MINIREVIEW / MINISYNTHÈSE

Activation of the glucose transporter GLUT4 by insulin

L. Michelle Furtado, Romel Somwar, Gary Sweeney, Wenyan Niu, and Amira Klip

Abstract: The transport of glucose into cells and tissues is a highly regulated process, mediated by a family of facilitative glucose transporters (GLUTs). Insulin-stimulated glucose uptake is primarily mediated by the transporter isoform GLUT4, which is predominantly expressed in mature skeletal muscle and fat tissues. Our recent work suggests that two separate pathways are initiated in response to insulin: (*i*) to recruit transporters to the cell surface from intracellular pools and (*ii*) to increase the intrinsic activity of the transporters. These pathways are differentially inhibited by wortmannin, demonstrating that the two pathways do not operate in series. Conversely, inhibitors of p38 mitogen-activated protein kinase (MAPK) imply that p38 MAPK is involved only in the regulation of the pathway leading to the insulin-stimulated activation of GLUT4. This review discusses the evidence for the divergence of GLUT4 translocation and activity and proposed mechanisms for the regulation of GLUT4.

Key words: glucose transporter 4 (GLUT4), glucose uptake, p38 MAPK, GLUT4 activity.

Résumé : Le transport du glucose dans les cellules et les tissus est un processus fortement réglé par l'intermédiaire de transporteurs de glucose facilitant (GLUT). La captation du glucose stimulée par l'insuline est principalement attribuable à l'isoforme GLUT4 du transporteur, qui est surtout exprimée dans les muscles squelettiques matures et le tissu adipeux. Nos travaux récents suggèrent que deux voies séparées sont activées à la suite d'une stimulation par l'insuline : (*i*) une voie de translocation des transporteurs d'un pool intracellulaire à la surface des cellules et (*ii*) une autre voie de stimulation de l'activité intrinsèque des transporteurs. Ces voies sont inhibées différemment par la wortmannine, ce qui démontre que les deux voies n'opèrent pas en série. Inversement, des inhibiteurs de la protéine kinase activée par un mitogène (MAPK) p38 indiquent que la MAPK p38 intervient seulement dans la régulation de la voie d'activation de GLUT4 stimulée par l'insuline. Cette revue discute des éléments de preuve concernant la divergence entre la translocation et la stimulation de l'activité de GLUT4 et des mécanismes proposés de régulation de GLUT4.

Mots clés : transporteur du glucose 4, GLUT4, captation du glucose, MAPK p38, activité de GLUT4.

[Traduit par la Rédaction]

Introduction

The cellular mechanism by which insulin stimulates glucose transport was unknown until approximately 20 years ago. In 1978, it was first demonstrated that insulin increases the number of functional glucose transporter units at the cell surface of isolated rat adipocytes (Wardzala et al. 1978). Following this initial description, two independent studies reported that these transporter units were recruited to the cell surface from an intracellular site (Cushman and Wardzala 1980; Suzuki and Kono 1980). GLUT4 is the predominant transporter isoform expressed in mature skeletal muscle and fat tissues. Insulin elicits translocation of GLUT4 to the surface of fat cells, and our laboratory has established that this phenomenon also occurs in skeletal muscle of rodents and humans (Douen et al. 1990; Klip et al. 1987; D. Li et al. 2001; Marette et al. 1992). Insulin-induced translocation of GLUT4 from an intracellular compartment to the cell surface has been further demonstrated in the insulin-responsive cell line 3T3-L1 adipocytes (Calderhead et al. 1990) and L6 skeletal myotubes (Mitsumoto and Klip 1992; Tsakiridis et al. 1995). Intriguingly, despite the implementation of diverse strategies to assess GLUT4 translocation to the cell surface, most studies report a consistent discrepancy between the fold stimulation in the rate of glucose uptake into the tissue and the fold translocation of GLUT4 to the plasma membrane (Table 1). To explain this difference between the magnitude of GLUT4 translocation and glucose transport, it has

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Table 1. Discrepancies between GLUT4 translocation and glucose uptake in response to insulin in skeletal muscle.

Glucose uptake fold increase by insulin	GLUT4 in plasma membrane fold increase by insulin	Reference(s)
Rat muscle		
2.7 (perfused hq, A/V glc)	1.8 (GLUT4 ib on PM)	Klip et al. 1990
3.6 (white muscle PM vesicles)	2.5 (GLUT4 ib on white muscle PM)	Goodyear et al. 1991
2.2 (red muscle PM vesicles)	1.6 (GLUT4 ib on white muscle PM)	
2.2 (perfused hq, 3OMG, white quadriceps)	1.5 (GLUT4 ibon white muscle PM)	Marette et al. 1992
2.5 (perfused hq, 3OMG, red quadriceps and soleus muscles)	1.7 (GLUT4 ib on red muscle PM)	
4.5 (PM vesicles)	1.8 (CB binding), 1.4 (GLUT4 ib on PM)	King et al. 1992
6.0 (isolated soleus muscle)	6.0 (ATB-BMPA photolabelling)	Lund et al. 1993
3.0 (PM vesicles)	2.0 (CB binding to PM)	Rosholt et al. 1994
17 (perfused hq, 2DG uptake)	1.5 (GLUT4 ib on PM)	Brozinick et al. 1994
8.6 (isolated soleus muscle)	8.0 (ATB-BMPA photolabelling)	Wilson and Cushman 1994
3.1 (isolated epitroclearis muscle)	3.2 (ATB-BMPA photolabelling)	Hansen et al. 1998
5.2 (isolated soleus muscle)	4.8 (ATB-BMPA photolabelling)	Brozinick et al. 1999
Mouse muscle		
3.0 (isolated soleus muscle)	1.4 (GLUT4 ib on PM)	Deems et al. 1994*a*, 1994*b*
8 (perfused hq)	4.1 (GLUT4 ib on PM)	Brozinick et al. 1996
4.8 (isolated soleus muscle)	7.7 (ATB-BMPA photolabelling)	Zierath et al. 1997
2.8 (isolated soleus muscle)	2.8 (ATB-BMPA photolabelling)	Ryder et al. 1999
Mouse muscle overexpressing GLUT4		
2.5 (hyperinsulinemic clamp)	3.1 (GLUT4 ib on PM)	Deems et al. 1994*b*; Ramlal et al. 1996
10 (perfused hq)	2.6 (GLUT4 ib on PM)	Brozinick et al. 1996
3.0 (isolated epitroclearis muscle)	3.0 (EM, ultrathin slices)	Wang et al. 1996
Human muscle		
3.4 (isolated muscle strips)	1.7 (GLUT4 ib on PM)	Guma et al. 1995

Note: perfused hq, glucose uptake into perfused hindquarter; A/V glc, arteriovenus glucose difference; ib on PM, isolated plasma membrane immunoblotted for GLUT4; 3OMG, 3-*O*-methyl-D-glucose; 2DG, 2-deoxy-D-glucose; CB, cytochalasin B (^{3}H) binding to isolated membranes; EM, electron microscopy.

been suggested that insulin further activates these transporters to take up glucose at a maximal rate.

