RESEARCH COMMUNICATION

Mitogen-activated protein kinase (MAP kinase), MAP kinase kinase and c-Mos stimulate glucose transport in *Xenopus* oocytes

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Mitogens and growth factors acutely stimulate glucose transport in all cells to supply energy for their growth and division, but little is known about the signalling mechanism by which these agonists promote sugar uptake. Here we show that the transport of deoxyglucose and 3-*O*-methylglucose into *Xenopus laevis* oocytes is stimulated about 2.5-fold when mitogen-activated protein kinase (MAP kinase) is microinjected into these oocytes. We also demonstrate that microinjection of the proto-oncogene product c-Mos (an activator of MAP kinase kinase, which activates MAP kinase in *Xenopus* oocytes), and purified MAP kinase kinase produce similar increases in deoxyglucose transport. Since the activation of MAP kinase is a general response to almost all mitogens and growth factors, we propose that one of its downstream effects is the stimulation of glucose-transport activity.

INTRODUCTION

The exposure of resting cells to mitogens activates numerous signal-transduction pathways that result in the growth and division of cells. Cells undergoing mitogenesis have an increased energy requirement, and therefore stimulation of glucose transport is a common action of all mitogens. Serum- and mitogen-induced stimulation of glucose transport is biphasic, an acute response within minutes being followed by a chronic effect between 4 and 12 h (Jiminez-de-Asua and Rozengurt, 1974; Kletzien and Perdue, 1974; Rollins et al., 1988; Hiraki et al., 1989; Kitagawa et al., 1989). Chronic exposure to a variety of polypeptide growth factors stimulates glucose transport by activation of glucose transporter (GLUT1) gene transcription in several fibroblast cell lines (Jiminez-de-Asua and Rozengurt, 1974; Kletzien and Perdue, 1974; Rollins et al., 1988; Hiraki et al., 1988, 1989; Kitagawa et al., 1989; Kahn and Flier, 1990), and hence the chronic effect on glucose transport is blocked by the protein-synthesis inhibitor cycloheximide (Rollins et al., 1988; Hiraki et al., 1989; Kitagawa et al., 1989). In addition, the expression of several oncogenes (v-*src*, v-*fps* and mutated forms of *ras*) which activate growth-factor-stimulated signal-transduction pathways elevate the level of GLUT1 and increase the rate of glucose transport (Birnbaum et al., 1987; Flier et al., 1987).

The acute effect of growth factors on glucose transport is not blocked by cycloheximide, and we and others have shown that it occurs independently of the activation of protein kinase C when fibroblasts are stimulated by insulin or platelet-derived growth factor (Kahn and Flier, 1990; Merrall et al., 1993). The mechanism of this acute effect has been shown to involve the movement of a pool of intracellular glucose transporters to the cell surface (Kitagawa et al., 1989; Yang et al., 1992). However, there is little or no information regarding the signalling mechanism by which mitogens acutely increase glucose transport.

It is well established that insulin-like growth factor-1 (IGF-1) elicits a 2–3-fold increase in the rate of deoxyglucose transport into *Xenopus* oocytes within minutes (Janicot and Lane, 1989; Hainaut et al., 1991). Thus oocytes provide a model system for the study of signalling events between the IGF-1 receptor and the activation of glucose transport (or other biochemical events). For example, potential roles for Ras (Korn et al., 1987) and tyrosine phosphorylation (Janicot and Lane, 1989) in IGF-1-induced oocyte maturation have been proposed on the basis of inhibitory effects of (anti-Ras and anti-phosphotyrosine) antibodies microinjected into the oocyte cytosol. Moreover, it is known that the oocyte glucose transporter is a GLUT1 homologue (Hainaut et al., 1991). Thus the activation of glucose transport in oocytes by IGF-1 involves the same glucose-transporter family member as that observed in fibroblasts or other cultured cells. In the present study we have examined the effect on glucose transport of microinjecting mitogen-activated protein kinase (MAP kinase), an important component of intracellular signalling pathways, which is activated after stimulation of many cells by mitogens or growth factors (see Ahn et al., 1991; Gomez and Cohen, 1991; Sturgill and Wu, 1991; Pelech and Sanghera, 1992; Thomas, 1992, and references therein). In addition, we have microinjected an oncogene product (Mos) which specifically activates MAP kinase in *Xenopus* oocyte extracts (Nebreda and Hunt, 1993; Posada et al., 1993), as well as purified MAP kinase kinase (MAPKK).

