of translation by a variety of stimuli. Physiologically, one of the most important of these is insulin [1] which activates overall protein synthesis within a few minutes of its administration. Several control points for mammalian translation have been identified and these may regulate either overall rates of protein synthesis or the translation of specific mRNAs [1–5]. The rapamycin-sensitive ('FRAP'/mTOR) signalling pathway appears to be involved in several of these [6,7]. However, rapamycin only slightly represses overall or insulin-stimulated rates of protein synthesis, especially in the short term [8,9]. This suggests that (a) further, insulin-activated but rapamycin-insensitive pathway mediates a high proportion of the stimulation of protein synthesis seen in response to insulin [8].

Eukaryotic initiation factor eIF2B mediates the recycling of the protein, eIF2, which binds the initiator Met-tRNA ($Met\text{-}tRNA_i$) to the 40S ribosomal subunit and is required for every initiation event. eIF2 binds GTP and the GTP is hydrolysed late in the initiation process, yielding the inactive [eIF2.GDP] complex. eIF2B acts on this to promote the release of the tightly bound GDP and allow eIF2 to bind GTP. Since [eIF2.GTP] is required for each initiation event, this rate-limiting eIF2B-mediated recycling step plays a key role in the regulation of mRNA translation [10]. We and others have shown that eIF2B is activated by insulin in several cell types [11–14], by glucose in pancreatic islets [15] and following mitogenic stimulation of T-lymphocytes [16].

eIF2B is a substrate for several protein kinases in vitro, including casein kinases (CKs)-1 and -2 and glycogen synthase kinase (GSK)-3 [10,17,18]. Phosphorylation by GSK-3 inhibits the activity of eIF2B and reverses the activation reported to be brought about by its phosphorylation by CK-1 or CK-2 [18]. GSK-3 is itself inactivated in response to insulin, by a mechanism which involves its phosphorylation at a conserved N-terminal Ser residue [19–21]. Data obtained using selective inhibitors point to an essential role for phosphatidylinositide 3-kinase (PI 3-kinase) in the regulation of GSK-3 by insulin [22–24], and recent findings suggest that the link between them may be provided by protein kinase B (PKB), which lies downstream of PI 3-kinase and can phosphorylate GSK-3 in vitro at the regulatory site [24]. Recent studies have shown that PI 3-kinase plays an essential role in the activation of protein synthesis by insulin and that this largely involves a signalling mechanism distinct from the rapamycin-sensitive p70 S6 kinase (mTOR/FRAP) pathway [8].

Here we report the results of the first studies addressing the signalling pathway through which insulin activates eIF2B. We show that the activation of eIF2B by insulin requires PI 3-kinase but is not mediated by the FRAP/mTOR pathway. eIF2B activation is also independent of MAP kinase. These data demonstrate that a distinct signalling pathway operates to activate eIF2B and are consistent both with (i) a key role for GSK-3 in the activation of eIF2B and hence translation initiation by insulin and (ii) an absolute requirement for PI 3-kinase for the activation of protein synthesis by insulin [8].

2. Materials and methods

2.1. Adenovirus vectors and treatment of cells

The dominant negative PI 3-kinase (Δp85) and a dominant negative Sos (ΔSos) mutants were as described previously [25,26]. Recombinant adenovirus vectors (termed pAxCAΔp85 or pAxCAΔSos) were generated by cloning the corresponding cDNAs into pAxCAwt [27] and cotransfection into 293 cells with DNA-TPC, as described previously [28]. An adenovirus vector encoding the lacZ gene (pAxCALacZ) [27] was a gift from I. Saito, Tokyo University. CHO-IR cells, Chinese hamster ovary cells overexpressing human insulin receptor [25], were infected with adenovirus vectors at a multiplicity of infection (MOI, plaque-forming units/cell) of 10, then the cells were used for experiments 48 h after infection. In some experiments, a similar cell line,

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CHO.T cells [29], which also overexpress the human insulin receptor, were employed. In all cases, cells were grown, treated with insulin and extracted as described previously [26,29].

2.2. Assays for enzymes and translation factors

MAP kinase assays were performed after immunoprecipitation with anti-MAP kinase antibodies (αC92) using myelin basic protein as substrate as described previously [26]. PI 3-kinase activity was assayed after immunoprecipitation with anti-phosphotyrosine antibodies (PY20; Transduction laboratories) as described previously [25]. GSK-3 and eIF2B were routinely assayed as described previously [14,17,30,31].