Development of L6 GLUT4myc cells to distinguish the effects of insulin on GLUT4 translocation versus GLUT4 activation

Until recently, it was not possible to determine experimentally whether GLUT4 *activation* occurs in addition to GLUT4 translocation. The available methodologies for measuring GLUT4 translocation could not account for the net changes in GLUT4 localization in intact cells. In addition, the only two cell lines that express GLUT4 and the machinery for its translocation, 3T3-L1 adipocytes and L6 myotubes (Mitsumoto and Klip 1992; Robinson et al. 1992), also abundantly express another transporter, GLUT1, which contributes significantly to glucose uptake. To circumvent these difficulties and allow valid comparisons of GLUT4 translocation vis a vis glucose uptake, our group developed and characterized an L6 muscle cell line, which stably expresses *myc*-tagged GLUT4 (Ueyama et al. 1999; Q. Wang et al. 1998; Wang et al. 1999), outnumbering endogenous GLUTs by up to 100 fold (Huang et al. 2002). Since there are no natural exofacial antigenic sites in GLUT4, the presence of the tag in its first exofacial loop is key to detect GLUT4 at the cell surface via enzyme-linked immunocytochemistry or immunofluorescence (Foster et al. 2001; D. Li et al. 2001).

In characterizing L6-GLUT4myc differentiated myotubes, we found the following.

(*i*) GLUT4myc behaves as the endogenous GLUT4 under basal conditions

In spite of overexpression, 90% of GLUT4myc resides intracellularly in the basal state (D. Li et al. 2001) and all GLUT4myc molecules participate in normal recycling to the cell surface (Foster et al. 2001).

(*ii*) GLUT4myc responds to insulin

Insulin stimulation elicits a rapid ($t_{1/2}$ = 2.5 min) externalization of GLUT4myc (Somwar et al. 2001*a*) to achieve a new steady state with 25% of GLUT4myc at the plasma membrane (D. Li et al. 2001).

(*iii*) Glucose uptake is mediated exclusively by GLUT4myc

An inhibitor of HIV proteases, indinavir, rapidly binds and inhibits GLUT4 but not other GLUTs (Murata et al. 2000). The presence of indinavir during a 5-min glucose transport assay inhibited both basal and insulin-stimulated glucose uptake in L6-GLUT4myc muscle cells but not in L6 cells overexpressing GLUT1myc (instead of GLUT4). Indinavir

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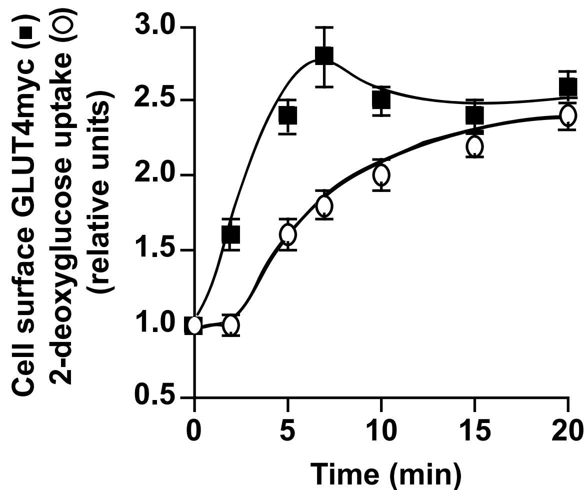
Fig. 1. GLUT4 translocation precedes the stimulation of glucose uptake by insulin. L6-GLUT4myc myotubes were treated for the indicated time periods with 100 nM insulin at 37°C. Cell surface GLUT4myc level (■) was then determined in intact cells. In parallel, 2-deoxyglucose uptake (○) was measured in the continued presence of insulin at 37°C. The time of insulin treatment shown does not include the time of the uptake assay. Results are the mean ± SE of nine (GLUT4 translocation) or five (2-deoxyglucose) experiments. Glucose uptake or GLUT4myc translocation is expressed relative to the respective basal values. (Adapted from (Somwar et al. 2001*a*.)



(50 μM) inhibited insulin-stimulated glucose uptake by 80% in L6-GLUT4myc cells but by less than 9% in L6-GLUT1myc cells (Huang et al. 2002). Thus, L6-GLUT4myc cells are ideal to study glucose exclusively through GLUT4, distinguishing them from all other cell lines available to study glucose uptake. Since GLUT4 is the major transporter responsible for glucose uptake in mature tissues, lessons learned with the L6-GLUT4myc cells may be applicable to these tissues.

From these studies, we concluded that GLUT4myc cells constitute a valuable instrument to differentiate the contribution of GLUT4 translocation and its activity to the final outcome of glucose uptake. We soon found conditions segregating GLUT4 translocation from glucose uptake.

Temporal shift between maximal GLUT4 translocation and glucose uptake

Through research in our laboratory, it was discovered that GLUT4myc translocation ($t_{1/2}$ = 2.5 min) precedes the stimulation of glucose uptake ($t_{1/2}$ = 6 min) (Fig. 1) in response to insulin. The temporal difference was enhanced when insulin action was studied at 22°C and compared with results obtained at 37°C, indicating that GLUT4 activation is more temperature sensitive than GLUT4 translocation (Somwar et al. 2001*a*).

Glucose uptake has a higher sensitivity to wortmannin than GLUT4 translocation

GLUT4 translocation requires phosphatidylinositol (PI) 3-kinase. Wortmannin inhibits PI 3-kinase by covalently modifying a conserved Lys residue within the catalytic domain of the p110 subunit. This residue is known to be crucial for the phosphate transfer reaction (Wymann et al. 1996). Interestingly, we found that wortmannin treatment abolished the stimulation of glucose uptake by insulin with an IC_{50} of 3 nM, whereas the gain in GLUT4myc at the plasma membrane was less sensitive to inhibition by wortmannin (IC_{50} = 43 nM). Similar results were obtained in 3T3-L1 adipocytes (Hausdorff et al. 1999). These results thus reveal that GLUT4 activity and GLUT4 translocation are regulated by signalling pathways with high and low sensitivities to wortmannin, respectively. Class IA PI 3-kinases are inhibited in vitro by wortmannin with IC_{50} values of 0.1–5 nM and in intact cells with IC_{50} values of 10–30 nM (Arcaro and Wymann 1993; Ui et al. 1995). PI 3-kinase-C2α is inhibited by wortmannin with an IC_{50} of 420 nM (Domin et al. 1997). PI 3-kinase-C2β, on the other hand, is much more sensitive to inhibition by wortmannin (IC_{50} = 2 nM), while full inhibition of PI 3-kinase-C2γ in vitro required 10 μM wortmannin (Misawa et al. 1998). Hence, inhibition of GLUT4myc translocation may arise from inhibition of class IA PI 3-kinases. Stimulation of glucose uptake may involve input from PI 3-kinase-C2β or from another process with very high sensitivity to wortmannin.

Possible participation of p38 mitogen-activated protein kinases (MAPKs) in GLUT4 activation

p38 MAPK: function, isoforms, and targets

p38 MAPK was first identified as an enzyme activated by stressors such as hypoxia, hypertonicity, UV light, oxidative radicals, and protein synthesis inhibitors (Jensen et al. 1997; Rappsilber et al. 2000). However, through our work and that of others, it is now clear that p38 MAPK is also activated by some hormones and growth factors (insulin, IGF-1, and FGF) (Kyriakis and Avruch 2001; Pawson and Scott 1997; Somwar et al. 2000) and by muscle contraction (Somwar et al. 2000). Four p38 MAPK cDNAs encoding proteins of similar size (360–372 amino acids) have been cloned in mammals (Kyriakis and Avruch 2001). The proteins p38α, p38β, p38γ, and p38δ are 60–70% homologous in their amino acid sequence, and splice variants of p38α (Mxi2) (Sanz et al. 2000) and p38β MAPK (p38β2) (Kumar et al. 1997) have been described. p38α is widely distributed in most tissues (mice) and cell lines, whereas p38β shows highest expression in heart and skeletal muscle (Kumar et al. 1997). p38γ MAPK is also present primarily in muscle (Li et al. 1996), and p38δ is enriched in lung, testis, pancreas, and small intestine (Hu et al. 1999). Emerging evidence suggests that each isoform has different substrate preference and function (Balasubramanian et al. 2002; W. Li et al. 2001; New et al. 1998; Y. Wang et al. 1998), and splice variants may also exhibit target specificity.

