Arch Pharm Res Vol 22, No 4, 329-334, 1999 Review

Glucose Transporters and Insulin Action: Some Insights into Diabetes Management

Chan Y. Jung and Wan Lee

The Biophysics Laboratory, VA Medical Center and the Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY 14215, USA

(Received February 5, 1999)

Insulin stimulates glucose uptake in muscle and adipose cells primarily by recruiting GLUT4 from an intracellular storage pool to the plasma membrane. Dysfunction of this process known as insulin resistance causes hyperglycemia, a hallmark of diabetes and obesity. Thus the understanding of the mechanisms underlying this process at the molecular level may give an insight into the prevention and treatment of these health problems. GLUT4 in rat adipocytes, for example, constantly recycles between the cell surface and an intracellular pool by endocytosis and exocytosis, each of which is regulated by an insulin-sensitive and GLUT4-selective sorting mechanism. Our working hypothesis has been that this sorting mechanism includes a specific interaction of a cytosolic protein with the GLUT4 cytoplasmic domain. Indeed, a synthetic peptide of the C-terminal cytoplasmic domain of GLUT4 induces an insulin-like GLUT4 recruitment when introduced in rat adipocytes. Relevance of these observations to a novel euglycemic drug design is discussed.

Key words : Diabetes, Insulin action, Glucose transporter

Glucose homeostasis and GLUT4 function

In humans and mammals, the blood glucose level is kept within a narrow concentration range, the regulation known as glucose homeostasis (DeFronzo, 1988). A derangement in this regulation results in hyperglycemia, which is responsible for many vascular and neurological complications seen in diabetes and obesity (Warram *et al.*, 1990; Garvey, 1992; Kahn, 1996). The results of the Diabetes Control and Complication Trial (DCCT) studies clearly show that more than 60% of diabetic complications can be prevented by more precise control of blood glucose levels (anonymous,1997)

Glucose utilization by muscle accounts for as much as 70% of the total body glucose consumption, thus representing a key control segment in glucose homeostasis (DeFronzo *et al.*, 1981). The muscle glucose utilization is regulated at the point of entry (transport) of glucose into the cell (Kahn, 1996). The process is mediated by two isoforms of the facilitative glucose transporter family, mostly by GLUT4 and in part by GLUT1 (Pessin and Bell, 1992; Mueckler, 1994), and further regulated by metabolites and hormones, most importantly by insulin (Birmbaum, 1992; Stephens and Pilch, 1995). GLUT4 in these cells are mostly (90% or more) kept idle in an intracellular compartment, and insulin recruits GLUT4 from this storage compartment to the plasma membrane (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). This GLUT4 recruitment is the major mechanism by which insulin stimulates glucose uptake in muscles and adipocytes, and represents a key control point for glucose homeostasis (Birmbaum, 1992; Stephens and Pilch, 1995). The dysfunction of this regulation is known to be responsible for the insulin resistance seen in diabetes and obesity (Garvey, 1992; Kahn, 1996).

Correspondence to: Chan Y. Jung, The Biophysics Laboratory, VA Medical Center and the Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY 14215
E-mail: cyjung@acsu.buffalo.edu

Transmembrane topology of GLUT proteins; the cytoplasmic domain and GLUT4 regulation

Five functional isoforms (GLUT1-5) are known in this glucose transporter family, which differ in terms of tissue expression, transport kinetics, substrate specificity, and metabolic and hormonal response (Pessin and Bell, 1992; Mueckler, 1994; Birmbaum, 1992). Molecular biological, biochemical and biophysical data (Pessin and Bell, 1992; Mueckler, 1994; Zheng *et al.*, 1996) indicate that all of these isoforms share a common transmembrane topology (Fig. 1). Each has a large, transmembrane domain (about 50% of the protein mass) made of twelve membrane-spanning alpha helices (TMHs 1-12) forming a