MATERIALS AND METHODS

Materials

Female wild-caught *Xenopus laevis* were purchased from the

Abbreviations used: MAP kinase, mitogen-activated protein kinase; MAPKK, MAP kinase kinase; IGF-1, insulin-like growth factor-1; ATP[S], adenosine 5′-[γ-thio]triphosphate; GST–MAPK, glutathione S-transferase–MAP kinase (fusion protein); deGlc, deoxyglucose; GLUT1, erythrocyte-type glucose transporter GLUT1; MAPKAP kinase-2, MAP kinase-2 activated protein kinase-2; MalE–Mos, *Escherichia coli* maltose-binding protein MalE linked to *Xenopus* Mos; ISPK, insulin-stimulated protein kinase.

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South African Xenopus Facility (Noordheok, South Africa). IGF-1 was from Calbiochem (Nottingham, U.K.). All other reagents were as described by Gould et al. (1991).

Isolation of oocytes from *Xenopus laevis*

The isolation and microinjection of oocytes was performed exactly as described previously (Gould et al., 1991). Stage V and VI oocytes were isolated using watchmaker's forceps and incubated in Barth's buffer [5 mM Hepes/NaOH (pH 7.4)/88 mM NaCl/1 mM KCl/2.4 mM $NaHCO_3$/0.82 mM $MgSO_4$/0.41 mM $CaCl_2$/0.33 mM $Ca(NO_3)_2$] overnight prior to experimentation. Oocytes were sorted to remove damaged oocytes and then microinjected as described (Gould et al., 1991; Colville et al., 1993).

Sugar-transport assays in oocytes

The transport of 2-deoxyglucose (2-deGlc) was assayed as described previously (Gould and Lienhard, 1989; Gould et al., 1991; Colville et al., 1993). Briefly, groups of eight oocytes were incubated in 0.5 ml of Barth's buffer in 13.5 ml centrifuge tubes. Transport measurements were initiated by the addition of an aliquot of deoxy-D-[2,6-^{3}H]glucose (de[2,6-^{3}H]Glc; 0.5 μCi/assay) to 0.1 mM. After incubation for 60 min at room temperature, the reaction was stopped by quickly aspirating the media and washing the oocytes with 3 ml of ice-cold PBS [10 mM sodium phosphate (pH 7.4)/150 mM NaCl] containing 0.1 mM phloretin, a potent transport inhibitor (Krupka, 1971). The oocytes were washed in this way twice more and dispensed into scintillation vials (one oocyte per vial). To each scintillation vial 0.5 ml of 1% SDS was added and the vials were incubated with agitation before addition of scintillant and measurement of radioactivity. Exactly the same assay was employed to measure 3-*O*-methyl-D-glucose transport, with the substrate at 0.1 mM, and with 1.0 μCi per assay; in this case, the time of exposure to isotope was varied as shown in the Figures.

Purification and activation of recombinant MAP kinase

The purified glutathione S-transferase–MAP kinase (GST–MAPK) fusion protein (Stokoe et al., 1992) (0.06 mg/ml) was maximally activated by incubation for 2 h at 30 °C with homogeneous MAPKK from rabbit skeletal muscle (purified as described in Nakielny et al., 1992) in 'activation buffer' [50 mM Tris/HCl (pH 7.3)/2 mM EDTA/2 mM EGTA/5% (v/v) glycerol/0.3 mM vanadate/0.03% (w/w) Brij 35/0.1% (v/v) β-mercaptoethanol/specific peptide inhibitor of cyclic AMP-dependent protein kinase (6 μM)], containing 10 mM magnesium acetate and 0.2 mM adenosine 5′-[γ-thio]triphosphate (ATP[S]). The activated GST–MAPK (800 units/mg) was then concentrated to 450 units/ml by centrifugation through a Centricon 30 membrane. One unit of MAP kinase catalyses the phosphorylation of 1 nmol of myelin basic protein in 1 min (Gomez et al., 1990).