All MAPKs are proline-directed kinases that phosphorylate their targets on S or T residues within the minimum consensus P/L-X-(**S**/**T**)-P. However, physiological MAPK substrates also have specific docking sites distant from the phosphorylated site that permit interaction with selective MAPKs (Barsyte-Lovejoy et al. 2002; Tanno et al. 2001). Proteins identified so far as targets of p38 MAPK in intact cells and (or) in vitro include transcription factors

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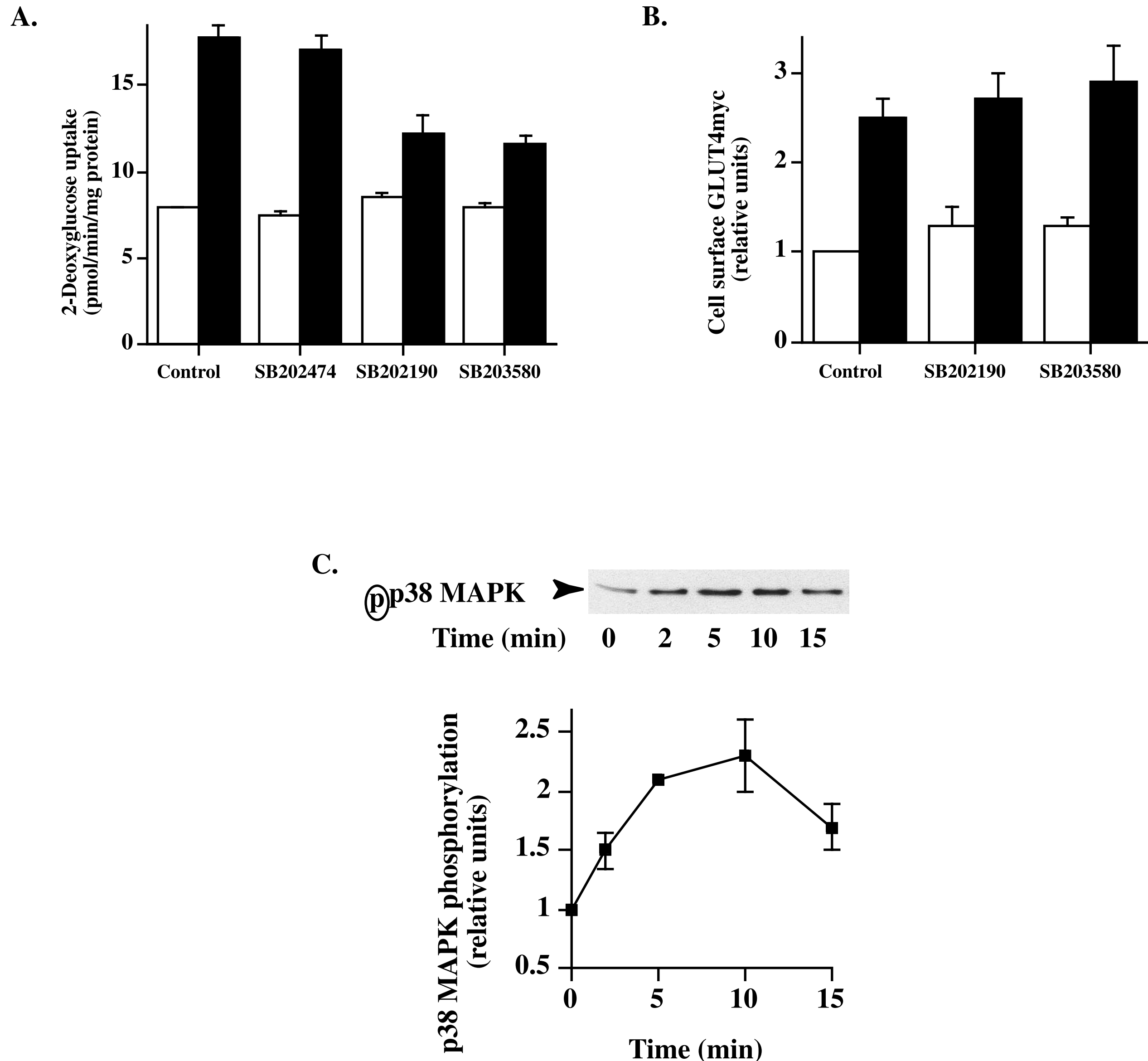
Fig. 2. SB202190 and SB203580 reduce insulin-stimulated glucose uptake but not GLUT4 translocation and increase p38 MAPK phosphorylation. (A) Cells were treated for 20 min with dimethyl sulfoxide vehicle alone or with 10 µM SB202474 (inactive analog), SB202190, or SB203580 prior to insulin treatment for an additional 20 min. 2-Deoxyglucose was then determined. Open bars, basal; solid bars, insulin treatment; $*P < 0.05$ compared with basal, $\#p < 0.01$ compared with insulin-treated control. Results are the mean ± SE of two to five experiments. Each condition was assayed in triplicate. (B) Surface GLUT4myc levels were determined in cells that were treated as described. Cell surface GLUT4myc levels are expressed relative to basal levels. Results are the mean ± SE of three experiments. Open bars, basal; solid bars, insulin. (C) Lysates were prepared from cells that were treated with 100 nM insulin for the indicated time periods. Fifty micrograms of total protein was resolved by 10% sodium dodecyl sulfate – polyacrylamide gel electrophoresis and immunoblotted with a phospho-specific p38 MAPK antibody. A representative immunoblot is shown. Phosphorylated p38 MAPK was quantitated and is shown in the accompanying graphs. All values are expressed relative to basal phosphorylation. Results are the mean ± SE of four experiments. (Adapted from Somwar et al. 2001*a*.)



(e.g., ATF2, MEF2A, and SAP1), certain protein kinases (MAPKAPK2, MAPKAPK3, MNK1, and PRAK) (Sharrocks et al. 2000), and the ion transporter NHE1 (Khaled et al. 2001).

Pharmacological inhibitors of p38 MAPK

The most widely used inhibitors of p38α and p38β MAPK and are of the pyridinylimidazole family, such as SB202190 and SB203580 (Lee et al. 1994). Several other categories of inhibitors that are structurally different from the pyridinylimidazoles have also been described (de Laszlo et al. 1998; Dumas et al. 2000; Salituro et al. 1999). In vitro, SB203580 and SB202190 inhibit p38α MAPK with IC_{50} values of 50–100 and 10–50 nM, respectively, and p38β2 MAPK with IC_{50} values of 80–500 and 20–100 nM, respectively (Davies et al. 2000; Kumar et al. 1997). In intact cells, however, higher concentrations are needed to prevent phosphorylation of p38 MAPK substrates such as ATF2 and

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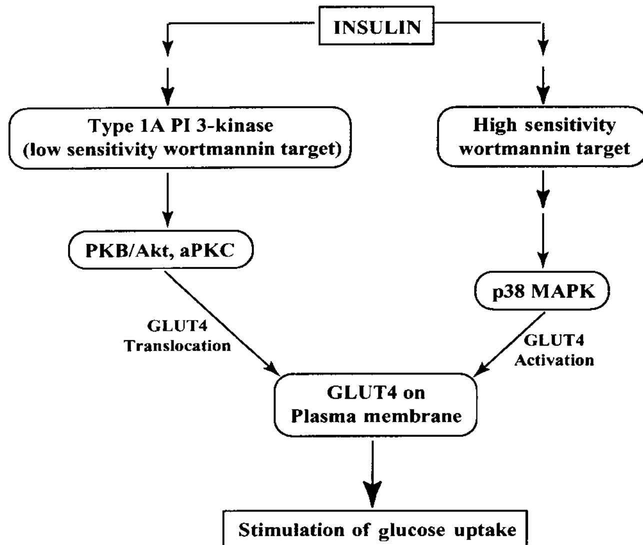
Fig. 3. Proposed model for the stimulation of glucose uptake by insulin. The studies summarized in this review suggest the following scenario. Exposure of muscle or adipose cells to insulin results in activation of the PI 3-kinase – PKB/Akt signalling pathway. Based on the use of wortmannin, LY294002, and dominant-negative mutants of PI 3-kinase and PKB/Akt, this pathway likely mediates GLUT4 translocation to the cell surface. Insulin also activates the p38 MAPK signalling pathway via a cellular target with higher sensitivity towards wortmannin. GLUT4 is then activated by a p38 MAPK dependent mechanism.