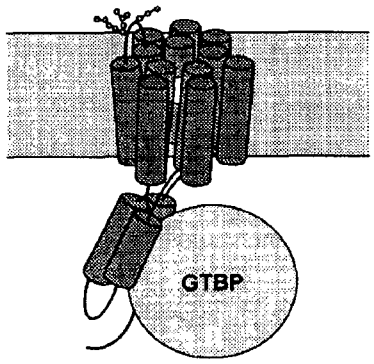

Fig. 1. GLUT1 transmembrane topology and tertiary structure :GLUT 4 structure illustrating putative catalytic and regulatory domains a GULT binding protein (GTBP)

water-filled glucose pathway (Mueckler, 1994; Zheng *et al.*, 1996; Chin *et al.*, 1987; Oka *et al.*, 1990) (the catalytic domain). Each isoform has two nonmembrane domains, the cytoplasmic domain and the exoplasmic domain (about 35 and 15% of the protein mass, respectively). The cytoplasmic domain is made of the N- and C-termini, and a large central loop between TMHs 6 and 7. The exoplasmic domain includes a fairly large, glycosylated loop between TMHs 1 and 2. These two nonmembrane domains show the isoform-specific amino acid sequence divergence (Mueckler, 1994) suggesting their roles in tissue-specific regulation of the transporter function. Indeed, a large body of molecular biological (Mueckler, 1994; Birnbaum, 1992; Oka *et al.*, 1990; Verhey and Birnbaum, 1994; Verhey *et al.*, 1993; James *et al.*, 1994) and biochemical (Lawrence *et al.*, 1990) data suggest that this cytoplasmic domain is important in isoform-specific regulation of GLUT function (the regulatory domain) (Fig. 1). Exactly how, at the molecular level, this domain participates in the regulation is yet to be elucidated. Our working hypothesis has been that a specific, cytosolic protein(s) is (are) involved in this regulation; this putative protein may interact with GLUT protein at the cytoplasmic domain and modulate GLUT function in response to hormonal or metabolic signals (Lee and Jung 1997; Jung, 1998).

GLUT4 constantly recycles via endocytosis and exocytosis

Steady state kinetic measurements have revealed that GLUT4 in rat adipocytes constantly recycles between the plasma membrane and an intracellular storage pool in a process essentially describable by two discrete first-order rate constants, one for internalization (k_{in}), and the other for externalization (k_{ex}) (Jhun *et al.*, 1992). The study also revealed that insulin affect both rate constants; it reduced k_{in} by 2.8 fold and increased k_{ex} by 3.3 fold. These findings indicate that insulin-induced GLUT4 recruitment results from modulation of GLUT4 endocytosis as well as its exocytosis, the biochemical basis of which may also be distinct, thus should be studied individually. Okadaic acid, a protein phosphatase inhibitor which stimulates glucose transport and induces insulin-like GLUT4 recruitment in basal adipocytes, increases k_{ex} without affecting k_{in} (Rampal *et al.*, 1995). Okadaic acid at higher concentrations interferes with the insulin effect (Lawrence *et al.*, 1990). These findings suggest that insulin may modulate GLUT4 recycling by changing the phosphorylation state of a specific serine/threonine phosphoprotein, if not GLUT4 itself, possibly by modulating a specific phosphatase including PP1 or PP2A (Begum, 1995; Ragolia and Begum, 1997).

Insulin signal transduction pathway that is responsible for GLUT4 recruitment

Isolated rat adipocytes, when incubated with insulin, increases glucose flux and the plasma membrane GLUT4 level typically by 6 and 10 fold, respectively, within 10 min. The insulin signal pathway leading to these effects is only incompletely understood: When insulin binds to its receptor, it activates the receptor tyrosine kinase activity by autophosphorylation (Kasuga *et al.*, 1982). This causes tyrosyl-phosphorylation of IRS-1 (Rothenberg *et al.*, 1991). The IRS-1 in turn activates PI3-kinase (Backer *et al.*, 1992). This IRS-1-PI3-kinase interaction has been suggested to be part of the signal transduction pathway for insulin-induced GLUT4 redistribution (Kanai *et al.*, 1993; Katagiri *et al.*, 1996). Recent studies also implicated protein kinase B/Akt as an essential component in this pathway (Kohn *et al.*, 1996). Further downstream elements in this pathway linking the signal to GLUT4, however, are yet to be identified. It is quite likely that this downstream pathway also involves a series of protein-protein interactions, including one that directly interacts with GLUT4 (GTBP) and modulates GLUT4 subcellular redistribution (Fig. 1) (Jung, 1998). This putative, GLUT-regulatory protein (GTRP) could be an enzyme such as a specific phosphokinase or phosphatase that covalently modifies GLUT4, or an adapter protein that indirectly participates in such a modulation.