3. Results and discussion

3.1. Insulin activates eIF2B and inactivates GSK-3 in CHO.IR cells

Two similar CHO cell lines, each expressing the human insulin receptor, have been used in the various experiments reported here. These are termed CHO.T [29] and CHO-IR [25] cells. Fig. 1A, shows that insulin treatment of CHO.T cells led to the 2-fold activation of eIF2B within 10 min of insulin treatment (Fig. 1A). Insulin also resulted in the inactivation of GSK-3, in this case to ≈40% of the activity in control cells (Fig. 1B) [17]. Essentially identical effects of insulin on eIF2B and on GSK-3 were also seen in CHO-IR cells (as described below, Sections 3.2 and 3.3).

The best studied mechanism for the regulation of eIF2B activity involves the phosphorylation of its substrate, eIF2, on its α-subunit, eIF2(αP) being a potent competitive inhibitor of eIF2B. In these experiments insulin did not affect the level of eIF2α phosphorylation as assessed by isoelectric focusing (≈15% with or without insulin; data not shown). This observation is consistent with other work in muscle [11,13,32,33] and in Swiss 3T3 cells [14] showing that insulin or diabetes do not affect the state of phosphorylation of eIF2α and points to an alternative mechanism regulating the activity of eIF2B.

3.2. Interference with PI 3-kinase prevents the activation of eIF2B by insulin

In CHO cells expressing the insulin receptor, wortmannin, an inhibitor of PI 3-kinase, inhibited the effects of insulin on the activities of both eIF2B and GSK-3 (Fig. 1A,B). In contrast, rapamycin, which blocks the FRAP/mTOR pathway, which may also lie downstream of PI 3-kinase [34], was without effect on the activation of eIF2B by insulin (Fig. 1A) and also had no effect on the inactivation of GSK-3 caused by this hormone (Fig. 1B and [22–24]). This is an important control given that effects of wortmannin or other treatments which affect PI 3-kinase could indicate the involvement of either the FRAP/TOR signalling pathway or of GSK-3 (or both).

Insulin potently activated PI 3-kinase in CHO.IR cells (Fig. 2A,B). In contrast, in cells infected with adenovirus expressing a dominant negative mutant of the p85 subunit of PI 3-kinase (Δp85), the activation of this enzyme by insulin was severely inhibited (Fig. 2A,B). On the other hand, insulin did activate PI 3-kinase in cells infected either with control virus encoding β-galactosidase or virus expressing a dominant interfering mutant of the GTP/GDP exchange factor Sos, which prevents the activation of Ras [26] (Fig. 2A,B). As expected, wortmannin also blocked the activity of PI 3-kinase (Fig. 2A). The interfering mutant of PI 3-kinase (Δp85) almost completely blocked the activation of eIF2B by insulin (Fig. 3A), whereas