MAPKAPK2 (Eyers et al. 1999; Somwar et al. 2001*a*; Tan et al. 1996). Intriguingly, SB202190 or SB203580 do not inhibit p38γ and p38δ MAPK at concentrations that fully inhibit p38α and p38β MAPK (Kumar et al. 1999).

SB202190 and SB203580 reduce the stimulation of 2-deoxyglucose and 3-*O*-methylglucose uptake by insulin

SB202474 is a structurally related analogue of SB202190 and SB203580 that is a poor inhibitor of p38 MAPK (Lee et al. 1994). Our laboratory has determined the effect of preincubation of myotubes with 10 μM SB202190, SB203580, or SB202474 on the stimulation of glucose uptake and GLUT4 translocation by insulin. This concentration of either SB202190 or SB203580 fully inhibits the enzymes in intact cells (Eyers et al. 1999). The effect of these drugs on 2-deoxyglucose uptake is shown in Fig. 2. Preincubation of myotubes with 10 μM SB202474 did not cause a significant change in basal or insulin-stimulated 2-deoxyglucose uptake. In contrast, SB202190 or SB203580 reduced insulin-induced 2-deoxyglucose uptake by about 60% (Somwar et al. 2001*a*).

To explore whether the inhibitory effect of SB203580 on 2-deoxyglucose uptake was due to inhibition of glucose transport activity per se and not to secondary effects on posttransport glucose utilization, we measured 3-*O*-methylglucose transport. This nonmetabolizable analog of glucose is transported but is not phosphorylated upon entry into the cell. Importantly, preincubation of cells with 10 μM SB203580 reduced the ensuing insulin-dependent 3-*O*-methylglucose uptake by 56%. These results suggest that the effect of the p38 MAPK inhibitors was due to inhibition of glucose transport activity and not to any alteration in posttransport glucose phosphorylation. Moreover, the action of SB203580 on insulin-stimulated hexose uptake was one of reducing the apparent V_{max} for transport of either 2-deoxyglucose or 3-*O*-methylglucose, without effect on the apparent K_m for either hexose (Somwar et al. 2001*a*).

To gain additional support for the proposed link between p38 MAPK activation by insulin and increased glucose uptake, two azaazulene inhibitors of p38 MAPK, which are structurally unrelated to SB203580, A291077 and A304000, were used. As with pyridinyl imidazoles, preincubation of L6 myotubes with A291077 and A304000 reduced insulin-stimulated hexose uptake without impinging on GLUT4 translocation (R. Somwar, S. Koteski, G. Sweeney, R. Sciotti, S. Djuric, J. Trevillyan, C. Berg, P.E. Scherrer, C.M. Rondinone, and A. Klip, personal communication).

Insulin-stimulated GLUT4 translocation is not reduced by SB202190 or SB203580

To determine if the reduction in glucose uptake observed in the presence of the p38 MAPK inhibitors was due to reduced GLUT4 translocation, cell surface GLUT4 levels were measured. Twenty minutes of insulin treatment caused a 2.5-fold increase in plasma membrane GLUT4myc, and this was unaffected by preincubation of cells with 10 μM SB202190 or SB203580. Similarly, the stimulation of GLUT4 translocation by insulin remained unaffected by up to 50 μM of either inhibitor. Hence, the reduction in insulin-stimulated glucose uptake did not arise from a decrease in the number of GLUT4 molecules at the cell surface. These results are consistent with the interpretation that, in addition to GLUT4 translocation, insulin increases glucose uptake by increasing GLUT4 activity and that such activation was prevented by inhibitors of p38 MAPK.

Insulin increases p38 MAPK phosphorylation and activity

Activation of p38 MAPK by cytokines requires dual phosphorylation by specific kinases (MKK3/6) on T180 and Y182 found within a TGY motif (Raingeaud et al. 1995). Detecting the phosphorylation on these sites has become a convenient way to measure p38 MAPK activation. To explore the effect of insulin on p38 MAPK, we monitored enzyme phosphorylation in extracts of L6-GLUT4myc myotubes stimulated with insulin using an antibody that recognizes only the doubly phosphorylated protein (Raingeaud et al. 1995). We observed that phosphorylation of p38 MAPK was rapid and transient, becoming elevated following 2 min of insulin stimulation (1.5-fold), reaching a maximum at 5–10 min (2.3 fold) and declining by 15 min (Fig. 2). The stimulation of p38 MAPK phosphorylation by insulin had a calculated $t_{1/2}$ of approximately 4 min. The EC_{50} for this effect of insulin was approximately 26 nM (R. Somwar, S. Koteski, G. Sweeney, R. Sciotti, S. Djuric, J. Trevillyan, C. Berg, P.E. Scherrer, C.M. Rondinone, and A. Klip, personal communication).

The phospho-specific p38 MAPK antibody is expected to recognize all isoforms of the enzyme. Since the two pyridinylimidazole derivatives that reduced glucose uptake inhibit only p38α and p38β MAPK, we also monitored activation of these two isoforms by insulin. This was achieved by selective immunoprecipitation of each isoform followed

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by an in vitro kinase activity assay using recombinant ATF2 as a substrate. Activation of each isoform was rapid and displayed similar time courses (R. Somwar, S. Koteski, G. Sweeney, R. Sciotti, S. Djuric, J. Trevillyan, C. Berg, P.E. Scherrer, C.M. Rondinone, and A. Klip, personal communication). Both enzymes were activated maximally (about 2.5 fold) within 5 min of addition of insulin, paralleling enzyme phosphorylation (Fig. 2).

Skeletal muscle is the principal site of glucose disposal in mammals; therefore, we examined whether p38 MAPK is also activated by insulin in the mature tissue. Four complementary approaches were used assaying quadriceps and soleus muscles isolated from rats after insulin administration in vivo via portal vein injection. First, insulin injection caused a 2.2-fold increase in p38 MAPK phosphorylation, detected in muscle extracts using a phospho-specific antibody. Second, insulin injection resulted in a 3.2-fold increase in the p38 MAPK signal relative to control when enzyme activation was determined by immunoprecipitation with anti-phosphotyrosine antibodies followed by immunoblotting using anti-p38α and -p38β MAPK antibodies. Third, in vivo insulin treatment for 3.5 min increased phosphorylation of the p38 MAPK effector CREB by 3.5 fold. Finally, activation of p38α and p38β MAPK was also measured in isolated muscle treated with insulin using an in vitro kinase assay (Somwar et al. 2000). Each isoform was immunoprecipitated with isoform-specific antibodies, and the ability of the immunopurified enzymes to phosphorylate myelin basic protein in vitro was determined. Insulin treatment in vivo (3.5 min) increased the activity of p38α MAPK by 2.1 fold. The kinase activity of p38β MAPK was stimulated by 2.4 fold by in vivo insulin treatment.