Identification of cytosolic proteins that bind to GLUT proteins (GTBP)

In an effort to identify this putative GTRP, we have found a number of cytosolic proteins that bind to GLUT proteins. These include an ATP-modulated association of glyceraldehyde-3-phosphate dehydrogenase with GLUT1 (Lachaal *et al.*, 1990) and an ADP- and glucose-6-phosphate-dependent association of bacterial glucokinase with GLUT1 (Lachaal and Jung, 1993). In the latter case, the interaction increased glucokinase activity, suggesting that a similar interaction may occur between GLUT2

and glucokinase, thus may generate glucose signal for insulin secretion in pancreatic beta cells. Using GST-fusion proteins of the cytoplasmic domain, Liu *et al.* (Liu *et al.*, 1995) have identified an ATP-sensitive interaction of a 70 kDa cytosolic protein (GTBP70) in rat adipocytes with GLUT4. GTBP70 was identical to HSC70, a clathrin-uncoating ATPase, and its role in GLUT4 regulation is yet to be demonstrated. Also using GST-fusion proteins, Shi *et al.* (1995) have identified the interaction of the GLUT4 C-terminus with two cytosolic proteins (GTBP28 and GTBP85) in Clone 9 cells, and presented the evidence that this interaction may be involved in azide-induced GLUT1 modulation. More recently, a yeast-based, two hybrid system (Yang *et al.*, 1992) screening of a human skeletal muscle library revealed an interactive protein with GLUT4 C-terminus (unpublished observation by Shi, Y.W. *et al.*). This protein (YP10) was 89% identical and 92% similar to pig heart mitochondrial L-3-hydroxyacyl CoA dehydrogenase (HAD), a fatty acid beta-oxidation enzyme. YP10 may regulate GLUT4 function via a protein acylation as a part of cross talk between glucose and fatty acid metabolism.

There is a number of interesting GTRP candidates that have been implicated in regulation of GLUT4 recycling. These include clathrin (Corvera *et al.*, 1994), clathrin-associated proteins, APs (Moore *et al.*, 1987), AP medium chain subunits (Schmid, 1997), protein kinase B/Akt (Kohn *et al.*, 1996; Calera *et al.*, 1998), protein kinase C-zeta (Standaert *et al.*, 1997), protein phosphatase-1 and 2A (Ragolia *et al.*, 1997; Tung *et al.*, 1985), NSF or SNAP (Warren, 1990), specific GTP binding proteins (Keller *et al.*, 1991) and specific kinases (Clancy *et al.*, 1990; Del Vecchio and Pilch, 1991). Whether any of these proteins directly interact with GLUT4 is yet to be studied.

A Novel assay system for GLUT4 regulatory protein (GTRP) function

Successful identification of a GTRP requires an unequivocal demonstration of its expected role in an *in vivo* assay system, ideally in a physiological insulin-target cell such as rat adipocytes. The use of transfected primary adipocytes to this end is currently limited by low transfection efficiency (5-10%). We have devised a versatile method to incorporate polypeptides into adipocytes and study their effects on endogenous GLUT4 in host cells. The method uses PEG-induced fusion of polypeptide-loaded rabbit erythrocyte ghosts with adipocytes (Fig. 2). The protein incorporation efficiency is close to 100%. The protein concentration in host cells can be readily adjusted. The rabbit erythrocytes ghosts contain very little GLUT1 with no other GLUT isoforms, and the introduction of GLUT1 is minimal in our standard fusion protocol. The fusion protocol by itself does not affect the GLUT4 function and regulation in host adipocytes.