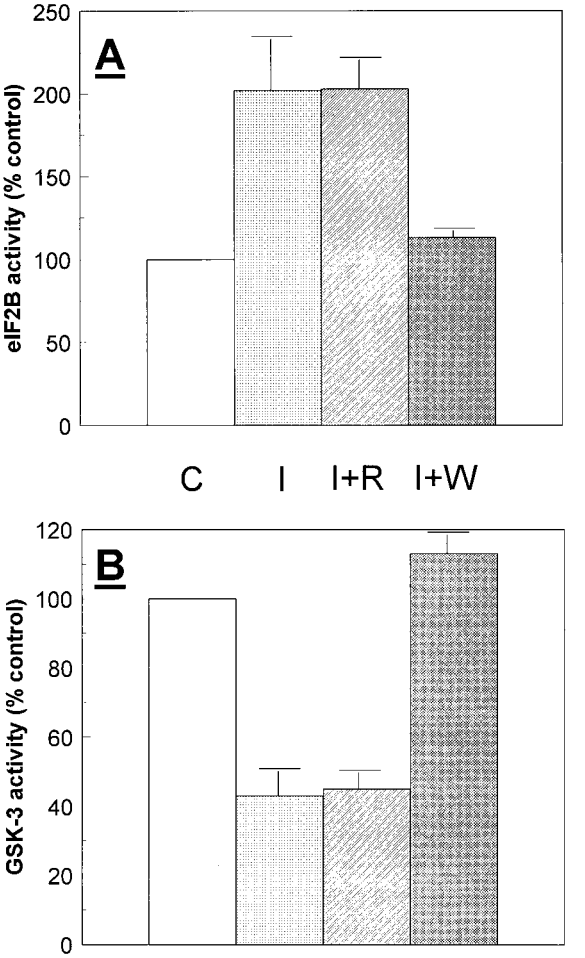

Fig. 1. Regulation of eIF2B and GSK-3 in Chinese hamster ovary cells expressing the insulin receptor. The activities of eIF2B (panel A) and GSK-3 (panel B) were measured in extracts from untreated CHO cells (C) or cells treated with insulin (I, 20nM) for 10 min in the absence or presence of wortmannin (W, 100nM) or rapamycin (R, 20nM), these inhibitors being added 30 min prior to insulin treatment of the cells. The data shown are from three separate experiments, assays being performed in duplicate in each case.

eIF2B was activated normally in cells infected with control virus or virus encoding dominant negative Sos.

The ability of insulin to activate MAP kinase was strongly suppressed in cells expressing the Sos mutant (ΔSos cells, [26]), whereas insulin did activate MAP kinase in control cells, and, to similar extents, in cells infected with control virus or virus expressing the Δp85 mutant (Fig. 2C). Thus, as expected, the Δp85 mutant did not interfere with MAP kinase activation, but blocked activation of PI 3-kinase, while the ΔSos mutation blocks MAP kinase activation but not that of PI 3-kinase. The expression of the dominant-negative ΔSos mutant did not interfere with the activation of eIF2B (Fig. 3A), indicating that this was independent both of Ras activation [26] and the MAP kinase cascade.

3.3. Regulation of GSK-3 by insulin requires PI 3-kinase

In CHO.IR cells infected with control virus or with virus expressing the ΔSos mutant, insulin treatment led to the inhibition of GSK-3 (Fig. 3B). In contrast, in cells expressing the Δp85 mutant, the ability of insulin to inhibit GSK-3 was sharply reduced relative to control cells (Fig. 3B). Thus the