Hence, in L6 myotubes and mature skeletal muscle, insulin rapidly stimulated p38α and p38β MAPK, challenging the concept that these enzymes are only activated by stress factors.

Activation of p38 MAPK by insulin is prevented by wortmannin with a potency that parallels the inhibition of glucose uptake

As discussed above, regulation of GLUT4 activity by insulin occurs via a wortmannin-sensitive mechanism (Somwar et al. 2001*b*). These observations led us to hypothesize that activation of p38 MAPK by insulin may involve a wortmannin-sensitive target. Interestingly, pretreatment of myotubes with wortmannin reduced the insulin-stimulated p38 MAPK phosphorylation with an IC_{50} of 7 nM. This effect was specific for insulin, as phosphorylation of p38 MAPK elicited by treating myotubes with anisomycin or mannitol was not affected by wortmannin pretreatment (C. Huang, R. Somwar, and A. Klip, personal communication). Moreover, the insulin-dependent activation of p38 MAPK towards endogenous CREB and exogenous ATF2 was highly sensitive to inhibition by wortmannin pretreatment of cells. The IC_{50} calculated for this effect was 6 nM.

From all of these studies, a correlation emerged between the high sensitivity to wortmannin of p38 MAPK activation and of glucose uptake. Instead, a lower sensitivity to wortmannin was displayed by GLUT4 translocation and to the insulin-stimulated activities of enzymes downstream of PI 3-kinase such as PKB/Akt and atypical PKC. Our current hypothesis is that the high sensitivity to wortmannin is due to a signal upstream of p38 MAPK, which is not PI 3-kinase. Future work is aimed at examining the nature of this signal.

Expression of a dominant-negative p38 MAPK mutant (p38AGF) to study the regulation of GLUT4 activity

A mutant p38 MAPK (p38AGF) in which the regulatory phosphorylation sites have been replaced by alanine and phenylalanine, respectively, has been used widely to investigate p38 MAPK function in vivo (Ludwig et al. 1998; Rincon et al. 1998; Y. Wang et al. 1998). We examined the participation of p38 MAPK in insulin action using a 3T3-L1 cell line that stably expressed this mutant p38 MAPK cDNA under the control of the *lac* operon (Engelman et al. 1998). The major advantage of this system is that is allows relatively tight repression of the transgene in the absence of the inducer isopropyl thio-β-D-galactoside (IPTG). Treatment of cells with IPTG overrides this transcriptional repression, thereby allowing expression of the construct. Insulin treatment (100 nM, 10 min) increased p38 MAPK and CREB phosphorylation by 2.2-fold relative to basal levels in these p38AGF-3T3-L1 adipocytes that were not treated with IPTG. Expression of p38AGF in response to IPTG treatment abrogated insulin-induced CREB phosphorylation without any significant effect on basal CREB phosphorylation, suggesting that p38AGF acts as a dominant-negative inhibitor of endogenous p38 MAPK signalling. Importantly, induction of p38AGF expression reduced insulin-stimulated glucose uptake by 40% without affecting basal glucose uptake. Moreover, there was no further reduction in insulin-stimulated glucose uptake when IPTG-treated p38AGF-3T3-L1 adipocytes were incubated with 10 μM SB203580 prior to insulin action. In contrast, the gain in surface GLUT4 was not altered. These results support the concept that insulin increased the intrinsic activity of GLUT4 via a p38 MAPK dependent signal (R. Somwar, S. Koteski, G. Sweeney, R. Sciotti, S. Djuric, J. Trevillyan, C. Berg, P.E. Scherrer, C.M. Rondinone, and A. Klip, personal communication).

Possible mechanisms for regulation of GLUT4

Phosphorylation of GLUT4

The implication of p38 MAPK in the regulation of GLUT4 activation by insulin begs an examination of the existing literature regarding the phosphorylation status of GLUT4. Results from sequential Edman degradation identified S488 as the major site of phosphorylation of GLUT4 in unstimulated cells (Lawrence et al. 1990*a*). Agents that increase cAMP or elevate intracellular calcium or inhibitors of phosphatases such as okadaic acid (Begum et al. 1993; Lawrence et al. 1990*b*; Reusch et al. 1991) increased GLUT4 phosphorylation at its C terminus. Treatment of isolated rat adipocytes with parathyroid hormone prior to an acute insulin challenge resulted in normal insulin-induced GLUT4 translocation, enhanced GLUT4 phosphorylation, and inhibition of insulin-induced glucose uptake (Reusch et al. 1993). Several other studies have confirmed the finding that agents that elevate GLUT4 phosphorylation reduce the stimulation of glucose uptake by insulin (Begum et al. 1993; Lawrence et al. 1990*b*; Reusch et al. 1991; Schurmann et al. 1992).

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Therefore, studies addressing the functional consequence of GLUT4 phosphorylation on GLUT4 intrinsic activity argue for a negative correlation between these two parameters (Lawrence 1994).

In untreated 3T3-L1 adipocytes, GLUT4 is more phosphorylated in the plasma membrane than in internal membranes. Insulin treatment does not cause any significant change in phosphorylation of total GLUT4. However, insulin reduced the amount of ^{32}P-labelled GLUT4 in the plasma membrane (James et al. 1989; Lawrence et al. 1990*a*; Reusch et al. 1993).

From the above summary of the literature, it is clear that additional studies are required to clarify the effect of insulin on the phosphorylation status of GLUT4 molecules that are properly inserted into the plasma membrane and to examine its consequence on insulin-dependent glucose uptake. We have also measured the level of surface GLUT4 phosphorylation in L6-GLUT4myc myotubes (L.M. Furtado and A. Klip, personal communication). Because the transporters were immunoprecipitated via the exofacially exposed myc epitope, this assay probes for phosphorylation of only those GLUT4 molecules fully inserted into the plasma membrane. The results confirm that, indeed, insulin treatment lowers the amount of phosphorylation of surface GLUT4 molecules. Further studies are required to establish if phosphorylation of GLUT4 directly controls its activity.

Translocation to the cell surface in response to insulin of a GLUT4 with an S488A mutation was normal (Marsh et al. 1998). Unfortunately, glucose uptake was not determined in that study, preventing any correlative relationship between GLUT4 phosphorylation and intrinsic activity of the transporter upon stimulation by insulin.

Regulatory proteins

It is conceivable that dephosphorylation of GLUT4 could be necessary to remove inhibitory protein(s) interfering with GLUT4 function or to permit binding of activator(s) that may enhance the catalytic activity of GLUT4. Alternatively, dephosphorylation of the GLUT4 C terminus could induce a conformational change in the molecule, thereby activating the transporter.

An important question emerges as to precisely how insulin-dependent p38 MAPK activation leads to enhanced GLUT4 activity. Although GLUT4 has a C-terminal phosphorylation site, it is unlikely that phosphorylation of this site by p38 MAPK would activate the transporter for two reasons. First, the sequence surrounding the phosphoacceptor site (AFRRTPSLL) does not conform to the minimum consensus required for phosphorylation by p38 MAPK (P/LXPS, where X is any small hydrophobic residue). Second, as summarized above, phosphorylation of GLUT4 in the plasma membrane correlates with inhibition of insulin-induced glucose uptake (Lawrence 1994). Instead, dephosphorylation of GLUT4 by a p38 MAPK activated phosphatase could conceivably result in activation of the transporter by alleviating any constraints imposed by the phosphorylated C terminus, as mentioned above.