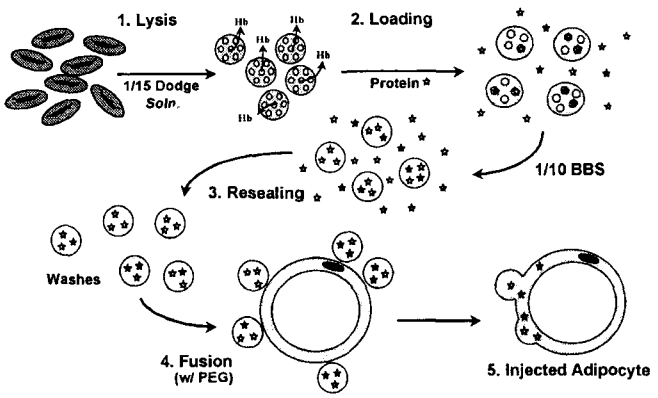

Fig. 2. Red-cell-mediated microinjection. A method to incorporate polypeptides into adipocytes. Cell sizes in this drawing are not to scale.

A GLUT4 C-terminus-derived peptide stimulates GLUT4 function in rat adipocytes

Using this ghost-adipocyte fusion procedure, we have introduced a synthetic peptide of GLUT4 C-terminus into adipocytes and shown that the peptide causes insulin-like effects in rat adipocytes (Lee and Jung, 1997). The C-terminal peptide was synthesized or prepared as recombinant protein (95% pure based on mass spectroscopy). Low millimolar concentrations of GLUT4 C-peptide in adipocyte cytosol greatly increased the native GLUT4 level in the host cell plasma membrane with a stoichiometric reduction in microsomal GLUT4 level. The effect was dose-dependent. GLUT1 levels are not affected. The effect is specific to GLUT4 C-peptide; incorporation of GLUT1 C-peptide exerted no effect on the levels of GLUT1 or GLUT4. Significant stimulation of 3-O-methyl-D-glucose flux was also observed after incorporation of GLUT4 C-peptide but not of GLUT1 C-peptide. These peptide effects on GLUT4 recruitment and 3OMG flux were not additive to the insulin effects.

These findings not only suggest the importance of the C-terminus in GLUT4-regulation in rat adipocytes, but also offer an insight into the molecular events underlying GLUT4 recycling (Fig. 3). The peptide may interfere with either GLUT4 internalization and/or GLUT4 sequestration, which may be mediated by specific GTRPs. It may be an endocytic adapter for the former case (Fig. 3, left panel), or an intracellular retention molecule for the latter (Fig. 3, right panel). In either case, GLUT4 C-peptide in excess may exert the effect by competing with native GLUT4 for the GTRP (dominant negative effect). This interpretation can be tested once an appropriate GTRP is identified. Steady-state kinetic measurements of GLUT4 recycling in host adipocytes may define which mechanism

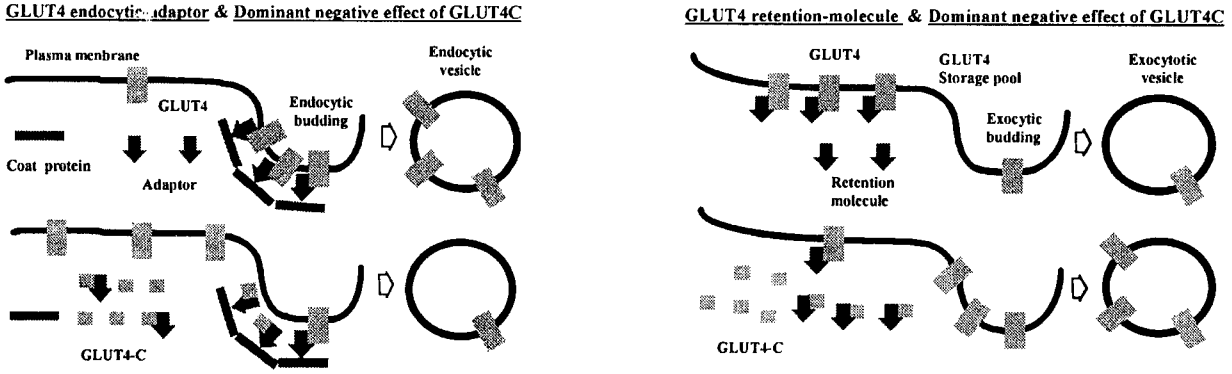

Fig. 3. Two possible interpretations of GLUT4C peptide-induced GLUT4 recruitment in adipocytes

(endocytosis or intracellular retention) is affected by the peptide.