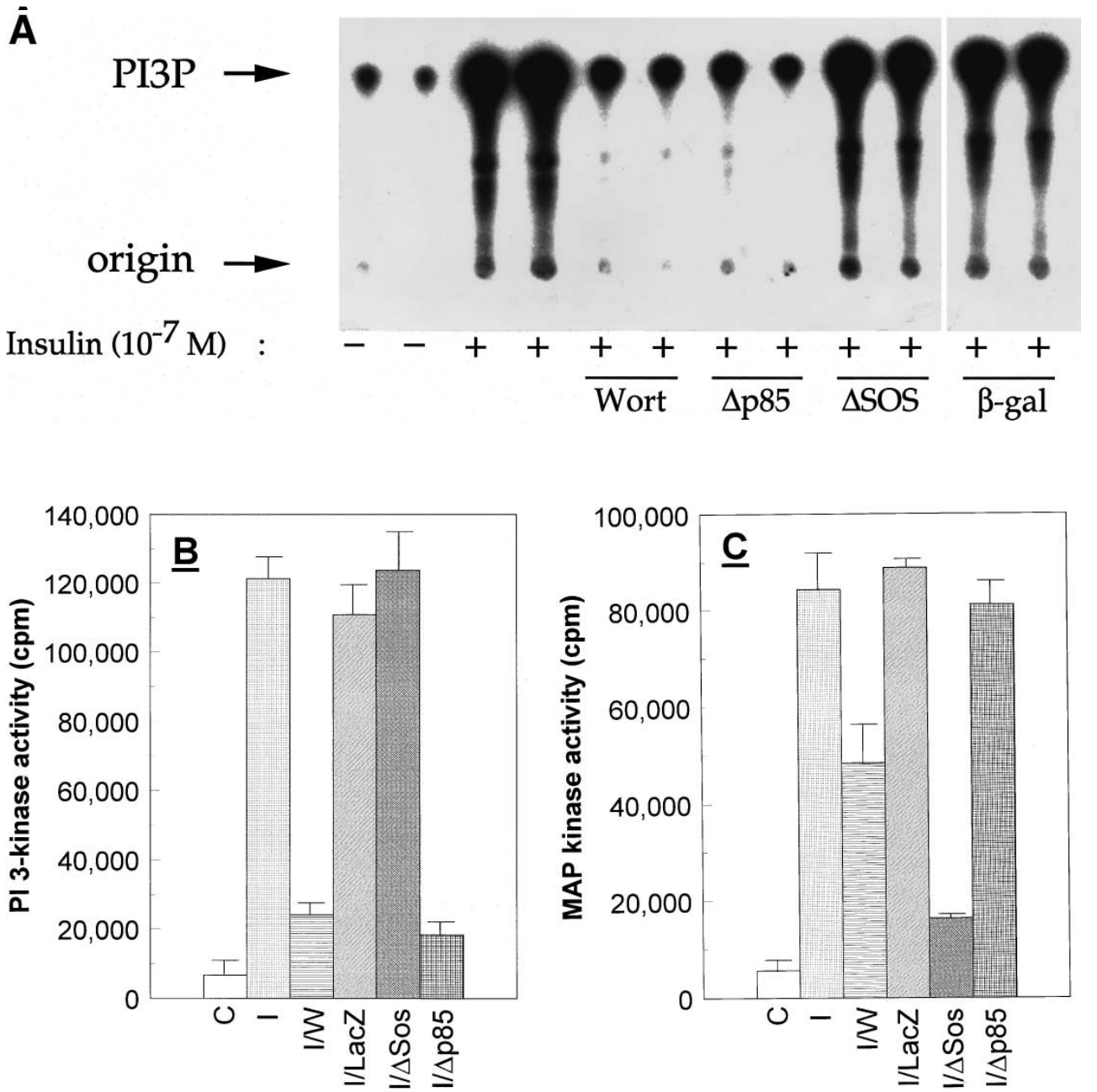

Fig. 2. Effects of wortmannin or overexpression of dominant negative molecules on PI 3-kinase and MAP kinase in CHO-IR cells. Control or virus-infected cells or uninfected cells pre-treated with 100 nM wortmannin for 20 min were incubated in the absence or presence of 0.1 μM insulin for 5 min. Cells were then extracted and samples prepared for assay of Pi 3-kinase or MAP kinase. PI 3-kinase was immunoprecipitated, and its activity was assayed (panels A and B) and, separately, MAP kinase was immunoprecipitated and then assayed (panel C). Panel A shows the data, in duplicate, from a single typical experiment measuring PI 3-kinase, and is an autoradiograph of a chromatogram corresponding to a PI 3-kinase assay: the positions of the origin and of PI 3-phosphate (PI3P) are indicated. Wort, cells pre-treated with wortmannin (100nM); Δp85, ΔSos and β-gal indicate cells infected with the pAxCAΔp85, pAxCAΔSos or pAxCALacZ viruses. Where no virus is indicated, cells were non-infected ones. Panel B shows cumulative PI 3-kinase assay data which are mean ± S.E. from at least three independent experiments. In panels B and C (see below), C, untreated cells; I, insulin (0.1 μM insulin for 10 min); W, wortmannin (100 nM); and LacZ, ΔSos and Δp85 indicate infection with the corresponding viruses. Panel C shows cumulative data for MAP kinase assays. Data are mean ± S.E. from at least three experiments and the labelling of the figure is as for panel B.

MAP kinase pathway does not appear to mediate the regulation of GSK-3 by insulin [24] while PI 3-kinase activity plays a key role in its control [22–24]. This corroborates other data obtained using alternative approaches employing selective (but not necessarily uniquely specific) inhibitors of MAP kinase activation or of PI 3-kinase [24]. These findings are at variance with the conclusions of Eldar-Finkelman et al. [35] who used a transfection-based approach to explore the regulation of GSK-3 by epidermal growth factors and concluded that this required the MAP kinase pathway.

3.4. Concluding remarks

The data described here lend further support to the model for the regulation of GSK-3 activity advanced by Cross et al.