The GLUT4 C terminus has indeed been implicated in regulating transport activity of the molecule. Expression of a GLUT4 mutant lacking 20 C-terminal amino acids in *Xenopus* oocytes enhanced the turnover number of the transporter by 3.5-fold (Dauterive et al. 1996). This result suggests that GLUT4 activity may be restricted by its C terminus. Consistent with this finding, the GLUT4 C terminus becomes more accessible to antibodies in response to insulin (Smith et al. 1991; Wang et al. 1996). This could be due to a conformational change or to dissociation of an inhibitory protein. Association of this inhibitor with GLUT4 in resting cells could be dependent or independent of GLUT4 phosphorylation.

Regrettably, of the proteins known to date to interact with GLUT4, none have been shown to dissociate from the transporter in an insulin-dependent manner. In addition, none of these proteins are known to be substrates of p38 MAPK. Most of the p38 MAPK substrates identified so far are either transcription factors or, with less frequency, protein kinases or phosphatases (Cohen 1997; Ono and Han 2000). We speculate that one of these protein kinases may act at an intermediate step to phosphorylate a GLUT4-binding protein, resulting in the dissociation of the GLUT4–inhibitor complex. Alternatively, p38 MAPK could allow a phosphatase to dephosphorylate S488 on GLUT4.

Conclusions

The studies summarized here indicate that insulin stimulates glucose uptake in muscle and fat cells by activating at least two signalling cascades (Fig. 3). One of these governs GLUT4 translocation and the other facilitates GLUT4 activation. Translocation of GLUT4 to the plasma membrane is regulated by the PI 3-kinase signalling network, whereas GLUT4 activation is possibly regulated by a p38 MAPK signalling pathway.

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References

Arcaro, A., and Wymann, M.P. 1993. Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem. J. **296**: 297–301.

Balasubramanian, S., Efimova, T., and Eckert, R.L. 2002. Green tea polyphenol stimulates a Ras, MEKK1, MEK3, and p38 cascade to increase activator protein 1 factor-dependent involucrin gene expression in normal human keratinocytes. J. Biol. Chem. **277**: 1828–1836.

Barsyte-Lovejoy, D., Galanis, A., and Sharrocks, A.D. 2002. Specificity determinants in MAPK signaling to transcription factors. J. Biol. Chem. **277**: 9896–9903.

Begum, N., Leitner, W., Reusch, J.E., Sussman, K.E., and Draznin, B. 1993. GLUT-4 phosphorylation and its intrinsic activity.

© 2002 NRC Canada

Mechanism of Ca(2+)-induced inhibition of insulin-stimulated glucose transport. J. Biol. Chem. **268**: 3352–3356.

Brozinick, J.T., Jr., Etgen, G.J., Jr., Yaspelkis, B.B., 3rd, and Ivy, J.L. 1994. Glucose uptake and GLUT-4 protein distribution in skeletal muscle of the obese Zucker rat. Am. J. Physiol. **267**: R236–R243.

Brozinick, J.T., Jr., Yaspelkis, B.B., 3rd, Wilson, C.M., Grant, K.E., Gibbs, E.M., Cushman, S.W., and Ivy, J.L. 1996. Glucose transport and GLUT4 protein distribution in skeletal muscle of GLUT4 transgenic mice. Biochem. J. **313**: 133–140.

Brozinick, J.T., Jr., Reynolds, T.H., Dean, D., Cartee, G., and Cushman, S.W. 1999. 1-[*N*, *O*-bis-(5-isoquinolinesulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62), an inhibitor of calcium-dependent camodulin protein kinase II, inhibits both insulin- and hypoxia-stimulated glucose transport in skeletal muscle. Biochem. J. **339**: 533–540.

Calderhead, D.M., Kitagawa, K., Tanner, L.I., Holman, G.D., and Lienhard, G.E. 1990. Insulin regulation of the two glucose transporters in 3T3-L1 adipocytes. J. Biol. Chem. **265**: 13 801 – 13 808.

Cohen, P. 1997. The search for physiological substrates of MAP and SAP kinases in mammalian cells. Trends Cell Biol. **7**: 353–361.

Cushman, S.W., and Wardzala, L.J. 1980. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J. Biol. Chem. **255**: 4758–4762.

Dauterive, R., Laroux, S., Bunn, R.C., Chaisson, A., Sanson, T., and Reed, B.C. 1996. C-terminal mutations that alter the turnover number for 3-*O*-methylglucose transport by GLUT1 and GLUT4. J. Biol. Chem. **271**: 11 414 – 11 421.

Davies, S.P., Reddy, H., Caivano, M., and Cohen, P. 2000. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem. J. **351**: 95–105.

Deems, R.O., Deacon, R.W., Ramlal, T., Volchuk, A., Klip, A., and Young, D.A. 1994*a*. Insulin action on whole body glucose utilization and on muscle glucose transporter translocation in mice. Biochem. Biophys. Res. Commun. **199**: 662–670.

Deems, R.O., Evans, J.L., Deacon, R.W., Honer, C.M., Chu, D.T., Burki, K., Fillers, W.S., Cohen, D.K., and Young, D.A. 1994*b*. Expression of human GLUT4 in mice results in increased insulin action. Diabetologia, **37**: 1097–1104.

de Laszlo, S.E., Visco, D., Agarwal, L., Chang, L., Chin, J., et al. 1998. Pyrroles and other heterocycles as inhibitors of p38 kinase. Bioorg. Med. Chem. Lett. **8**: 2689–2694.

Domin, J., Pages, F., Volinia, S., Rittenhouse, S.E., Zvelebil, M.J., Stein, R.C., and Waterfield, M.D. 1997. Cloning of a human phosphoinositide 3-kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin. Biochem. J. **326**: 139–147.

Douen, A.G., Ramlal, T., Rastogi, S., Bilan, P.J., Cartee, G.D., Vranic, M., Holloszy, J.O., and Klip, A. 1990. Exercise induces recruitment of the “insulin-responsive glucose transporter”. Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. J. Biol. Chem. **265**: 13 427 – 13 430.

Dumas, J., Sibley, R., Riedl, B., Monahan, M.K., Lee, W., Lowinger, T.B., Redman, A.M., Johnson, J.S., Kingery-Wood, J., Scott, W.J., et al. 2000. Discovery of a new class of p38 kinase inhibitors. Bioorg. Med. Chem. Lett. **10**: 2047–2050.

Engelman, J.A., Lisanti, M.P., and Scherer, P.E. 1998. Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. J. Biol. Chem. **273**: 32 111 – 32 120.

Eyers, P.A., van den, I.P., Quinlan, R.A., Goedert, M., and Cohen, P. 1999. Use of a drug-resistant mutant of stress-activated protein kinase 2a/p38 to validate the in vivo specificity of SB 203580. FEBS Lett. **451**: 191–196.

Foster, L.J., Li, D., Randhawa, V.K., and Klip, A. 2001. Insulin accelerates inter-endosomal GLUT4 traffic via phosphatidylinositol 3-kinase and protein kinase B. J. Biol. Chem. **276**: 44 212 – 44 221.

Goodyear, L.J., Hirshman, M.F., Smith, R.J., and Horton, E.S. 1991. Glucose transporter number, activity, and isoform content in plasma membranes of red and white skeletal muscle. Am. J. Physiol. **261**: E556–E561.

Guma, A., Zierath, J.R., Wallberg-Henriksson, H., and Klip, A. 1995. Insulin induces translocation of GLUT-4 glucose transporters in human skeletal muscle. Am. J. Physiol. **268**: E613–E622.

Hansen, P.A., Han, D.H., Marshall, B.A., Nolte, L.A., Chen, M.M., Mueckler, M., and Holloszy, J.O. 1998. A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J. Biol. Chem. **273**: 26 157 – 26 163.