CONCLUISION

Understanding at the molecular level how insulin causes GLUT4 recruitment in normal cells would give an insight into the identification of the nature of the defect underlying the insulin resistance. In this report we showed an evidence that the cytoplasmic domain of GLUT4 is involved in this insulin-induced GLUT4 recruitment by interacting with a specific cellular protein (GTRP) or proteins. These putative regulatory proteins are expected to modulate the exocytosis, the endocytosis, or both during GLUT4 recycling. The identification, cloning, and characterization of this GLUT4 regulatory protein or proteins will greatly enhance our understanding how insulin regulates glucose transport in normal muscles and adipose tissue. More importantly, it may lead to a novel drug design and development in the prevention, diagnosis and treatment of diabetes and obesity. Dissection of the GLUT4 C-peptide to identify the minimal amino acid sequence that may be responsible for its GLUT4 recruiting effect is currently in progress.

ACKNOWLEDGEMENTS

Work described here were supported in part by grants from National Institutes of Health, American Heart Association, and Buffalo VA Medical Center.

REFERENCES CITED

Backer, J. M., Myers, M. G. Jr., Shoelson, S. E., Chin, D. J., Sun, X. J., Miralpeix, M., Hu, P., Margolis, B., Skolnik, E. Y. and Schlessinger, J., Phosphatidylinositol 3'-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J.*, 11, 3469-3479 (1992).

Begum, N., Stimulation of protein phosphatase-1 activity by insulin in rat adipocytes. Evaluation of the role of mitogen-activated protein kinase pathway. *J. Biol. Chem.*, 270, 709-714 (1995).

Birnbaum, M. J., The insulin-sensitive glucose transporter *Int. Rev. Cytol.*, 137A, 239-297 (1992).

Calera, M. R., Martinez, C., Liu, H. Z., El Jack, A. K., Birnbaum, M. J. and Pilch, P. F., Insulin increases the association of Akt-2 with GLUT4-containing vesicles. *J. Biol. Chem.*, 273, 7201-7204 (1998).

Chin, J. J., Jung, E. K. Y. and Jung, C. Y., Structural basis of human erythrocyte glucose transporter function in reconstituted vesicles. Circular dichroism studies. *Proc. Natl. Acad. Sci. USA*, 84, 4113-4116 (1987).

Clancy, B. M. and Czech, M. P., Hexose transport stimulation and membrane redistribution of glucose transporter isoforms in response to cholera toxin, dibutyryl cyclic AMP, and insulin in 3T3-L1 adipocytes. *J. Biol. Chem.*, 265, 12434-12443 (1990).

Corvera, S., Chawla, A., Chakrabarti, R., Joly, M., Buxton, J. and Czech, M. P., A double leucine within the GLUT4 glucose transporter COOH terminal domain functions as an endocytosis signal. *J. Cell Biol.*, 126, 979-989 (1994).

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

DeFronzo, R. A., Jacto, E., Jequire, E., Meader, E., Wahren, J. and Felber, J. P., The effect of insulin on the disposal of intravenous glucose. *Diabetes*, 30, 1000-1007 (1981).

DeFronzo, R. A., The triumvirate: beta cell, muscle, liver; a collusion responsible for NIDDM. *Diabetes*, 37, 667-684 (1988).

Del Vecchio, R. L. and Pilch, P. F., Phosphatidylinositol 4-kinase is a component of glucose transporter (GLUT4)-containing vesicles. *J. Biol. Chem.*, 266, 13278-13283 (1991).

Garvey, W. T., Glucose transport and NIDDM. *Diabetes Care*, 15, 396-417 (1992).