Purification of recombinant c-Mos fusion protein

MalE–Mos fusion protein (*Escherichia coli* maltose-binding protein MalE linked to *Xenopus* c-Mos) was purified exactly as described (Nebreda and Hunt, 1993).

RESULTS

A GST–MAPK fusion protein was incubated with MgATP[S] and MAPKK to produce an active thiophosphorylated species with a specific activity similar to that of MAP kinase isolated from mammalian tissues (results not shown). This thiophosphorylated GST–MAPK, which should be more resistant to dephosphorylation (and hence inactivation) *in vivo*, stimulated deGlc transport into oocytes about 2.5-fold within 75 min of microinjection, similar to the stimulation produced by IGF-1 (Table 1). No stimulation of deGlc transport was observed upon microinjection of buffer alone, but a small stimulation was consistently observed when a control containing buffer, MgATP[S] and MAPKK (but no GST–MAPK) was microinjected. This is presumably the result of partial activation of the oocyte MAP kinase by the small amount of MAPKK activity introduced during microinjection. A similar stimulation of transport rate was observed using the non-metabolizable glucose analogue 3-*O*-methyl-D-glucose (Figure 1), demonstrating that the effect of the MAP kinase was exerted at the level of transport and not at a subsequent step (i.e. glucose phosphorylation by hexokinase).

Accurate assessment of the rate of deGlc transport in oocytes required incubations with deGlc of 1 h. Thus the times indicated in the legend to Table 1 include this period. Thus 'MAP kinase, 120 min' represents injection of MAP kinase and a 1 h incubation prior to initiation of deGlc uptake for a further 1 h. Owing to the

Table 1 Microinjection of MAP kinase or MAPKK stimulates glucose transport in *Xenopus* oocytes

Thiophosphorylated GST–MAPK (450 units/ml; 40 nl/oocyte) was microinjected into oocytes and, after incubation at room temperature for the times indicated, the rate of transport of deGlc was determined over a 1 h period as described below. 'Basal' and 'IGF-1' show the transport rate 1 h after injection of oocytes with water or exposure of the oocyte to 250 nM IGF-1 respectively. 'Buffer' and 'Buffer + activator' show the transport rate 1 h after injection of oocytes with MAP kinase-activating buffer ± MAPKK (see the Materials and methods section). 'MAPK 75', '90', '120' and '180' show the transport rates observed when transport was initiated 15, 30, 60 or 120 min after microinjection of GST–MAPK. 'MAPK + IGF-1', oocytes microinjected with GST–MAPK and incubated for 60 min in buffer containing 250 nM IGF-1 prior to initiation of deGlc transport; 'MAPK + CHx', oocytes microinjected with GST–MAPK and 10 μg/ml cycloheximide for 60 min prior to initiation of deGlc transport; 'MAPKK', oocytes microinjected with MAPKK from rabbit skeletal muscle (1.4 units/oocyte; 44 800 units/mg) and incubated at room temperature for 1 h prior to initiation of deGlc uptake. The results are means ± S.D. ($n = 8$) from a typical experiment. Note that results from two separate oocyte preparations (1 and 2) are presented. In four separate experiments, MAP kinase stimulated deGlc transport 2.5 ± 0.5-fold. The levels of activated MAP kinase injected in these experiments represents about 25–50% of the endogenous MAP kinase activity (Gotoh et al., 1991). * Indicates a statistically significant increase in transport rate compared with buffer-injected oocytes.

Preparation	Addition(s)	Transport rate (pmol/min per oocyte)
1	Basal	0.42 ± 0.03
	IGF-1 (250 nM)	1.26 ± 0.09*
	Buffer	0.46 ± 0.03
	Buffer + activator	0.66 ± 0.03
	MAPK 75	1.23 ± 0.08*
	MAPK 90	1.20 ± 0.06*
	MAPK 180	1.17 ± 0.02*
2	Basal	0.24 ± 0.03
	IGF-1 (250 nM)	0.60 ± 0.05*
	MAPK 120	0.54 ± 0.05*
	MAPK + IGF-1	0.57 ± 0.06*
	MAPK + CHx	0.53 ± 0.03*
	MAPKK	0.61 ± 0.05*