Hausdorff, S.F., Fingar, D.C., Morioka, K., Garza, L.A., Whiteman, E.L., Summers, S.A., and Birnbaum, M.J. 1999. Identification of wortmannin-sensitive targets in 3T3-L1 adipocytes. Dissociation of insulin-stimulated glucose uptake and glut4 translocation. J. Biol. Chem. **274**: 24 677 – 24 684.

Hu, M.C., Wang, Y.P., Mikhail, A., Qiu, W.R., and Tan, T.H. 1999. Murine p38-delta mitogen-activated protein kinase, a developmentally regulated protein kinase that is activated by stress and proinflammatory cytokines. J. Biol. Chem. **274**: 7095–7102.

Huang, C., Somwar, R., Niu, W., Patel, N., Khayat, Z., and Klip, A. 2002. Sustained exposure of L6 myotubes to high glucose and insulin decrease insulin-stimulated GLUT4 translocation but upregulates GLUT4 activity and p38 MAP kinase. Diabetes, **51**: 2090–2098.

James, D.E., Hiken, J., and Lawrence, J.C., Jr. 1989. Isoproterenol stimulates phosphorylation of the insulin-regulatable glucose transporter in rat adipocytes. Proc. Natl. Acad. Sci. U.S.A. **86**: 8368–8372.

Jensen, O.N., Podtelejnikov, A.V., and Mann, M. 1997. Identification of the components of simple protein mixtures by high-accuracy peptide mass mapping and database searching. Anal. Chem. **69**: 4741–4750.

Khaled, A.R., Moor, A.N., Li, A., Kim, K., Ferris, D.K., Muegge, K., Fisher, R.J., Fliegel, L., and Durum, S.K. 2001. Trophic factor withdrawal: p38 mitogen-activated protein kinase activates NHE1, which Induces intracellular alkalinization. Mol. Cell. Biol. **21**: 7545–7557.

King, P.A., Horton, E.D., Hirshman, M.F., and Horton, E.S. 1992. Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation. J. Clin. Invest. **90**: 1568–1575.

Klip, A., Ramlal, T., Young, D.A., and Holloszy, J.O. 1987. Insulin-induced translocation of glucose transporters in rat hindlimb muscles. FEBS Lett. **224**: 224–230.

Klip, A., Ramlal, T., Bilan, P.J., Cartee, G.D., Gulve, E.A., and Holloszy, J.O. 1990. Recruitment of GLUT-4 glucose transporters by insulin in diabetic rat skeletal muscle. Biochem. Biophys. Res. Commun. **172**: 728–736.

Kumar, S., McDonnell, P.C., Gum, R.J., Hand, A.T., Lee, J.C., and Young, P.R. 1997. Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. Biochem. Biophys. Res. Commun. **235**: 533–538.

© 2002 NRC Canada

Kumar, S., Jiang, M.S., Adams, J.L., and Lee, J.C. 1999. Pyridinylimidazole compound SB 203580 inhibits the activity but not the activation of p38 mitogen-activated protein kinase. Biochem. Biophys. Res. Commun. **263**: 825–831.

Kyriakis, J.M., and Avruch, J. 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol. Rev. **81**: 807–869.

Lawrence, J. 1994. Role of GLUT4 phosphorylation in controlling glucose transport. *In* Molecular biology of diabetes. *Edited by* B. Draznin and D. LeRoith. Humana Press Inc., Totowa, N.J. pp. 473–495.

Lawrence, J.C., Jr., Hiken, J.F., and James, D.E. 1990*a*. Phosphorylation of the glucose transporter in rat adipocytes. Identification of the intracellular domain at the carboxyl terminus as a target for phosphorylation in intact-cells and in vitro. J. Biol. Chem. **265**: 2324–2332.

Lawrence, J.C., Jr., Hiken, J.F., and James, D.E. 1990*b*. Stimulation of glucose transport and glucose transporter phosphorylation by okadaic acid in rat adipocytes. J. Biol. Chem. **265**: 19 768 – 19 776.

Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Heys, J.R., Landvatter, S.W., et al. 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature (London), **372**: 739–746.

Li, Z., Jiang, Y., Ulevitch, R.J., and Han, J. 1996. The primary structure of p38 gamma: a new member of p38 group of MAP kinases. Biochem. Biophys. Res. Commun. **228**: 334–340.

Li, D., Randhawa, V.K., Patel, N., Hayashi, M., and Klip, A. 2001. Hyperosmolarity reduces GLUT4 endocytosis and increases its exocytosis from a Vamp2-independent pool in L6 muscle cells. J. Biol. Chem. **276**: 22 883 – 22 891.

Li, W., Nadelman, C., Henry, G., Fan, J., Muellenhoff, M., Medina, E., Gratch, N.S., Chen, M., Han, J., and Woodley, D. 2001. The p38-MAPK/SAPK pathway is required for human keratinocyte migration on dermal collagen. J. Invest. Dermatol. **117**: 1601–1611.

Ludwig, S., Hoffmeyer, A., Goebeler, M., Kilian, K., Hafner, H., Neufeld, B., Han, J., and Rapp, U.R. 1998. The stress inducer arsenite activates mitogen-activated protein kinases extracellular signal-regulated kinases 1 and 2 via a MAPK kinase 6/p38-dependent pathway. J. Biol. Chem. **273**: 1917–1922.

Lund, S., Holman, G.D., Schmitz, O., and Pedersen, O. 1993. Glut 4 content in the plasma membrane of rat skeletal muscle: comparative studies of the subcellular fractionation method and the exofacial photolabelling technique using ATB-BMPA. FEBS Lett. **330**: 312–318.

Marette, A., Richardson, J.M., Ramlal, T., Balon, T.W., Vranic, M., Pessin, J.E., and Klip, A. 1992. Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. Am. J. Physiol. **263**: C443–C452.

Marsh, B.J., Martin, S., Melvin, D.R., Martin, L.B., Alm, R.A., Gould, G.W., and James, D.E. 1998. Mutational analysis of the carboxy-terminal phosphorylation site of GLUT-4 in 3T3-L1 adipocytes. Am. J. Physiol. **275**: E412–E422.

Misawa, H., Ohtsubo, M., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Yoshimura, A. 1998. Cloning and characterization of a novel class II phosphoinositide 3-kinase containing C2 domain. Biochem. Biophys. Res. Commun. **244**: 531–539.

Mitsumoto, Y., and Klip, A. 1992. Development regulation of the subcellular distribution and glycosylation of GLUT1 and GLUT4 glucose transporters during myogenesis of L6 muscle cells. J. Biol. Chem. **267**: 4957–4962.

Murata, H., Hruz, P.W., and Mueckler, M. 2000. The mechanism of insulin resistance caused by HIV protease inhibitor therapy. J. Biol. Chem. **275**: 20 251 – 20 254.

New, L., Jiang, Y., Zhao, M., Liu, K., Zhu, W., Flood, L.J., Kato, Y., Parry, G.C., and Han, J. 1998. PRAK, a novel protein kinase regulated by the p38 MAP kinase. EMBO J. **17**: 3372–3384.

Ono, K., and Han, J. 2000. The p38 signal transduction pathway: activation and function. Cell. Signalling, **12**: 1–13.

Pawson, T., and Scott, J.D. 1997. Signaling through scaffold, anchoring, and adaptor proteins. Science (Washington, D.C.), **278**: 2075–2080.

Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J., and Davis, R.J. 1995. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J. Biol. Chem. **270**: 7420–7426.