James, D. E., Piper, R. C. and Slot, J. W., Insulin stimulation of GLUT4 translocation: a model for re-

gulated recycling. *Trends in Cell Biol.*, 4, 120-126 (1994).

Jhun, B. H., Rampal, A. L., Liu, H. Z., Lachaal, M. and Jung, C. Y., Effects of insulin on steady state kinetics of GLUT4 subcellular distribution in rat adipocytes: Evidence of constitutive GLUT4 recycling. *J. Biol. Chem.*, 267, 17710-17715 (1992).

Jung, C. Y., Proteins that interact with facilitative glucose transporters: Implication for function. *Exptl. Physiol.*, 83, 267-273 (1998).

Kahn, B. B., Glucose transport: Pivotal step in insulin action. Lilly Lecture 1995 Diabetes, 45, 1644-1654 (1996).

Kanai, F., Ito, K., Todaka, M., Hayashi, H., Kamohara, S., Ishii, K., Okada, T., Hazeki, O., Ui, M. and Ebina, Y., Insulin-stimulated GLUT4 translocation is relevant to the phosphorylation of IRS-1 and the activity of PI3-kinase. *Biochem. Biophys. Res. Commun.*, 195, 762-768 (1993).

Kasuga, M., Zick, Y., Blith, D. L., Karlsson, F. A., Haring, H. U. and Kahn, C. R., Insulin stimulation of phosphorylation of the beta subunit of the insulin receptor. Formation of both phosphoserine and phosphotyrosine. *J. Biol. Chem.*, 257, 9891-9894 (1982).

Katagiri, H., Asano, T., Ishihara, H., Inugkai, K., Shibasaki, Y., Kikuchi, M., Yazaki, Y. and Oka, Y., *J. Biol. Chem.*, 271, 16987-16990 (1996).

Keller, S. R., Kitagawa, K., Aebersold, R., Lienhard, G. E. and Garner, C. W., Isolation and characterization of the 160,000-Da phosphotyrosyl protein, a putative participant in insulin signaling. *J. Biol. Chem.*, 266, 12817-12820 (1991).

Kohn, A. D., Summers, S. A., Birnbaum, M. J. and Roth, R. A., Expression of a constitutively active Akt ser/thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J. Biol. Chem.*, 271, 31372-31378 (1996).

Lachaal, M. and Jung, C. Y., Interaction of facilitated glucose transporter with glucokinase and its modulation by glucose-6-phosphate. *J. Cell Physiol.*, 156, 326-332 (1993).

Lachaal, M., Berenski, C. J., Kim, J. and Jung, C. Y., An ATP-modulated specific association of glyceraldehyde-3-phosphate dehydrogenase with human erythrocyte glucose transporter. *J. Biol. Chem.*, 265, 15449-15454 (1990).

Lawrence, J. C. Jr., Hiken, J. F. and James, D. E., Stimulation of glucose transport and glucose transporter phosphorylation by okadaic acid in rat adipocytes. *J. Biol. Chem.*, 265, 19768-19776 (1990).

Lee, W. and Jung, C. Y., A synthetic peptide corresponding to the GLUT4 C-terminal cytoplamic domain causes insulin-like glucose transport stimulation and GLUT4 recruitment in rat adipocytes. *J. Biol. Chem.*, 272, 21427-21431 (1997).

Liu, H. Z., Xiong, S. H., Samuel, S. J., Shi, Y. W., Lachaal, M. and Jung, C. Y., ATP sensitive binding of a 70kDa cytosolic protein to glucose transporter in rat adipocytes. *J. Biol. Chem.*, 270, 7869-7875 (1995).

Moore, M. S., Mahaffey, D. T., Brodsky, F. M. and Anderson, R. G. W., Assembly of clathrin-coated pits onto purified plasma membranes. *Science*, 338, 558-563 (1987).

Mueckler, M., Facilitative glucose transporters. *Eur. J. Biochem.*, 219, 713-725 (1994).

Office of the Director, NIH, NIDDKD. 1997, Diabetes mellitus: Challenges and Opportunities, Final Report and Recommendations. Natcher Conference Center, NIH, September 4-5.