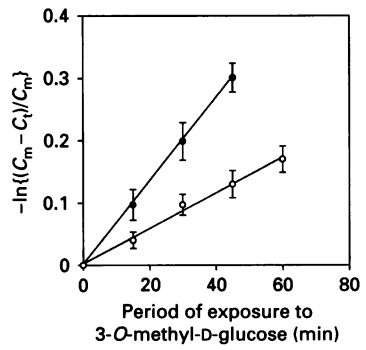

Figure 1 MAP kinase stimulates 3-*O*-methyl-D-glucose transport in *Xenopus* oocytes

Shown is a first-order plot of the equilibration of the oocyte water space by 3-*O*-methyl-D-glucose in buffer-injected (○) or GST–MAPK-injected (450 units/ml; 40 nl/oocyte) oocytes (●). Assays were performed as described in the Materials and methods section 1 h after injection, and each point represents the mean ± S.D. for five oocytes. For clarity, not all data points are shown. C_m is c.p.m./oocyte at $t = 8$ h and C_t is c.p.m./oocyte at time t.

Table 2 MalE–Mos stimulates glucose transport in *Xenopus* oocytes

The transport of deGlc was determined, exactly as described in the Materials and methods section, in oocytes microinjected with 50 nl of purified MalE–Mos protein (250 μg/ml) or buffer. Shown is the result from a typical experiment, with each rate expressed as the mean ± S.D. ($n = 8$). From three independent experiments, the mean fold stimulation observed in response to MalE–Mos injection for 1 h prior to initiation of deGlc uptake was 2.3 ± 0.3-fold. * Indicates a statistically significant increase in transport rate compared with buffer-injected oocytes.

Conditions	Transport rate (pmol/min per oocyte)
Basal	0.42 ± 0.07
IGF-1 (250 nM)	1.31 ± 0.09*
Buffer	0.46 ± 0.04
MalE–Mos	
30 min	0.70 ± 0.02*
60 min	0.81 ± 0.12*
120 min	1.05 ± 0.09*
180 min	1.15 ± 0.09*

very low endogenous deGlc and 3-*O*-methylglucose transport rates observed in oocytes, we have not attempted to evaluate kinetically the rate of stimulation of transport after microinjection of MAP kinase or any other protein. Thus it should be appreciated that the uptake of deGlc is unlikely to be linear over the 60 min period of incubation with deGlc.

Co-injection of MAP kinase with 10 μg/ml cycloheximide produced quantitatively similar stimulations of deGlc transport as MAP kinase alone (Table 1); in addition, IGF-1 stimulation of MAP kinase-injected oocytes produced similar stimulations to IGF-1 treatment alone (Table 1). Microinjection of purified MAPKK also stimulated deGlc transport into oocytes (Table 1). Note that the MAPKK used in these experiments was purified from rabbit skeletal muscle and thus is purified in an active form. Hence activation with MgATP[S] was not performed.

Note that we have observed some variation in basal oocyte deGlc uptake rates between different oocyte preparations, typically between 0.2 and 0.5 pmol/min per oocyte (see Tables 1 and 2). However, the fold increases observed in response to either IGF-1 stimulation or MAP kinase injections were similar between preparations (2.5 ± 0.5-fold; results from four independent preparations of oocytes).

The product of the proto-oncogene c-*mos* is a protein serine/threonine kinase that is synthesized *de novo* during the maturation of *Xenopus* oocytes. The addition to *Xenopus* oocyte extracts of a MalE–Mos fusion protein leads first to the activation of MalE–Mos and then to the activation MAP kinase (Nebreda and Hunt, 1993). Hence we have examined the effect of microinjection of purified MalE–Mos on oocyte deGlc transport, and the results are presented in Table 2.

DISCUSSION

The activation of glucose transport by mitogens is one of the earliest detectable cellular changes which precedes cell growth and division. The increase in glucose transport observed in response to mitogens is biphasic, an early phase occurring within minutes of exposure to hormones or mitogens, and a late phase occurring over 4–24 h which is accompanied by an increase in GLUT1-gene transcription. Studies of the early phase of transport stimulation have suggested that the increase is mediated by an increased level of cell-surface glucose transporters (GLUT1), at least in several fibroblast cell lines (3T3-L1, Swiss 3T3 cells and BC3H-1 cells; Kitagawa et al., 1989; Calderhead et al., 1990; Yang et al., 1992). However, others have suggested that modulation of the intrinsic activity of GLUT1 may account for the stimulations observed (Czech et al., 1992). In the present study we have examined the role of the MAP kinase signalling cascade in the stimulation of glucose transport using *Xenopus* oocytes as a model system.