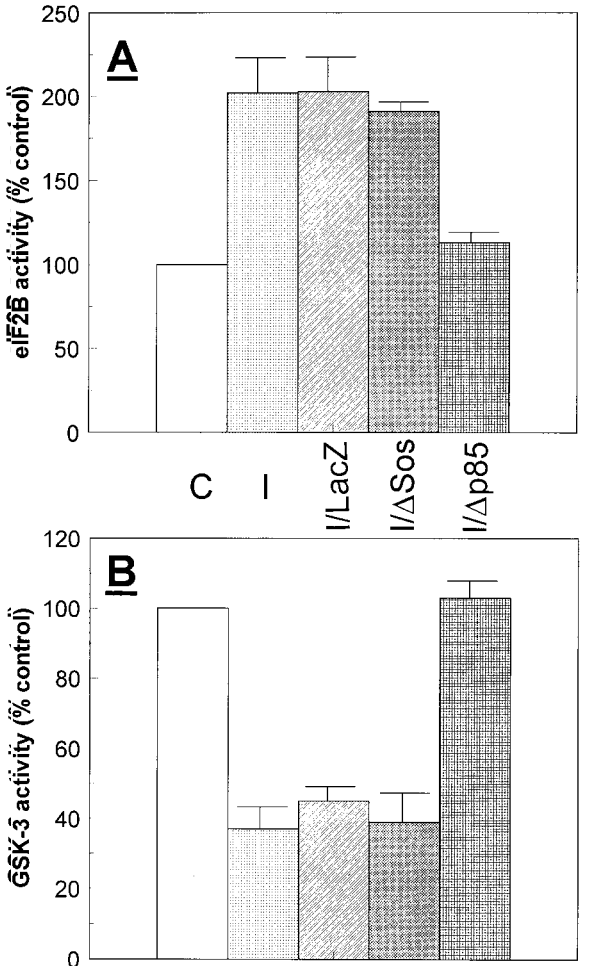

Fig. 3. PI 3-kinase but not MAP kinase is required for the activation of eIF2B by insulin. The activities of eIF2B (panel A) and GSK-3 (panel B) were determined in extracts of control CHO cells, treated with or without insulin, or cells infected with control adenovirus (LacZ) or adenovirus encoding Δp85 or ΔSos interfering mutants (as indicated), treated with insulin (0.1 μM for 10 min).

[24]. Any of three manipulations designed either to inhibit PI 3-kinase or block its activation sharply reduced or eliminated the ability of insulin to bring about the inactivation of GSK-3. Similar effects have been reported before for the inhibitors [24], but this is the first occasion where interfering mutants of PI 3-kinase have been employed. Use of these mutants has previously been shown to block the insulin-induced translocation of glucose transporters and activation of glucose transport [25]. The data obtained here underline the requirement for PI 3-kinase for the insulin-induced regulation of GSK-3. In particular, this approach avoids the potential problems which may be encountered using inhibitors, which may also affect other targets in the cell. This is especially relevant in the case of wortmannin, which, since the earlier studies were carried out, has been shown to inhibit other enzymes [36–38].

The most important new results reported here concern the signalling mechanisms through which insulin regulates eIF2B. We and others have previously shown that insulin activates this key step in translation [13,14,39]. We have also shown that eIF2B is a substrate for GSK-3, at least in vitro, and phosphorylation of eIF2B causes its inactivation (Welsh and Proud, unpublished; see also [18]). The present data show that insulin activates eIF2B through a signalling pathway which involves PI 3-kinase, but is independent of the FRAP/mTOR pathway (as shown by the lack of inhibition by rapamycin of insulin's effect on eIF2B). The activation of eIF2B is also independent of MAP kinase, as demonstrated by the absence of an effect of the ΔSos mutant on the activation of eIF2B by insulin. Mendez et al. [8] showed that wortmannin caused a profound inhibition of the activation of protein synthesis by insulin and that this was largely independent of the FRAP/mTOR pathway. Thus, a major element of the insulin-induced activation of protein synthesis involves a PI 3-kinase-dependent but FRAP/mTOR-independent regulatory pathway. All the available data are consistent with the idea that this involves the GSK-3 mediated regulation of eIF2B, whose activation would increase the availability of active [eIF2.GTP] complexes, which are required for every initiation event. The present data fully support the idea that the stimulation of eIF2B resulting from the inactivation of GSK-3 by insulin plays a major role in the overall activation of translation by insulin. Other insulin activated signalling events – e.g. MAP kinase and phosphorylation of eIF4E [40], the FRAP/mTOR pathway and regulation of S6 phosphorylation [41], 4E-BP1 [9,42–44] or eEF2 [6] – are likely to make quantitatively lesser contributions to the activation of translation although their inputs may be especially important for the regulation of the translation of specific mRNAs, e.g. those with structured 5′-UTRs [9,45] or which possess 5′-terminal polypyrimidine tracts [46]. We are grateful to Dr. A.J. Loughlin (Kent) for preparing the eIF2 used in this work.

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