Oka, Y., Asano, T., Shibasaki, Y., Lin, J. L., Tsukuda, K., Katagiri, H., Akanuma, Y. and Takaku, F., C-Terminal truncated glucose transporter is locked into an inward-facing form without transport activity. *Nature*, 345, 550-553 (1990).

Pessin, J. E. and Bell, G. I., Mammalian facilitative glucose transporter family: Structure and molecular regulation. *Annu. Rev. Physiol.*, 54, 911-930 (1992).

Ragolia, L. and Begum, N., The effect of modulating the glycogen-associated regulatory subunit of protein phosphatase-1 on insulin action in rat skeletal muscle cells. *Endocrin.*, 138, 2398-2404 (1997).

Rampal, A. L., Jhun, B. H., Kim, S. S., Liu, H. Z., Manka, M., Lachaal, M., Spangler, R. A. and Jung, C. Y., Okadaic acid stimulates glucose transport in rat adipocytes by increasing the externalization rate constant of GLUT4 recycling. *J. Biol. Chem.*, 270, 3938-3943 (1995).

Rothenberg, P. L., Lane, W. S., Karasik, A., Baker, J., White, M. and Kahn, C.R., Purification and partial sequence analysis of pp185, the major cellular substrate of the insulin receptor tyrosine kinase. *J. Biol. Chem.*, 266, 8302-8311 (1991).

Schmid, S. L., Clathrin-coated vesicle formation and protein sorting: an integrated process. *Annu. Rev. Biochem.*, 66, 511-548 (1997).

Shi, Y. W. H., Liu, H. Z., Vanderburg, G., Samuel, S. J., Ismail-Beigi, F. and Jung, C. Y., Modulation of GLUT1 intrinsic activity in Clone 9 cells by inhibition of oxidative phosphorylation. *J. Biol. Chem.*, 270, 21772-21778 (1995).

Standaert, M. L., Galloway, L., Karnam, P., Bandyopadhyay, G., Moscat, J. and Farese, R. V., Protein kinase C-zeta as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation I rat adipocytes. *J. Biol. Chem.*, 272, 30075-30082 (1997).

Stephens, J. M. and Pilch, P. F., The metabolic regulation and vesicular transport of GLUT4, the major insulin-responsive glucose transporter. *Endocrinology Reviews*, 16, 529-546 (1995).

Suzuki, K. and Kono, T., Evidence that insulin cause

translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc. Natl. Acad. Sci. USA*, 77, 2542-2545 (1980).

Tung, H. Y. L., Pelech, S., Fisher, M. J., Pogson, C. I. and Ohen, P., The protein phosphatases involved in cellular regulation. Influence of polyamines on the activities of protein phosphatase-1 and protein phosphatase-2A. *Eur. J. Biochem.*, 149, 305-313 (1985).

Verhey, K. J. and Birnbaum, M. J., A leu-leu sequence is essential for COOH-terminal targeting signal of GLUT4 glucose transporter in fibroblasts. *J. Biol. Chem.*, 269, 2353-2356 (1994).

Verhey, K. J., Hausdorff, S. F. and Birnbaum, M. J., Identification of the carboxy terminus as important for the isoform-specific subcellular targeting of glucose transporter proteins. *J. Cell Biol.*, 123, 137-147 (1993).

Warram, J. H., Martin, B. C., Krolewski, A. S., Soeldner, J. S. and Kahn, C. R., Slow glucose removal rate and hyperinsulinemia precede the development of type II in the offspring of diabetic parents. *Ann. Intern. Med.* 113, 909-915 (1990).

Warren, G., Intracellular transport: Vesicular consumption *Nature* 345, 382-383 (1990).

Yang, X., Hubbard, E. J. A. and Carlson, M., A protein kinase substrate identified by the two-hybrid system. *Science*, 257, 680-682 (1992).

Zheng, H., Rampal, A. L., Parthasarathy, R. and Jung, C. Y., Proposed structure of putative glucose channel in GLUT1 facilitative glucose transporter. *Biophysical J.*, 70, 14-21 (1996).