It is well established that oocytes exhibit a 2–3-fold increase in deGlc transport in response to IGF-1 (Janicot and Lane, 1989; Hainaut et al., 1991). This increase is mediated by a glucose transporter isoform immunologically similar to the erythrocyte-type glucose transporter GLUT1 (Hainaut et al., 1991), which is the transporter identified as regulated by mitogens and growth factors in eukaryotic cells (Kahn and Flier, 1990; Flier et al., 1987; Birnbaum et al., 1987). In other results, not shown here, we have also identified the native oocyte glucose transporter as GLUT1, based upon cross-reactivity with antibodies against the human GLUT1 species (H. M. Thomas and G. W. Gould, unpublished work). Thus we have examined the effect of microinjection of several protein kinases, known to be activated in response to many, if not all, mitogens on the rate of deGlc transport into oocytes.

MAP kinase is an important component of intracellular signalling pathways activated in response to mitogens or growth factors (see Ahn et al., 1991; Gomez and Cohen, 1991; Sturgill and Wu, 1991; Pelech and Sanghera, 1992; Thomas, 1992, and references cited therein).

Microinjection of thiophosphorylated GST–MAP kinase fusion protein into oocytes resulted in a consistent stimulation of deGlc transport (2.5 ± 0.5-fold; $n = 4$) (Table 1). The stimulation was maximal within 75 min. Under the conditions of the assay, GST–MAPK was microinjected for times from 15 min to 2 h, and the oocytes then incubated with deGlc for a further 60 min. Table 1 shows that microinjection of GST–MAPK stimulated glucose transport maximally within 75 min, and that further incubations with MAP kinase did not significantly increase the rate of glucose transport. The levels of activated MAP kinase injected in these experiments represents about 25–50% of the endogenous oocyte MAP kinase activity (Gotoh et al., 1991) and thus should not be considered supraphysiological.

Further analysis of the GST–MAPK-induced stimulation of deGlc transport showed that the stimulations were independent of new protein synthesis, since co-injection of 10 μg/ml cycloheximide was without effect, and further show that the IGF-1- and MAP kinase-induced stimulations were not additive (Table 1).

A small stimulation of deGlc uptake was consistently observed when a control containing buffer, MgATP[S] and MAPKK (but no GST–MAPK) was microinjected (Table 1). This was assumed to reflect the partial activation of the native oocyte MAP kinase by the small amount of MAPKK activity introduced during microinjection. Thus we have examined the effect of microinjection of a homogeneous preparation of MAPKK from rabbit skeletal muscle (Table 1). We have observed that the microinjection of homogeneous MAPKK also stimulated oocyte glucose transport (1.4 units microinjected/oocyte gave a 2–3-fold increase in transport rate). Note, however, that the residual MAPKK present in the MAP kinase preparations used above was 20-fold lower than the range which activated transport in oocytes.

The product of the proto-oncogene c-*mos* is a protein serine/threonine kinase that is synthesized *de novo* during the maturation of *Xenopus* oocytes. The addition to *Xenopus* oocyte extracts of MalE–Mos fusion protein leads first to the activation of MalE–Mos and then to the activation MAP kinase (Nebreda and Hunt, 1993; Posada et al., 1993). MalE–Mos (immunoprecipitated from oocyte extracts) phosphorylates and potently activates MAPKK *in vitro* (Posada et al., 1993; A. R. Nebreda, N. Gomez, P. Cohen and T. Hunt, unpublished work) and this MAPKK kinase activity presumably explains how MalE–Mos activates MAP kinase in oocyte extracts (Nebrada and Hunt, 1993). Microinjection of MalE–Mos into oocytes stimulates deGlc transport more than 2-fold, maximal effects occurring in about 2 h (Table 2). This is in good correlation with the time required for the activation of MAP kinase by MalE–Mos in oocyte extracts (Nebreda and Hunt, 1993). Taken together, the results are consistent with an important role for MAP kinase in mediating the activation of glucose transport.