Ramlal, T., Ewart, H.S., Somwar, R., Deems, R.O., Valentin, M.A., Young, D.A., and Klip, A. 1996. Muscle subcellular localization and recruitment by insulin of glucose transporters and Na^+-K^+-ATPase subunits in transgenic mice overexpressing the GLUT4 glucose transporter. Diabetes, **45**: 1516–1523.

Rappsilber, J., Siniossoglou, S., Hurt, E.C., and Mann, M. 2000. A generic strategy to analyze the spatial organization of multi-protein complexes by cross-linking and mass spectrometry. Anal. Chem. **72**: 267–275.

Reusch, J.E., Begum, N., Sussman, K.E., and Draznin, B. 1991. Regulation of GLUT-4 phosphorylation by intracellular calcium in adipocytes. Endocrinology, **129**: 3269–3273.

Reusch, J.E., Sussman, K.E., and Draznin, B. 1993. Inverse relationship between GLUT-4 phosphorylation and its intrinsic activity. J. Biol. Chem. **268**: 3348–3351.

Rincon, M., Enslen, H., Raingeaud, J., Recht, M., Zapton, T., Su, M.S., Penix, L.A., Davis, R.J., and Flavell, R.A. 1998. Interferon-gamma expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway. EMBO J. **17**: 2817–2829.

Robinson, L.J., Pang, S., Harris, D.S., Heuser, J., and James, D.E. 1992. Translocation of the glucose transporter (GLUT4) to the cell surface in permeabilized 3T3-L1 adipocytes: effects of ATP insulin, and GTP gamma S and localization of GLUT4 to clathrin lattices. J. Cell Biol. **117**: 1181–1196.

Rosholt, M.N., King, P.A., and Horton, E.S. 1994. High-fat diet reduces glucose transporter responses to both insulin and exercise. Am. J. Physiol. **266**: R95–R101.

Ryder, J.W., Kawano, Y., Chibalin, A.V., Rincon, J., Tsao, T.S., Stenbit, A.E., Combatsiaris, T., Yang, J., Holman, G.D., Charron, M.J., and Zierath, J.R. 1999. In vitro analysis of the glucose-transport system in GLUT4-null skeletal muscle. Biochem. J. **342**: 321–328.

Salituro, F.G., Germann, U.A., Wilson, K.P., Bemis, G.W., Fox, T., and Su, M.S. 1999. Inhibitors of p38 MAP kinase: therapeutic intervention in cytokine-mediated diseases. Curr. Med. Chem. **6**: 807–823.

Sanz, V., Arozarena, I., and Crespo, P. 2000. Distinct carboxy-termini confer divergent characteristics to the mitogen-activated protein kinase p38alpha and its splice isoform Mxi2. FEBS Lett. **474**: 169–174.

Schurmann, A., Mieskes, G., and Joost, H.G. 1992. Phosphorylation of the adipose/muscle-type glucose transporter (GLUT4) and its relationship to glucose transport activity. Biochem. J. **285**: 223–228.

Sharrocks, A.D., Yang, S.H., and Galanis, A. 2000. Docking domains and substrate-specificity determination for MAP kinases. Trends Biochem. Sci. **25**: 448–453.

© 2002 NRC Canada

Smith, R.M., Charron, M.J., Shah, N., Lodish, H.F., and Jarett, L. 1991. Immunoelectron microscopic demonstration of insulin-stimulated translocation of glucose transporters to the plasma membrane of isolated rat adipocytes and masking of the carboxyl-terminal epitope of intracellular GLUT4. Proc. Natl. Acad. Sci. U.S.A. **88**: 6893–6897.

Somwar, R., Perreault, M., Kapur, S., Taha, C., Sweeney, G., Ramlal, T., Kim, D.Y., Keen, J., Cote, C.H., Klip, A., and Marette, A. 2000. Activation of p38 mitogen-activated protein kinase alpha and beta by insulin and contraction in rat skeletal muscle: potential role in the stimulation of glucose transport. Diabetes, **49**: 1794–1800.

Somwar, R., Kim, D.Y., Sweeney, G., Huang, C., Niu, W., Lador, C., Ramlal, T., and Klip, A. 2001*a*. GLUT4 translocation precedes the stimulation of glucose uptake in L6 cells: potential activation of GLUT4 by p38 mitogen activated protein kinase. Biochem. J. **359**: 639–649.

Somwar, R., Niu, W., Kim, D., Sweeney, G., Rhandawa, V., Huang, C., Ramlal, T., and Klip, A. 2001*b*. Differential effects of PI 3-kinase inhibition on signals GLUT4 translocation and glucose uptake. J. Biol. Chem. **276**: 46 079 – 46 087.

Suzuki, K., and Kono, T. 1980. Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc. Natl. Acad. Sci. U.S.A. **77**: 2542–2545.

Tan, Y., Rouse, J., Zhang, A., Cariati, S., Cohen, P., and Comb, M.J. 1996. FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. EMBO J. **15**: 4629–4642.

Tanno, S., Mitsuuchi, Y., Altomare, D.A., Xiao, G.H., and Testa, J.R. 2001. AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. Cancer Res. **61**: 589–593.

Tsakiridis, T., Vranic, M., and Klip, A. 1995. Phosphatidylinositol 3-kinase and the actin network are not required for the stimulation of glucose transport caused by mitochondrial uncoupling: comparison with insulin action. Biochem. J. **309**: 1–5.

Ueyama, A., Yaworsky, K.L., Wang, Q., Ebina, Y., and Klip, A. 1999. GLUT-4myc ectopic expression in L6 myoblasts generates a GLUT-4-specific pool conferring insulin sensitivity. Am. J. Physiol. **277**: E572–E578.

Ui, M., Okada, T., Hazeki, K., and Hazeki, O. 1995. Wortmannin as a unique probe for an intracellular signalling protein, phosphoinositide 3-kinase. Trends Biochem. Sci. **20**: 303–307.

Wang, W., Hansen, P.A., Marshall, B.A., Holloszy, J.O., and Mueckler, M. 1996. Insulin unmasks a COOH-terminal Glut4 epitope and increases glucose transport across T-tubules in skeletal muscle. J. Cell Biol. **135**: 415–430.

Wang, Q., Khayat, Z., Kishi, K., Ebina, Y., and Klip, A. 1998. GLUT4 translocation by insulin in intact muscle cells: detection by a fast and quantitative assay. FEBS Lett. **427**: 193–197.

Wang, Y., Huang, S., Sah, V.P., Ross, J., Jr., Brown, J.H., Han, J., and Chien, K.R. 1998. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J. Biol. Chem. **273**: 2161–2168.

Wang, Q., Somwar, R., Bilan, P.J., Liu, Z., Jin, J., Woodgett, J.R., and Klip, A. 1999. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. Mol. Cell. Biol. **19**: 4008–4018.

Wardzala, L.J., Cushman, S.W., and Salans, L.B. 1978. Mechanism of insulin action on glucose transport in the isolated rat adipose cell. Enhancement of the number of functional transport systems. J. Biol. Chem. **253**: 8002–8005.

Wilson, C.M., and Cushman, S.W. 1994. Insulin stimulation of glucose transport activity in rat skeletal muscle: increase in cell surface GLUT4 as assessed by photolabelling. Biochem. J. **299**: 755–759.

Wymann, M.P., Bulgarelli-Leva, G., Zvelebil, M.J., Pirola, L., Vanhaesebroeck, B., Waterfield, M.D., and Panayotou, G. 1996. Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. Mol. Cell. Biol. **16**: 1722–1733.

Zierath, J.R., Houseknecht, K.L., Gnudi, L., and Kahn, B.B. 1997. High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. Diabetes, **46**: 215–223.

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