A number of likely physiological substrates for MAP kinase have been identified, including cytosolic proteins [tyrosine hydroxylase (Haycock et al., 1992), stathmin (Leighton et al., 1993), nuclear transcription factors (c-*jun*; Pulverer et al., 1991) and Elk1 (Hill et al., 1993)] and two distinct types of protein kinase [ribosomal S6 kinases (Rsk) (Sturgill et al., 1988; Sutherland et al., 1993) and MAPKAP kinase-2 (Stokoe et al., 1992)]. A member of the Rsk family of protein kinases, termed the insulin-stimulated protein kinase (ISPK), is implicated in the activation of glycogen synthesis by insulin in skeletal muscle (Sutherland et al., 1993; Dent et al., 1990). To investigate whether any of these downstream kinases were responsible for the effects of MAP kinase on glucose transport, we microinjected highly purified preparations of ISPK and MAPKAP kinase-2 after their conversion into active thiophosphorylated species by incubation with activated GST–MAPK and MgATP[S]. No stimulation of glucose transport was observed following microinjection of either kinase over periods ranging from 1 to 4 h (results not shown), suggesting that neither ISPK nor MAPKAP kinase-2 mediates stimulation of glucose transport. Technical considerations preclude assay of these kinases after microinjection, and thus we cannot be certain that the kinases remain active in the cell.

Recent studies have suggested a role for phosphorylation in the regulation of insulin-stimulated glucose transport in adipocytes (Haystead et al., 1989; Fingar et al., 1993; Inoue et al., 1993); however, this is a distinct type of regulated transport, since it is mediated by a different glucose transporter isoform, the insulin-responsive glucose transporter, GLUT4 [see Gould and Holman (1993) for review]. The activation of glucose transport by mitogens is generally accepted to be mediated by the GLUT1 isoform (Birnbaum et al., 1987; Flier et al., 1987; Hiraki et al., 1988; Rollins et al., 1988; Kitagawa et al., 1989; Kahn and Flier, 1990; Merrall et al., 1993). In addition, phosphorylation of GLUT1 does not appear to be involved in the stimulation of glucose transport by mitogens in either human (Allard et al., 1987) or murine fibroblasts (G. W. Gould, unpublished work). Thus the effect of MAP kinase reported here is likely to modulate either the machinery involved in translocation of GLUT1 to the oocyte surface or modulation of the intrinsic activity of this transporter.

In conclusion, we have shown that microinjection of MAP kinase, MAPKK or MalE–Mos (a MAPKK kinase) all increase the transport of glucose into oocytes. The present study provides the first data implicating a well-characterized cellular-signalling mechanism in the activation of glucose transport observed in response to mitogens, and thus represents an important step towards gaining a molecular understanding of this response.

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REFERENCES

Ahn, N. G., Seger, R., Bratlien, R. L., Diltz, C. D., Tonks, N. K. and Krebs, E. G. (1991) J. Biol. Chem. **266**, 4220–4227

Allard, W. J., Gibbs, E. M., Witters, L. A. and Lienhard, G. E. (1987) Biochim. Biophys. Acta **929**, 288–295

Birnbaum, M. J., Haspel, H. C. and Rosen, O. M. (1987) Science **235**, 1495–1498

Calderhead, D. M., Kitagawa, K., Lienhard, G. E. and Gould, G. W. (1990) Biochem. J. **269**, 597–601

Colville, C. A., Seatter, M. J., Jess, T. J., Gould, G. W. and Thomas, H. M. (1993) Biochem. J. **290**, 701–706

Czech, M. P., Clancy, B. M., Pessino, A., Woon, W. and Harrison, S. A. (1992) Trends Biochem. Sci. **17**, 197–201

Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F. B., Watt, P. and Cohen, P. (1990) Nature (London) **348**, 302–308

Fingar, D. C., Hausdorff, S. F., Blenis, J. and Birnbaum, M. J. (1993) J. Biol. Chem. **268**, 3005–3008

Flier, J. S., Mueckler, M. M., Usher, P. and Lodish, H. F. (1987) Science **235**, 1492–1495

Gomez, N. and Cohen, P. (1991) Nature (London) **353**, 170–173

Gomez, N., Tonks, N. K., Morrison, C., Harmar, T. and Cohen, P. (1990) FEBS Lett. **271**, 119–122

Gotoh, Y., Nishida, E., Matsuda, S., Shiina, N., Kosako, H., Shiokawa, K., Akiyama, T., Ohta, K. and Sakai, H. (1991) Nature (London) **349**, 251–254

Gould, G. W. and Holman, G. D. (1993) Biochem. J., in the press

Gould, G. W. and Lienhard, G. E. (1989) Biochemistry **28**, 9447–9452

Gould, G. W., Thomas, H. M., Jess, T. J. and Bell, G. I. (1991) Biochemistry **30**, 5139–5145

Hainaut, P., Giorgetti, S., Kowalski, A. and Van Obberghen, E. (1991) Cell Regul. **2**, 317–327

Haycock, J. W., Ahn, N. G., Cobb, M. H. and Krebs, E. G. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2365–2369

Haystead, T. A. J., Sim, A. T. R., Carling, D., Honnor, R. C., Tsukitani, Y., Cohen, P. and Hardie, D. G. (1989) Nature (London) **337**, 78–81

Hill, C. S., Marais, R. M., John, S., Wynne, J., Dalton, S. and Treismann, R. (1993) Cell, in the press

Hiraki, Y., Rosen, O. M. and Birnbaum, M. J. (1988) J. Biol. Chem. **263**, 13655–13662

Hiraki, Y., de Herreros, A. G. and Birnbaum, M. J. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8252–8256

Inoue, G., Kuzuya, H., Hayashi, T., Okamoto, M., Yoshimasa, Y., Kosaki, A., Kono, S., Okamoto, M., Maeda, I., Kubota, M. and Imura, H. (1993) J. Biol. Chem. **268**, 5272–5278
Janicot, M. and Lane, M. D. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 2642–2646
Jimenez-de-Asua, L. and Rozengurt, E. (1974) Nature (London) **251**, 624–626
Kahn, B. B. and Flier, J. S. (1990) Diabetes Care **13**, 548–564
Kitagawa, T., Tanaka, M. and Akamatsu, Y. (1989) Biochim. Biophys. Acta **980**, 100–108
Kletzien, R. and Perdue, J. F. (1974) J. Biol. Chem. **249**, 3366–3374
Korn, L. J., Siebel, C. W., McCormick, F. and Roth, R. A. (1987) Science **236**, 840–843
Krupka, R. M. (1971) Biochemistry **10**, 1143–1153
Leighton, I., Curmi, P., Campbell, D. G., Cohen, P. and Sobel, A. (1993) Mol. Cell. Biochem., in the press
Merrall, N. W., Plevin, R. J., Wakelam, M. J. O. and Gould, G. W. (1993) Biochim. Biophys. Acta **1177**, 191–198
Nakielny, S., Campbell, D. G. and Cohen, P. (1992) FEBS Lett. **308**, 183–189
Nebreda, A. R. and Hunt, T. (1993) EMBO J. **12**, 1979–1986
Pelech, S. L. and Sanghera, J. S. (1992) Trends Biochem. Sci. **17**, 233–238
Posada, J., Yew, N., Ahn, N. G., Vande Woude, G. F. and Cooper, J. A. (1993) Mol. Cell. Biol. **13**, 2546–2553
Pulverer, B. J., Kyriakis, J. M., Avruch, J., Nikolakaki, E. and Woodgett, J. R. (1991) Nature (London) **353**, 670–674
Rollins, B. J., Morrison, E. D., Usher, P. and Flier, J. S. (1988) J. Biol. Chem. **263**, 16523–16526
Stokoe, D., Campbell, D. G., Nakielny, S., Hidaka, H., Leevers, S. J., Marshall, C. and Cohen, P. (1992) EMBO J. **11**, 3985–3994
Sturgill, T. W. and Wu, J. (1991) Biochim. Biophys. Acta **1092**, 350–357
Sturgill, T. W., Ray, L. B., Erikson, E. and Maller, J. L. (1988) Nature (London) **334**, 715–718
Sutherland, C., Campbell, D. G. and Cohen, P. (1993) Eur. J. Biochem. **212**, 581–588
Thomas, G. (1992) Cell **68**, 1–4
Yang, J., Clark, A., Kozka, I. J., Cushman, S. W. and Holman, G. D. (1992) J. Biol. Chem. **267**, 10393–10399

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